Horticulture Innovation Australia

Final Report

National Potato Breeding Program: Strategic Trait Development

Tony Slater
The Dept of Economic Development & Resources

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Media Summary

Potatoes are the fourth-largest food crop internationally, and account for 20% of all vegetable production in Australia. Unfortunately, the current main Australian commercial cultivars suffer from a number of production and quality issues, which this project has investigated.

The techniques developed in this project will change the way potato breeding is conducted. This project has investigated the rapidly advancing area of molecular genetic technologies. As a result of reviewing the publicly available resources, undertaking economic cost comparisons between alternatives and through establishing significant international links with the leading research groups, the project has validated and implemented DNA markers, and opened significant opportunities to develop improved potato cultivars for Australia. These efforts have enabled this Australian research program to become internationally recognised, within a very short period.

We have developed a greater understanding of potato genetics, which provides understanding of the biology and how to control two major problems the Australian industry has had over recent years, potato cyst nematodes (PCN) and *Potato virus Y* (PVY). By understanding resistance and the resistance mechanisms controlling PCN and PVY we can now provide answers for all members of the industry to aid in their control.

We have also adapted a genetic analysis technique used in livestock breeding that could be widely applied to potato research, producing significant advances. This technique could also be widely applied to other horticultural research in Australia, and we expect that it will be adopted globally.

The Australian program is now positioned to continually improve cultivars, build significantly on previous efforts, and have populations grown in remote areas to the central program, which will enable cultivars to be bred in specific regions such as Tasmania and Western Australia.

Breeding once involved comparing characteristics of potential new cultivars. Numbers and population size once mattered in the search to find a superior cultivar. Now the populations can be designed and tested to determine the better families, parents and cultivars, due to a better understanding of their genetics. Today computing and technological advances are far more important than numbers in breeding, and this will change the progressive potato breeding programs globally.

Technical Summary

Potatoes are the fourth-largest food crop internationally, and account for 20% of all vegetable production in Australia. Although potatoes are a valuable food crop for Australia, the current main commercial cultivars suffer from a number of production and quality issues. Prior to the start of this project the Potato Breeding Program was reviewed twice, which saw the old program develop into two distinct projects. The first continued to develop improved cultivars, but in partnership with industry investors, while this project investigated the genetics and biology of important traits for the Australian industry.

Understanding potato genetics is not just about potato breeding, there are much wider applications.

This project has developed a much greater understanding of potato genetics, which has led to an understanding of the biology and how to control two major pest and disease problems the Australian industry has had over recent years, potato cyst nematodes (PCN) and *Potato virus Y* (PVY). By understanding resistance and the resistance mechanisms controlling PCN and PVY we can now provide some answers for all parties in the Australian potato industry.

We have also been able to adapt a genetic analysis technique used in livestock breeding that could be widely applied to potato research and produce significant advances. This technique could also be widely adapted to other horticultural research in Australia, as we expect that it will be adopted globally.

Understanding potato genetics has led to a better understanding of molecular genetics and quantitative genetics which can be applied into breeding programs. Breeding programs can operate without the application of molecular techniques and quantitative genetic analysis, however to deliver results and products with greater assurance, rates of genetic gain and improvement these tools are essential. The rate of adoption and integration of these tools and techniques will determine and dictate the most viable and successful programs internationally.

This project has:

- Reviewed the literature and identified the relevant publicly available genetic and genomic resources.
- Established significant international linkages with the most progressive research groups globally, to ensure the most advanced techniques were adopted.
- Developed a potato genotype identity kit, to enable DNA fingerprinting of Australian cultivars.
- Prioritised the traits for marker-assisted selection based on their importance for the Australian industry.
- Phenotyped the main cultivars for PCN, PVY, and PVX resistance, and validated markers for these traits, which identified current resistant cultivars.
- Sequenced the genome of 16 isolates of PVY, and identified that there have been two introductions of PVY, including a recent introduction of PVY^{NTN}.
- Analysed the genetics of PVY resistance, which provides an explanation to the recent increase in crop infections.
- Developed a multiplexed assay for the validated markers to ensure cost-effectiveness when applied into a breeding program.
- Costed conventional screening and marker-assisted selection, compared the methods, and found MAS to be very cost-effective, once validated.

- Investigated various statistical analysis packages and databases to improve the computational ability of a breeding program.
- Investigated the analysis of quantitative traits using analyses employed in livestock breeding and adapted them for use in potato breeding.
- Developed the ability to calculate Estimated Breeding Values (EBVs) for potatoes.
- Estimated the heritability of nine important traits, including breeder's visual preference and yield, and calculated the expected genetic gain.
- Used EBVs to improve cross generation prediction for traits with low heritability.
- Published our results so that progressive breeding programs globally will be able to adopt these protocols.
- Developed significant molecular and genetic solutions that will greatly accelerate the breeding cycle and has the potential to change potato breeding globally.

As a result of the last five years work, the techniques developed in this project will change the way potato breeding is conducted in Australia. Now the populations can be designed and tested to determine the better families, parents and cultivars. This change is directly due to a better understanding of the genetics and biology of the traits, quantitative genetics and molecular genetics.

Breeding once involved comparing characteristics of potential new cultivars. Numbers and population size matter in the search to find a superior cultivar. Now computing and technological advances are far more important than numbers, and this will change the progressive potato breeding programs globally. The Australian program is now in a position to continually improve cultivars, build significantly on previous efforts, and have populations grown in remote areas to the central program, which will enable cultivars to be bred in specific regions such as Tasmania and Western Australia.

Introduction

Potatoes are the fourth-largest food crop internationally, and account for 20% of all vegetable production in Australia. Approximately 1.2 million tonnes of potatoes are produced annually in Australia, valued at $557 million *per annum* at the farm gate (ABS 2009). The retail value is estimated to be worth $550 million for fresh market sales, $600 million processed as French fry style product, and $600 million processed as crisps, which represents 3% of supermarket sales. Processing potatoes account for 56%, fresh potatoes 36%, and seed production make up the remaining 8%. This production is spread across all Australian states (but not Territories) with Victoria, Tasmania and South Australia each accounting for almost one quarter of the crop.

Although potatoes are a valuable food crop for Australia, the current main commercial cultivars still suffer from a number of production and quality issues, of which disease susceptibility, tuber distortion, internal disorders, and storage problems are just a few. Disease susceptibility, in particular, can be potentially catastrophic for the industry, as major outbreaks do occur worldwide. Abiotic factors such as water availability, temperature, nutrition and photoperiod also affect crops across the various production areas in Australia.

Agricultural production has made significant advances over the last two centuries due to the development of improved cultivars, as well as advances in agrochemical and agricultural production. Mendel's discovery of the principles of genetic inheritance, and Darwin's description of hybrid vigour contributed to the accelerated development of new cultivars by plant breeders during the twentieth century. Despite this progress, factors such as changing agricultural practices, population growth, climate change and economic factors are also driving the need for new cultivars. Secondarily, pests and diseases can overcome genetic resistance factors, and urbanisation is resulting in more marginal land being used for farming. These factors place pressure on plant breeders to develop new cultivars in order to maintain and improve productivity despite these issues. Towards the end of the twentieth century, a greater understanding of quantitative genetics and advances in molecular biology have seen further understanding of Mendel and Darwin's discoveries, which will assist plant breeders to accelerate genetic gain in crops. Genetic markers based on variation in DNA, derived from research in molecular genetics, provide great potential to assist plant breeders to identify genes of interest for the development of new cultivars to address these needs.

Prior to the start of this project, the Potato Breeding Program was reviewed twice to identify a direction for the future. The scientific direction of the program was reviewed (Brennan et al., 2004), as well as the interaction with industry members, to establish a firm route to market for the cultivars developed in the program (DArT, 2006). These reviews have seen the program develop a strong pre-breeding focus on developing technologies, more effective screening techniques and the construction of a germplasm collection that will underpin the future Australian potato industry. The second area will adopt these technologies to support conventional breeding techniques in order to accelerate the development of improved commercial cultivars, which will be rapidly transferred to investors for commercial evaluation and subsequent commercialisation.

The Brennan review of the National Potato Breeding Program identified that the program would be enhanced by the use of gene markers (Brennan et al., 2004). At that time, the Victorian DPI was already using this technology in other plant breeding programs such as those directed to wheat, canola, and both temperate pasture grasses and legumes. It was seen that the potato breeding program would benefit from this enhancement, as a number of key breeder's traits may be efficiently selected using molecular markers, including disease resistance and tolerance to abiotic

stresses (particularly water limitation). Molecular markers will allow the detection of pre-existing genetic variation for these factors at the glasshouse seedling stage or in early field generation trials.

DNA-based markers have great potential to assist plant breeders to identify genes of interest for the development of new cultivars. Marker-assisted breeding in potato has not yet been applied to the degree observed in other crops, such as the cereals (Collard and Mackill, 2008). This is presumably because of the complexities of potato (autotetraploid) genetics, the absence of detailed functional maps and lack of genetic markers linked with the numerous qualitative and quantitative traits of interest. The majority of studies to identify markers have also been performed on diploid cultivars and populations, which do not always prove useful for tetraploid breeding populations.

The program also has the potential to be enhanced by the adoption of analyses undertaken in livestock breeding programs of complex quantitative traits. These analyses have developed Estimated Breeding Values (EBVs) for a number of important traits within dairy cattle, beef cattle, fat lambs, poultry and salmon breeding programs and have resulted in a substantial improvement in these important traits for those industries.

Use of DNA markers and EBVs will have the potential to greatly accelerate the breeding cycle in potatoes, and even change the breeding model used in Australia.

Over the last few years, the Australian Potato Breeding Program has established collaborative links with the James Hutton Institute (JHI, Scotland), United States Department of Agriculture (USDA), Teagasc (Ireland) and Wageningen University (the Netherlands). We now have interactions with potato breeding companies and research groups around the world, and we have visited a number of these programs in order to adopt the most advanced breeding techniques used anywhere in the world.

Objectives

This project aimed to develop more effective screening techniques through the development and application of molecular markers, to develop the computational ability to more rapidly combine desirable traits, and strengthen global links to more rapidly introduce international advances into the Australian program.

Materials and Methods

1. Review of Literature and the publicly available resources.

A review of the literature was undertaken using the main literature databases of Agricola, Cab Abstracts, Scopus and Google Scholar. Articles were also obtained from on-line journal resources. The internet was also searched for the publicly available genetic and genomic resources.

2. International linkages.

During the course of the project, members of the project team regularly attended international potato and relevant genetic and genomic research conferences. Attendance at these conferences enabled members of the team to understand the current research being undertaken around the globe, as well as identifying other researchers active in the field. While travelling to attend these conferences, the team members also undertook to visit research laboratories in the vicinity of the conference. During these conferences and trips, formal and informal discussions were held with leading researchers from around the globe.

3. Potato Genotype Identity Kit

The application of molecular genetic markers to develop a potato genotype identity (PGI) kit, relevant to the Australian potato industry has been undertaken. A collection of 12 specific highly polymorphic molecular markers were identified and experimentally validated (Table 3.1.1).

Table 3.1.1. Molecular marker identity validated in the developed PGI kit

Pool	MM	Marker	Allele Size Range (bp)	Dye Label
1	1	STM0006	102-126	FAM/Blue
1	2	STM2005	153-213	TAMRA/Yellow
1	3	STM2022	190-252	FAM/Blue
1	4	STM5127	248-294	ROX/Red
2	5	STG0016	137-172	ROX/Red
2	6	STG3012	179-227	FAM/Blue
2	7	STM3023	185-215	Hex/green
2	8	SSR1	169-236	TAMRA/Yellow
3	9	STI0004	90-127	HEX/Green
3	10	STM3009	157-191	TAMRA/Yellow
3	11	STM0019	180-252	FAM/Blue
3	12	STM5136	220-294	HEX/Green

All of the generated marker profiles and products were recorded and have been documented (an example of one of the twelve assays is provided in Figure 3.1.1) for ease of future analysis, along with the development of a comprehensive standard operating procedure (SOP) that has been attached to this report.

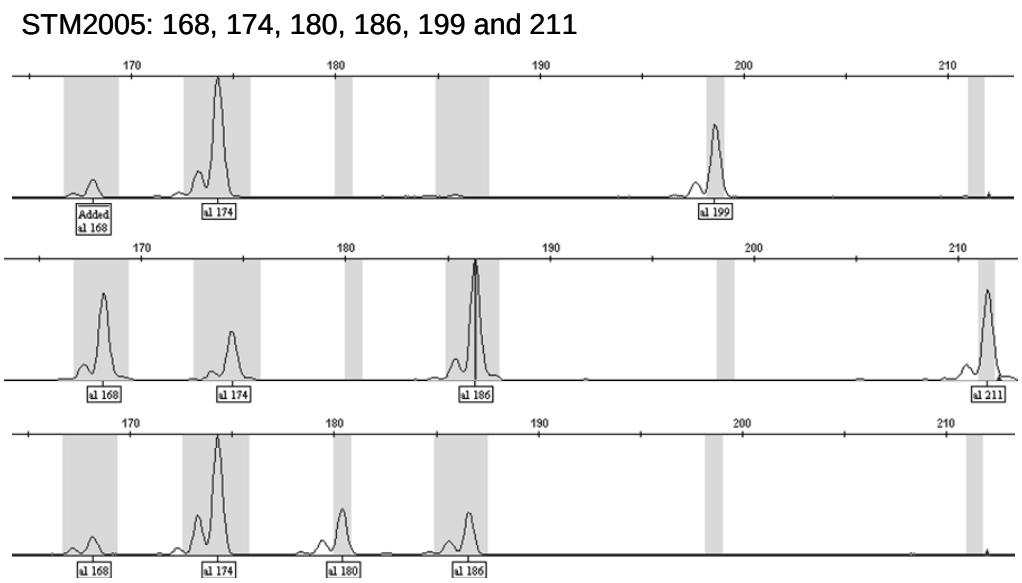

Figure 3.1.1. Example of typical marker resolution profiles and products obtained from PCR using the molecular marker STM2005, values indicated under the amplified product are in base pair size. Analysis was performed using the GeneMapperv3.7 software application (Applied Biosciences).

4. Priorities for MAS

Following the review of the literature and publicly-available genomic resources, the priorities for marker-assisted selection were determined based on the priority of traits for development. These were ranked on a five point scale from low to high priority. Then the ease of phenotyping and genotyping were compared in order to determine where the best gains could be made from replacement of conventional screening with MAS.

5. Potato Cyst nematode resistance marker development

The potato cyst nematodes (PCN), *Globodera rostochiensis* (Woll.) and *G. pallida* (Stone), are major pests of potato crops world-wide. They cause significant yield reductions and severely impact the movement of potatoes. In Australia, *G. rostochiensis* has been discovered in only a few areas, and they are the subject of strict regulation and quarantine procedures, which create severe market access issues. To date, the only species and pathotype detected has been the golden potato cyst nematode, *G. rostochiensis* pathotype Ro1 (Hinch et al., 1998, Faggian et al., 2011).

Resistance to *G. rostochiensis* has been attributed to a number of genes, which confer near absolute (*H1, Gro1, GroVI*) or partial (*Gro1.2, Gro1.3, Gro1.4, Grp1*) resistance (Gebhardt and Valkonen, 2001, Finkers-Tomczak et al., 2011). The *Grp1* locus also confers partial resistance to the pale cyst nematode (*G. pallida*) (Finkers-Tomczak et al., 2009). The *H1* gene was introduced from *S. tuberosum* ssp. *andigena* (Toxopeus and Huijsman, 1953). This gene confers resistance against 2 of the 5 known pathotypes, Ro1 and Ro4 (Kort et al., 1977), and has been extensively introgressed into commercial potato cultivars (Simko et al., 2007, Finkers-Tomczak et al., 2011).

H1 was mapped to chromosome 5 and is located close to the markers TG689 (De Koeyer et al., 2010, Milczarek et al., 2011) and 57R (Finkers-Tomczak et al., 2011, Schultz et al., 2012).

Plant Material

Plant materials used in this study were from tetraploid potato cultivars and breeding lines selected from the Australian potato breeding program's germplasm collection. All planting material for phenotyping trials consisted of small tubers produced by the breeding program during the previous summer. Leaf material for DNA extraction was collected from plants growing in the breeding program's annual parental material multiplication trial. The leaf samples consisted of approximately 1.5 cm^2 of leaf material placed directly into 96 well plates, with a duplicate sample placed in a resealable plastic bag for freezing at -70^oC.

PCN phenotyping assay

A quarantine facility was established to conduct all experiments using PCN. The facility consisted of a glasshouse modified to eliminate the risk of PCN escape, and quarantine procedures to ensure strict adherence to specific hygiene protocols, including the proper disposal of soil and other waste materials. Briefly, access to the glasshouse was restricted by lock and key and entry was via a separate anteroom, with those entering the glasshouse-proper required to wear protective spray suits and watertight plastic boot covers. Before exiting the glasshouse, protective clothing was discarded, hands were washed with 1% chlorhexidine and footwear was treated in a Biogram (Ecolab, Castle Hill) footbath. In the glasshouse, 75 μm mesh traps were installed in all drains to capture cysts in run-off water. All waste, including potting media, soil and plant material, were double-autoclaved before disposal by deep burial. The quarantine facility and associated protocols were developed with, and approved by, Biosecurity Victoria, a government agency that delivers programs to preserve market access for Victoria's livestock, plant, fisheries and forestry industries.

PCN soil sampling and inoculum preparation

PCN infested soil was sampled from infection 'hotspots' within the Koo Wee Rup PCN control area in Victoria, Australia. Soil-sampling and hotspot-identification was carried out with Biosecurity Victoria staff. A total of 40 L of infested soil was sampled from an infested paddock using a 10 m by 10 m grid sampling regime. Soil was sampled with a shovel in each grid section to a depth of approximately 30cm after removing the upper pasture layer. Soil was placed in one of two 20 L plastic drums and transported directly to the laboratory for cyst isolation, identification and quantification. Cysts were extracted from 500g sub-samples of soil using the Fenwick can flotation method (Fenwick, 1940, Turner, 1998), and counted. The concentration of cysts in the sub-sample was 525 cysts/500g of soil.

The number of eggs per cyst, and egg viability, were also determined. Eggs were counted by taking a sub-sample of ten extracted cysts, soaking them in sterile distilled water for 24 hours and breaking each open with a set of fine tweezers. Eggs were scraped out of the body of the cyst onto a glass slide containing a 3 mm grid on which all eggs and hatched juvenile nematodes were counted. Egg-viability was assessed by soaking ten cysts in a 0.01% Nile Blue stain for 48 hours, breaking them open on to the 3 mm grid slide, and counting stained (non-viable) and unstained (viable) eggs. Each cyst contained an average of 323.5 eggs, and viability was 65%.

The PCN species identity was confirmed to be *G. rostochiensis* using a species-specific polymerase chain reaction (PCR) assay (Bulman and Marshall, 1997). DNA was extracted from soil-extracted cysts that were sampled from potential infestation-sites, and also from cysts that developed on test cultivars in this study. DNA was extracted from the cysts according to the method outlined by Bulman and Marshall (1997) and also using a FastDNA™ Spin kit for soil (Qbiogene, California,

USA), as per the manufacturer's instructions. The extracted DNA was subjected to PCR amplification using the specific primers PITSr3 and PITSp4 (Bulman and Marshall, 1997), and the generic Internal Transcribed Spacer region primer ITS5 (White et al., 1990), in a Hybaid PCR Express ver. 2.0 thermocycler according to the conditions described by Bulman and Marshall (1997).

Planting material and growth conditions

Selected potato cultivars were sown in 100 mm pots, in the aforementioned quarantine facility, during August 2005, 2007 and 2008 with three replicates of each cultivar. Pots were filled with approximately 100 g of a pine-based potting mix followed by a layer of infested soil. The volume of soil used was 45 ml, which was determined to achieve a final egg-per-pot concentration of approximately 9500 eggs. One small tuber of each cultivar was then partially imbedded in the soil, and the pots were topped up with additional potting mix as required, with an average pot weight (soil and potting mix) of 250 g. Pots were placed on raised benches in a randomised block design and watered by hand. The tubers were fertilised initially with 10g of Osmocote Plus and then periodically throughout the trial's duration with Nitrosol. Positive control and negative control pots were also included: positive controls consisted of the susceptible cultivar Ilam Hardy inoculated as described above, and negative controls consisted of un-inoculated Ilam Hardy. Positive control pots were also harvested at regular intervals in order to monitor the development of cysts, and thereby to predict the optimum time of harvest for the whole experiment. Plants were allowed to continue to grow post-assessment in order for the cysts to complete their life-cycle, and the potting mix and cysts were retained for subsequent trials.

Cyst assessment

Harvest was carried out approximately 10 weeks after planting. Each plant was removed from the pot, and its roots were visually assessed for the presence of PCN cysts, and counted. By this stage the plants were beginning to appear 'pot-bound' (that is, root growth filled the pot and were circling the root ball), and it was therefore easy to observe the root ball and also to replace them in the pots if further incubation was required. Plants with 3 or more cysts were considered susceptible, and plants with no cysts were considered resistant (John Anderson, pers comm). A cultivar was classified as resistant or susceptible if all replicates were consistent. If an inconsistent result was observed, such as two resistant plants and one susceptible, the cultivar was reassessed. Cultivars that allowed development of greater numbers of cysts than the susceptible control, Ilam Hardy, were classified as very susceptible.

When possible, harvesting of plants was conducted several days earlier than optimum (as determined on Ilam Hardy plants) to avoid cysts becoming dark brown and therefore difficult to distinguish from the surrounding soil. In those cases where white (presumably immature) cysts were encountered, plants were replaced in their pots and left in the glasshouse for an additional week for reassessment, until such time as golden coloured cysts were observed.

Once the data was collated, it was compared to the characteristics listed on the European Cultivated Potato Database to determine the likely pathotype of the PCN inoculum used in this study.

PCN marker assays

TG689 marker assay

Polymerase chain reaction (PCR) was carried out using a GeneAmp® PCR System 9700 (Applied Biosystems, Foster City, CA, USA). Amplicon-specific primers were designed based on the TG689 haplotype sequences described by De Koeyer *et al*. (2010). A semi-nested PCR approach was adopted, with conserved forward (5' -GGGCTTATCCTGGTTGAAGTA- 3') and reverse (5' –

CAATAGAATGTGTTGTTTCACCAA- 3') primers amplifying a 196 bp fragment in all samples as an internal control for DNA integrity, and nested *H1*-specific primer (5' –TAAAACTCTTGG TTATAGCCTATAG- 3') amplifying a 139 bp fragment in samples with *H1*-mediated PCN resistance. The 5'-terminus of the conserved forward primer and *H1*-specific nested primer were fluorescently labelled with the fluorochromes HEX (4,7,2',4',5',7'-hexachloro-6carboxyfluorescein) and 6-FAM (6-carboxyfluorescein), respectively, to enable high-throughput, automated detection. PCR reactions were performed based on a total volume of 10 µl containing 1x ImmoBuffer (Bioline), 0.25 mM dNTPs (Bioline), 0.25 µM of each primer and 0.5 Unit Immolase™ DNA Polymerase (Bioline). The thermocycling program consisted of an initial denaturation step of 95°C for 10 min, followed by 30 cycles of denaturation at 95°C for 45 s, primer annealing at 55°C for 45 s, and extension at 72°C for 45 s; followed by a final extension at 72°C for 10 min. Aliquots of 2 µl of 100 X diluted PCR amplicon was added to 8 µl of a mixture containing 5000 X diluted GeneScan™ -500LIZ® size standard in Hi-Di™ formamide (Applied Biosystems). Amplicons were separated by capillary electrophoresis using an ABi3730xl DNA Analyzer (Applied Biosystems) and resultant FSA files analysed with GeneMapper V3.7 (Applied Biosystems).

57R marker assay

PCR reaction conditions for amplification of the 57R SCAR marker were similar to those used for TG689, with minor modifications. The non-fluorescently labelled primer pairs, forward (5' –TGC CTGCCTCTCCGATTTCT- 3') and reverse (5' –GGTTCAGCAAAAGCAAGGACGTG- 3') as developed by Finkers-Tomczak *et al*. (2011) were used for screening. These primers were validated by Schultz et al. (2012) to be potentially diagnostic for the *H1* gene, during the course of this project. The 57R marker was combined in a duplex assay with the simple sequence repeat (SSR) marker STM1051 (Milbourne et al., 1998). A single tube assay protocol was devised that multiplexed the two reactions together. The optimised reaction conditions were as follows; PCR reactions were performed in a total volume of 10 µl containing 20 ng of template DNA, all other reaction components as described for the TG689 reaction, with the exception that the four requisite primers were combined together in equal molar ratios. The forward primers for both 57R and STM1051 were fluorochrome labelled with FAM for capillary electrophoresis. The thermocycling program consisted of an initial denaturation step of 95°C for 10 min, followed by 30 cycles of denaturation at 95°C for 45 s, primer annealing at 63°C for 45 s, and extension at 72°C for 45 s; followed by a final extension at 72°C for 10 min. The amplified products were prepared for resolution on an ABi3730xl DNA Analyzer (Applied Biosystems) and resultant FSA files analysed with GeneMapper V3.7 (Applied Biosystems). The marker STM1051 acts as an internal control for DNA quality, but has also been shown to be linked to a locus controlling quantitative resistance to *Verticillium dahliae* (Simko et al., 2004a). Presence of a diagnostic 452 bp band for 57R was compared to the PCN resistance phenotype in order to determine effectiveness of marker prediction.

6. *Potato virus Y* and *Potato virus X* resistance marker development

Potato virus Y

Potato virus Y (PVY) is found world wide and is one of the most important viral problems for seed and commercial potato growers (Gebhardt and Valkonen, 2001, Solomon-Blackburn and Barker, 2001a, Singh et al., 2008). In Australia, PVY has been recorded in all states, and is currently absent or successfully controlled only in WA and Tasmania. PVY can be transmitted by aphids and mechanical damage from machinery, tools and by brushing plants while walking through the field. Symptom expression is cultivar and virus strain dependent, but can include vein necrosis, mottling, yellowing of leaflets, leaf-dropping, plant dwarfing and premature plant death (Ottoman et al., 2009). Yield loss can be as high as 80% (Jefferies, 1998). There are also cultivars, such as Shepody, where PVY can be symptomless rather than producing the typical mosaic symptoms on the leaves, and this can create a major risk for the spread of the virus in seed schemes (Nolte et al., 2004). Furthermore, the lack of symptoms in infected plants ensures that visual inspection will be generally ineffective and that such plants can provide a source of PVY in production areas.

There are several strains of this virus, and the main strains are: common (PVY^{O}), tobacco veinal necrosis (PVY^{N}), and stipple streak (PVY^{C}). PVY^{NTN} was first detected as an N strain that caused Tuber Necrosis (NTN) in Europe (Eu-PVY^{NTN}) in the late 1980's, and has since spread to most continents, including North America, and to Australia in 2003.

There are two types of single dominant resistance genes for PVY, namely hypersensitive response or *Ny* genes and extreme resistance or *Ry* genes. The hypersensitive response results in cell death and inhibits virus spread from cell to cell and through the vascular system, while extreme resistance is characterised by a strong reduction in virus replication (Ponz and Bruening, 1986, Solomon-Blackburn and Barker, 2001b, Song and Schwarzfischer, 2008).

A number of PVY resistance genes have been identified due to different symptoms, their infection of differential cultivars, as well as serological and molecular data (Singh et al., 2008, Fomitcheva et al., 2009). These include the hypersensitive resistance genes *Ny, Nc*, the proposed gene *Nz*, and the extreme resistance genes Ry_{adg}, Ry_{sto} and Ry_{chc} (Cockerham, 1970, Gebhardt and Valkonen, 2001). The European potato breeding programs have used Ry_{sto} rather than Ry_{adg} or Ry_{chc} (Ross, 1986, Valkonen et al., 2008), while Ry_{adg} is the main resistance gene used in North and South America.

In order to develop markers for PVY resistance it is necessary to understand which strains of PVY are present. An extreme resistance gene (Ry_{adg}) was mapped to chromosome 11 and linked to the markers M45 (Brigneti et al., 1997) and RYSC3 (Kasai et al., 2000, Whitworth et al., 2009), while the extreme resistance gene Ry_{sto} was mapped to chromosome 12 and linked to the marker STM0003 (Song et al., 2005).

Potato virus X

Potato virus X (PVX) has been separated into two types, PVX^{O} (common) and PVX^{A} (Andean). PVX^{O} is found worldwide, while PVX^{A} is restricted to Peru and Bolivia (Moreira et al., 1980, Jefferies, 1998). PVX infection causes mild mosaics or can be symptomless, but occasionally severe mosaics can occur. Infection will result in a yield reduction of usually 5–20%, and tuber necrosis can occur in some cultivars (Jefferies, 1998, Nyalugwe et al., 2012). Mixed infections with other viruses, particularly *Potato virus A* and *Potato virus Y* may have a reaction and cause a 'rugose mosaic' or 'crinkle' and can result in considerable yield loss (Solomon-Blackburn and Barker, 2001a, Nyalugwe et al., 2012). PVX can be readily transmitted mechanically (e.g. by machinery), which includes plant-to-plant contact (Jefferies, 1998).

PVX^O has four strains which differ in their virulence to the hypersensitive resistance genes *Nx* and *Nb* (Cockerham, 1970, Cox and Jones, 2010), while two extreme resistance genes, *Rx1* and *Rx2*, have also been identified (Cockerham, 1970, Gebhardt and Valkonen, 2001).

The genes *Rx1* and *Rx2* have been mapped to chromosomes 12 and 5 respectively (Ritter et al., 1991), and they have subsequently been identified (Kanyuka et al., 1999, Bendahmane et al., 2000).

PVY sequencing

Virus isolates

Sixteen *Potato virus Y* (PVY) isolates that were found in various Australian locations during the period 2005 to 2010 or were sourced from virus reference collections in Queensland and Victoria, were selected for genome sequencing (Table 6.1.1). Isolates 1-11 came from infected potato leaves; isolate 12 was extracted from a potato tuber and isolates 13-16 were extracted from freeze-dried potato leaves.

Table 6.1.1. Geographic location, strain and year of collection of the PVY isolates sequenced in this study

Isolate	Virus ID	Strain	Location collected	Year collected
1	624	N	Gatton	2007
2	759	N	Thorpdale	2008
3	850	N	Warragul	2009
4	879	N	Ballarat	2008
5	908	N	Ballarat	2009
6	908	N	Ellinbank	2009
7	908	N	Thorpdale	2009
8	1068	N	South Australia	2010
9	PVY^C	C	QLD	unknown
10	PVY^O	O	QLD	unknown
11	PVY^N	N	Victoria	2005
12	Nicola tuber	NTN	Victoria	2003
13	55	unknown	QLD	unknown
14	72	O	QLD	unknown
15	1473	NTN	QLD	unknown
16	1622	NTN	QLD	unknown

Nucleic acid extraction

Total RNA was extracted from fresh potato leaves and tubers of isolates 1-12 using the ZR Plant RNA Miniprep Kit (ZYMO research, USA) and a standard ethanol precipitation. Specifically, total RNA was isolated from approximately 0.2 g of frozen and finely ground leaf material or scrapings from the eye of the potato tuber following the manufacturer's protocol, including the in-tube DNase treatment (Appendix B of manufacturer's instruction manual). The RNA was eluted in 50 µL of nuclease-free water, then precipitated using 5M NaCl and 100% ethanol. The precipitate was allowed to air dry and then resuspended in 50 µL of 1x TE buffer (10 mM Tris-Cl (pH 7.5), 1 mM EDTA (pH 8.0)).

Isolates 13-16 were obtained as freeze-dried plant material and total RNA was extracted from approximately 0.1 g of the freeze-dried tissue using the RNeasy Plant Mini Kit (Qiagen) and a modified lysis buffer (MacKenzie et al., 1997). RNA extracts were eluted in a final volume of 50 µL nuclease-free water and stored at -20°C until use.

Primer design

An alignment of 36 full-length PVY genomes (representing all PVY strains) which were available in the NCBI database as of 31 March, 2011 was used for the purpose of designing PVY-specific primers. Highly conserved regions that are located between 1kb-1.5kb apart in the alignment were targeted for their suitability as primer sites based on their sequence information. To obtain the 5′ end of the PVY genome, primers GSP1 (5′ -GGMTCTCCACCAGC- 3′) and GSP2 (5′ -CGAATC TGGRCATCAGTCTTGTATCG- 3′) located at c. 500 and c. 400 base pairs distant from the 5′-terminus, respectively, were designed. To obtain the 3′-terminus of the PVY genome, primer (8iF) (which is located 300-500 bp distant from the poly-A tail of PVY) was designed. Degeneracy was largely avoided in the primer design, and the most dominant base in the sequences was used. All primers designed were tested *in silico* with OligoAnalyzer 1.2 (Gene-Link software) and OligoCalc (http://www.basic.northwestern.edu/biotools/oligocalc.html). The primers were predicted to have minimal self-hybridisation, hairpin loops and primer dimer formations according to the OligoAnalyzer 1.2 and OligoCalc analysis outputs.

In total, 22 primers were designed to sequence the PVY genome (Figure 6.1.1; Table 6.1.2). Sixteen of the 22 primers amplified a fragment of >900 bp; 4 of the 22 primers targeted the 5′ and 3′ terminal end of the genome and 2 were inner primers (3iF-3iR) that are located in the CI region of the PVY polyprotein.

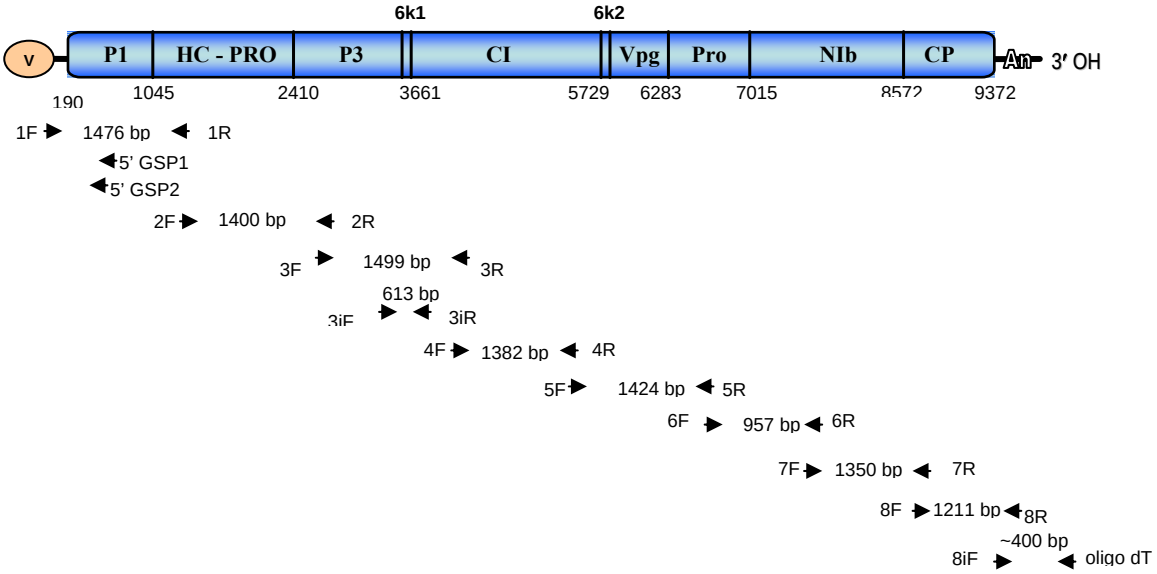

Figure 6.1.1. Schematic diagram of the Potato virus Y genome based on a PVY^{NTN} isolate (AB331516.1) Arrows indicate positions of the genome-walking primers in relation to the orientation of the PVY genome.

Table 6.1.2. Positions, nucleotide sequences, product sizes and annealing temperatures of primers designed to sequence the PVY genomes.

Name	Position in alignment	Sequence (5' – 3')	Product size (bp)	Annealing temp. (^{o}C)
1F(2)	10 - 29	CAACTCAATACAACATAAG	1476	50
1R	1462 – 1485	GAGCTGTATTTTCCTTTCCTTTCA		
2F	1396 – 1417	TCAAGTCTATAGGGGAGAAGCA	1400	58
2R	2775 – 2795	GAACCCATCACACGTAGCATC		
3F	2704 – 2723	TACGAGTGTCAGCGGCAGAA	1499	60
3R	4180 – 4202	TCCAGAACATGGCACTCATCAAA		
3iF	3168 – 3188	TAAGTGTGTAAATATTTCATC	613	50
3iR	3760 – 3779	CACCAATCACTAAACTTTGT		
4F	4056 – 4077	AACACTGCGTATGCGTGGAAAT	1382	55
4R	5413 – 5437	TGTAATCTTTTGCATATCTAGCTCT		
5F	5327 – 5347	AGCGAGTGAAGCAGAGCCAAT	1424	55
5R	6733 – 6750	CTCACTACTGGTAATCCACAATG		
6F	6646 – 6668	GCATCGTCAATCATCACAGAAAC	957	60
6R	7583 – 7602	GGTGCAGCAGTGAATGTCCT		
7F	7402 – 7422	TGCTGTCGGAGCTATGTATGG	1350	55
7R	8729 – 8751	GGCATTCTCATTTTGGACGTGAT		
8F	8586 – 8606	ATGACACAATCGATGCAGGAG	1211	55
8R	9774 – 9795	GTCTCCTGATTGAAGTTTACAG		
8iF	9443 – 9662	TTTTCCTGTACTACTTTTAT	>400	50
potyvirid 1	Poly-A	CACGGATCCCGGG$(T)_{17}$VGC		

cDNA synthesis

Total RNA obtained from isolates 1-12 were converted to cDNA using the SMARTer PCR cDNA synthesis Kit (Clontech Laboratories; Japan) following the manufacturer's protocols. An aliquot of 1 µl of total RNA was used for cDNA synthesis, and the resultant cDNA was diluted to a concentration of 50 ng/µL for use in downstream applications.

Total RNA obtained from isolates 13-16 were converted to cDNA using the SuperScript III First-Strand Synthesis System (Life Technologies, USA) and three different primers, 3R, 6R and 'potyvirid 1', a modified oligo d(T) primer (Gibbs and Mackenzie, 1997). Three pools of cDNA were therefore available for each of the isolates 13-16.

PCR, cloning and sequence assembly

PCR was performed in a 25 µL volume reaction containing 2 µL of cDNA, 10 x reaction buffer (200 mM Tris-HCl (pH 8.4), 500 mM KCl), 1.5 mM $MgCl_2$, 0.2 mM dNTPs, 0.2 µM of each primer and 1 U Platinum *Taq* DNA polymerase (Life Technologies, USA). Amplification was performed using a GeneAmp thermocycler (Applied Biosystems, USA) under the following cycling conditions: one cycle of denaturation at 94^{o}C for 2 minutes, 35 cycles of denaturation at 94^{o}C for 30 seconds, various annealing temperatures (Table 6.1.2) for 30 seconds and elongation at 72^{o}C for 1 minute per kb, followed by a final elongation step of 72^{o}C for 5 minutes. The amplified products were separated by electrophoresis on an ethidium bromide (EtBr) stained agarose gel and visualised by trans-illumination at 313 nm.

Fragments from the 5′-terminal end of the PVY genome were amplified using the 5′-RACE system for rapid amplification of cDNA ends, Version 2.0 (Life Technologies, USA) and the primers GSP 1 and GSP 2, following the manufacturer's instructions.

PCR products of the expected size were purified using either the QIAquick PCR purification kit or the QIAquick gel purification kit (Qiagen, USA) and then cloned into the pGEMT Easy Vector 2 (Promega, USA) following the manufacturers' instructions. Bacterial colonies suspected of carrying the insert were amplified using TempliPhi (GE healthcare, USA) following the manufacturer's instructions in an overnight reaction. Inserts were confirmed by restriction enzyme analysis and sequenced using BigDye 3.1 chemistry on an ABi3730xl DNA analyzer (Applied Biosystems) resulting in a 3-fold sequencing coverage for the majority of the PVY isolates.

Sequence analysis

Sequence data was assembled by use of DNA baser V3 (Heracle Biosoft SRL) and the integrity of the consensus sequence was manually curated by translation into a continuous amino acid sequence of approximately 3000 bp in length. Phylogenetic analysis was conducted on the PVY sequences obtained from this study in comparison to published PVY genomes (Table 6.1.3).

Table 6.1.3. Accession, country of origin, strain information and references of the PVY isolates used as reference genomes for phylogenetic analysis in the study

NCBI accession	Country of origin	Strain/variation	Reference
AB331515.1	Japan	NTN	(Ogawa et al., 2008)
AB331516.1	Japan	NTN	(Ogawa et al., 2008)
AB270705.1	Syria	Recombinant between PVY^{NTN} and $PVY^{N}W$	(Chikh Ali et al., 2007)
AJ585197.1	UK	N	NCBI
AJ585196.1	UK	O	NCBI
EF558545.1	Poland	NW	NCBI
AF522296.1	Egypt	N	NCBI
EF026075.1	USA	NTN	NCBI
FJ204165.1	USA	NTN	(Hu et al., 2009)
AJ889866.1	Poland	NTN	(Schubert et al., 2007)
AY166866.1	Canada	NTN	(Nie and Singh, 2003)
AJ890342.1	Poland	N	(Schubert et al., 2007)
AM268435.1	NZ	NTN	(Schubert et al., 2007)
AY166867.1	Canada	N	(Nie and Singh, 2003)
AJ584851.1	UK	N	NCBI
EF026074.1	USA	O	NCBI
AJ890349.1	Poland	O	(Schubert et al., 2007)
AJ889868.1	Germany	Wilga	(Schubert et al., 2007)
AM113988.1	Germany	Wilga	(Schubert et al., 2007)
U09509.1	Cananda	O	(Singh and Singh, 1996)
D00441.1	N/A	N	(Robaglia et al., 1989)

PVY and PVX phenotyping assays

Trials were run separately for PVY^{O}, PVY^{NTN} and PVX, although the same phenotyping protocol was used. There were 4 trials for PVY^{O} and PVY^{NTN} during 2010, 2011 and 2012, while there were 2 trials for PVX during 2011 and 2012.

Planting material and growth conditions

Selected potato cultivars were sown in either 150mm or 180mm pots depending on the trial. Pots were filled to two-thirds full with a pine-based potting mix. One small tuber or cut tuber of each cultivar was partially imbedded in the potting mix, and the pots were topped up with additional potting mix as required. Pots were placed on raised benches in a glasshouse, and watered by hand. The tubers were fertilised initially with 10 g of Osmocote Plus. Known resistant and susceptible cultivars were included in the trials as positive and negative controls.

Four replicates of each cultivar were separately inoculated with either PVY^{O}, PVY^{NTN} or PVX. Leaves from infected PVY^{O}, PVY^{NTN} or PVX potato or tobacco plants were ground in chilled extraction buffer, at the rate of approximately 1 in 10 dilution of leaf material to the buffer, to create the inoculum. The extraction buffer was prepared by adding 7.5 g $NaH_2PO_42H_2O$ (0.05 M phosphate buffer) with 1% (w/v) Na_2SO_3 to 1 L distilled water, and the pH adjusted to 7.4. Plant leaves to be inoculated were powdered with carborundum powder and the PVY^{O}, PVY^{NTN} or PVX inoculum was then rubbed across the leaves. The carborundum would graze the leaf surface to facilitate virus transmission into the leaf. Two plants of each cultivar were inoculated with buffer containing uninfected potato leaf material, and two plants were left uninoculated. Plants were tested for virus infection using ELISA by taking leaf samples after 30 days, and then cultivars with negative results were re-tested after 40 days. Young fully expanded leaves were sampled from the growing tip, to avoid inoculated leaves that could contain a trace of the virus from the inoculum. Cultivars were considered susceptible if virus was detected, but were considered resistant if no virus was detected over three independent trials.

ELISA testing for virus

A stock solution (5x) of Phosphate Buffered Saline (PBS 5x) was prepared by combining 200 g NaCl, 5.0 g KH_2PO_4, 28.8g HNa_2O_4P and 5.0g KCl, and was made up to 5 L with distilled water. The pH of the 5x PBS stock solution was adjusted to pH 7.4. From the stock solution, PBS Tween 1x was prepared by combining 1 L PBS 5x, 4 L distilled water and 2.5 ml Tween 20. Leaf samples (approximately 3 g) were crushed between two rollers in a motorised leaf press. 10 ml of extraction buffer (1L PBS Tween 1x, 20.0 g polyvinylpyrrolidine and 4.0 g skim milk powder) was run down the rollers and the run off, comprising sap and extraction buffer, was collected in a 30 ml sample cup. Rollers were washed with tap water and wiped clean between samples to prevent cross-contamination of samples.

Antiserum for PVY and PVX was obtained from Agdia Inc and a double antibody sandwich ELISA technique was used (Clark and Adams, 1977). The antiserum (100 µl/well) was pipetted into 96 well microtitre plates (Nunc) at 1/200 dilution in carbonate coating buffer (1.59 g Na_2CO_3 and 2.93 g $NaHCO_3$, and made up to 1 L with distilled water and adjusted to pH 9.6) as stated in the supplier instructions for each ELISA kit, followed by incubation at 37°C for 4 hours or at 4°C overnight and washing. Microtitre plates were washed between each step by filling each well with wash buffer (100 ml PBS 10x stock, 900 ml distilled water, 0.5 ml Tween 20 and 1.0 g milk powder, made up to 1 L and adjusted to pH 7.4) and soaking for at least 3 minutes. This step was repeated three times. After the final wash, plates were emptied of wash buffer, and allowed to drain upside down over paper towels for approximately 5 minutes before the next step.

Samples, negative and positive controls were added to 96 well micrtitre plates in duplicate wells located next to each other. The plates with sample extracts were incubated overnight at 4°C, as recommended by the manufacturer's instructions. Antiserum conjugated with alkaline phosphatase was prepared at the same dilution that was used to coat the plate. Conjugated antiserum was diluted in conjugation buffer (100 ml PBS 10x, 2.0 g Bovine serum albumin, 20.0 g Polyvinylpyrrolidine, made up to 1 L with distilled water and adjusted to pH 7.4, added 100 µl dilute conjugated

antiserum to each well and microtitre plates incubated at 37°C for 4 hours. Plates were washed as described above. Substrate tablets, each containing 5 mg p-nitrophenyl phosphate, were added at a rate of 1 tablet per 10 ml of substrate buffer, (48.5 ml Diethanolamine and 400 ml of distilled water adjusted to pH 9.8) and 100 µl of substrate buffer was added to each well. Plates were incubated for 30-60 minutes at room temperature to allow colour development, and absorbance of each well was read at 405 nm using a Titertek photometer (Flow Laboratories, Helsinki, Finland). Samples were considered positive if the optical density reading was above 0.160, and negative if below 0.120. Readings between these values were considered ambiguous and the cultivar was re-tested.

PVY marker assays

RYSC3 marker assay

The SCAR marker RYSC3 has been shown to produce a 321 bp band in the presence of the PVY resistance gene Ry_{adg}. Forward (5' – ATACACTCATCTAAATTTGATGG - 3') and reverse (5' – AGGATATACGGCATCATTTTTCCGA - 3') RYSC3 primers were designed by Kasai et al. (2000) with the forward primer labelled with the fluorochrome FAM for detection following capillary electrophoresis. PCRs were carried out in a total volume of 10 µl containing 20 ng of template DNA, 1x ImmoBuffer (Bioline), 0.25 mM dNTPs (Bioline), 0.25 µM of each primer and 0.5 Unit Immolase™ DNA Polymerase (Bioline). Cycling conditions for PCR were; 94°C for 10 min followed by 30 cycles of 94°C for 45 s, 60°C for 45 s, 72°C for 45 s and a final step of 72°C for 10 min. The amplified products were prepared for resolution on an ABi3730xl DNA Analyzer (Applied Biosystems) and analysed with GeneMapper V3.7 (Applied Biosystems) as described in the TG689 marker assay protocol.

M45 marker assay

The M45 marker with forward (5' – GACTGCGTACATGCAGCT – 3') and reverse (5' – GATGA GTCCTGAGTAAGGA – 3') primer sequences were developed by Brigneti et al. (1997). The M45 marker is linked to the Ry_{adg} gene (Valkonen et al., 2008). PCR reaction conditions for amplification of the M45 marker were similar to those used for RYSC3, with minor modifications. PCRs were carried out in a total volume of 10 µl containing 20 ng of template DNA, 1 x ImmoBuffer (Bioline), 0.25 mM dNTPs (Bioline), 0.25 µM of each primer and 0.5 Unit Immolase™ DNA Polymerase (Bioline). Cycling conditions for PCR were: 94°C for 10 min followed by 35 cycles of 94°C for 45 s, 60°C for 45 s, 72°C for 45 s and a final step of 72°C for 10 min. The forward primer was fluorochrome-labelled with HEX for capillary electrophoresis as described in the TG689 marker assay protocol. Cultivars that amplified a 493 bp fragment are Ry_{adg} positive. The 116 bp band that is present in all lanes is an internal control for DNA quality.

STM0003 marker assay

The simple sequence repeat (SSR) marker STM0003 (Milbourne et al., 1998) has been shown to detect a 111 bp allele diagnostic for potato cultivars that express extreme resistance to PVY and carry Ry_{sto} (Song et al., 2005). The forward (5' – GGAGAATCATAACAACCAG – 3') primer was fluorochrome labelled with HEX for capillary electrophoresis for use with reverse (5' – AATTGTA ACTCTGTGTGTGTG – 3') primer by Milbourne et al. (1998). PCR reaction conditions for amplification and analysis of the STM0003 marker were similar to those used for RYSC3, with minor modifications. PCRs were carried out in a total volume of 10 µl containing 20 ng of template DNA, 1x ImmoBuffer (Bioline), 0.25 mM dNTPs (Bioline), 0.25 µM of each primer and 0.5 Unit Immolase™ DNA Polymerase (Bioline). Cycling conditions for PCR were: 94°C for 10 min followed by 30 cycles of 94°C for 45 s, 58°C for 45 s, 72°C for 45 s and a final step of 72°C for 10 min. The amplified products were prepared for resolution on an ABi3730xl DNA Analyzer

(Applied Biosystems) and analysed with GeneMapper V3.7 (Applied Biosystems) as described in the TG689 marker assay protocol.

PVX marker assays

'PVX' marker assay

The PVX resistance gene *Rx1*, was mapped to chromosome 12 (Ritter et al., 1991). The *Rx1* locus was identified through a series of fine mapping and transgene-based cloning activities (Bendahmane et al., 1997, Bendahmane et al., 1999, Kanyuka et al., 1999). Based on *Rx1* sequence information, Ohbayashi et al. developed an STS marker designated 'PVX' (Mori et al., 2011). The marker with forward (5' - ATCTTGGTTTGAATACATGG – 3') and reverse (5' – CACAATATTGGAAGG ATTCA – 3') primers was combined in a duplex assay with a granule-bound starch synthase I gene (GBSS) marker. The GBSS marker, with forward (5' –ATGGCAAGCATCACAG - 3') and reverse (5' – CAAAACTTTAGGTGCCTC - 3') primers, functioned as an internal control and produced a fragment of 980 bp (Mori et al., 2011). PCR reactions were performed in a total volume of 10 µl containing 20 ng of template DNA, 1x ImmoBuffer (Bioline), 0.25 mM dNTPs (Bioline), 0.25 µM of each primer and 0.5 Unit Immolase™ DNA Polymerase (Bioline), with both forward primers fluorochrome labelled with FAM for capillary electrophoresis. Cycling conditions for PCR were: 94°C for 10 min followed by 30 cycles of 94°C for 45 s, 57°C for 45 s, 72°C for 90 s and a final step of 72°C for 10 min. Aliquots of 2 µl of 100 X diluted PCR amplicon was added to 8 µl of a mixture containing 5000 X diluted GeneScan™ -2500ROX® size standard in Hi-Di™ formamide (Applied Biosystems). Amplicons were separated by capillary electrophoresis using an ABi3730xl DNA Analyzer (Applied Biosystems) and resultant FSA files analysed with GeneMapper V3.7 (Applied Biosystems). Presence of a 1230 bp band diagnostic for 'PVX' was compared to the confirmed PVX resistance phenotype to determine effectiveness of marker prediction.

RXSC1 marker assay

To develop a PVX assay with conditions favourable for multiplex combination with other diagnostic markers, a reverse primer to amplify a smaller fragment of *Rx1* was designed in the variable leucine-rich repeat (LRR) region of the *Rx1* gene. The reverse primer (5' – CCTGTACAA TACCGAGGGTTC – 3') was used with the FAM fluorochrome labelled forward primer (5' – ATCTTGGTTTGAATACAT GG – 3') of the 'PVX' marker to develop a marker we have named RXSC1. PCR reaction conditions for amplification and analysis of the RXSC1 marker were the same as those used for RYSC3.

7. *Tomato Spotted Wilt Virus*

Tomato spotted wilt virus (TSWV) was first described in Australia in 1915, and has been reported from all Australian States. TSWV has a wide host range, including native plants and other closely related Solanaceae including tomato and capsicum. Symptoms include leaf mottling and necrosis, stunting and tuber ringspots and necrosis. It is transmitted by 4 types of thrips species.

Resistance has been attributed to a number of genes, both dominant and recessive. Resistance in tomato is controlled by a single gene (Stevens et al., 1995, Brommonschenkel and Tanksley, 1997), and this gene is the most widely used source of resistance. Putative orthologues of this gene have been mapped in pepper and tomato (Jahn et al., 2000). Resistance manifested as a hypersensitive response has been observed in capsicum and is controlled by a different single dominant gene. Resistance to TSWV in potato could hence be determined by a single resistance gene. Resistance

has not been identified in potato, and disease in potato associated with TSWV is a unique problem for Australia with only a few other reports globally.

TSWV phenotyping assay

Screening trials for TSWV resistance were conducted over three seasons on selected potato cultivars. Tubers from the cultivars were planted in a 200 mm pot and placed in a glasshouse for inoculation. Two mechanical inoculation methods were used, by hand and by pressure spray, to enable inoculation of a larger number of plants.

The host plants used as a source of virus inoculum were TSWV infected potato or tomato plants. The virus presence was confirmed in each TSWV infected plant by ELISA using a TSWV specific 1000 DAS ELISA kit (Agdia) and by following the manufacturer's protocol. Actively growing tips from these plants were used for inoculation.

Inoculum was prepared by grinding TSWV-infected potato leaves using a mortar and pestle in chilled inoculation buffer (7.5 g Na_2PO_4 $2H_2O$ + 1% Na_2SO_3 in 1 L of DH_2O, pH 7.4). The leaf to buffer ratio was approximately 1:5 (1 g infected leaf to 5 ml buffer) for the hand inoculation and 1:10 for the spray inoculation. One stem in each pot was selected, dusted with carborundum and 2 – 4 marked leaves were inoculated with TSWV infected sap. The inoculated leaves were marked to assist with differentiation between local and systemic symptom expression. After inoculation, the carborundum was rinsed off the leaves with water to minimise leaf tissue damage. Tomato plants were also challenged with the TSWV inoculum and used as a positive control. Another set of plants was used as a negative control. These potato plants were challenged solely using mechanical inoculation buffer and carborundum.

As the spray inoculation method was considered to be the most efficient for mass inoculation, 5 different inoculation rates (0, 1, 2, 3, 4 sprays) were compared on 4 susceptible cultivars. The plants were then monitored for symptom expression. Using a four spray inoculation protocol, four families that had a resistant and susceptible parent were inoculated.

All plants were maintained under glasshouse or shadehouse conditions for the duration of the trials of c. 10 weeks. The presence of TSWV symptoms was monitored on the challenged plants and a TSWV susceptibility/resistance score was recorded.

The following 'resistance scale' was used to score plant response to TSWV inoculation on a scale of 0–5, with 5 as the highest resistance:

0 - Susceptible: chronic symptoms, systemic spread, stunting, tip necrosis.
1 - Severe stunting, necrotic stems and necrosis of tips. Widespread systemic distribution.
2 - Widespread distribution of 2 or more of the TSWV symptoms. Symptoms may include leaf distortion and stunting. Same symptoms on other than inoculated stem were scored 2 when mild or 1 if severe.
3 - More than one symptom of: necrotic lesions in leaves other than that inoculated, which can be described as systemic or systemic spread, scattered black lesions, stem lesions, leaf cupping / curling / distortion, mosaic are signs of systemic spread. Same symptoms on other parts of inoculated stem (petioles, other than inoculated leaves etc.) were scored as 3 when mild or 2 if severe.
4 - One symptom of: black circle around inoculated wound, local lesions, mild stem lesions, upward curling / cupping of leaves, mosaic or graft necrosis. Symptoms observed on inoculated leaf or grafted stem only.
5 - Resistance: No symptoms, no infection, only mechanical damage on the inoculated leaf.

8. Verticillium wilt

Verticillium wilt is caused by the soil borne fungi *Verticillium dahliae* and *V. albo-atrum*. Verticillium wilt is also known as early dying disease. Symptoms include wilting, leaf chlorosis and necrosis, and the tubers can exhibit necrosis in the vascular tissue, which reduces quality (Simko et al., 2003). In heavily infested soils, susceptible cultivars can have their yield reduced by up to 50% (Goth and Haynes, 2000). Cultivars are known to vary in their level of resistance, with early maturity cultivars usually more susceptible (Jansky and Rouse, 2000, Simko et al., 2004b).

Resistance in the closely related tomato has been associated with the tomato genes *Ve1* and *Ve2*, which are closely linked on the tomato chromosome 9. Simko et al. (2003) located a resistance gene, *StVe1*, in a similar location in potato. They used SSR markers that had been mapped to potato chromosome 9, and found that there was a high level of concordance between the marker STM1051 and expected Verticillium resistance response, although the expression of phenotype was confounded by maturity.

Presence of the linked marker was necessary for resistance, but some cultivars that had the marker were susceptible. All cultivars that did not have the marker were susceptible. The intermediate to late maturing cultivars containing the marker had a significantly increased chance of being resistant, while early maturing cultivars were usually susceptible (Simko et al., 2003). In a further study, all North American cultivars that are highly resistant were shown to contain the allele (Simko et al., 2004b).

As some of the markers that were used are SCAR markers, a simple sequence repeat (SSR) marker was used as a positive DNA control. The SSR marker STM1051 had been determined by Simko et al. (2003) to be associated with quantitative resistance to Verticillium wilt. So this SSR was used as the positive DNA control, as the $STM1051_{190}$ amplicon would provide an indication of Verticillium wilt resistance.

STM1051 marker assay

The presence of the 193 bp amplicon from the SSR marker STM1051 is diagnostic for Verticillium wilt resistance, based on the forward (5' – TCCCCTTGGCATTTTCTTCTCC - 3') and reverse (5' –TTTAGGGTGGGGTGAGGTTG - 3') primers designed by Milbourne et al. (1998). The forward primer was labelled with the fluorochrome FAM for detection following capillary electrophoresis. PCRs were performed in a total volume of 10 µl containing 20 ng of template DNA, 1x ImmoBuffer (Bioline), 0.25 mM dNTPs (Bioline), 0.25 µM of each primer and 0.5 Unit Immolase™ DNA Polymerase (Bioline). Cycling conditions for PCR were: 94°C for 10 min followed by 10 cycles of 94°C for 30 s, 70°C for 30 s decreasing 1°C per cycle, 72°C for 30 s, then an additional 30 cycles of 94°C for 30 s, 60°C for 30 s, 72°C for 30 s and a final step of 72°C for 10 min. The amplified products were prepared for resolution on an ABi3730xl DNA Analyzer (Applied Biosystems) and analysed with GeneMapper V3.7 (Applied Biosystems) as described in the TG689 marker assay protocol.

9. Multiplexed marker assays

To deliver molecular marker technology with optimal performance at lowest cost, the validated diagnostic molecular markers were combined in multiplex ratio into a single tube marker assay. The collection of markers that were selected was: 57R, RYSC3, RXSC1, M45 and STM1051. The SSR marker STM1051 was included as both a positive control capable of being amplified from all samples as well as generating information for Verticillium wilt resistance. To accommodate the marker assay conditions into a single reaction, several alterations to the initial reaction conditions were made. PCRs were performed in a total volume of 10 µl containing 20 ng of template DNA, 1 x ImmoBuffer (Bioline), 0.25 mM dNTPs (Bioline), 0.25 µM of each primer and 0.5 Unit Immolase™ DNA Polymerase (Bioline). Cycling conditions for PCR were: 94°C for 10 min followed by 30 cycles of 94°C for 45 s, 60°C for 45 s, 72°C for 45 s and a final step of 72°C for 10 min. The amplified products were prepared for resolution on an ABi3730xl DNA Analyzer (Applied Biosystems) and analysed with GeneMapper V3.7 (Applied Biosystems) as described in the TG689 marker assay protocol. All of the generated marker profiles and products were recorded and have been documented for ease of future analysis along with the development of a comprehensive standard operating procedure (SOP) that has been attached to this report.

10. Cost of conventional screening compared to MAS.

In order for MAS to be adopted by a breeding program, the cost of the sampling and analysis must be attractive to the program. In order to assess the comparative costs of the two methods, a conventional field trial was costed in detail for standard agronomic and postharvest traits that are measured for a typical potato breeding population. Field and glasshouse screening were also costed for disease resistance. All activities, labour and consumables were costed for these trials. These activities included establishment and maintenance of the site and plants for the duration of the trial, harvest, rehabilitation of the trial site, data collection and analysis.

Genotyping activities were also costed in detail. All activities, labour and consumables were again costed. These included sample collection, DNA extraction, PCR amplification, product detection and analysis. These costs were also analysed for single and multiplexed assays.

11. Progeny testing and the development of estimated breeding values

Most of the traits of interest in plant breeding are under the control of multiple genes, or are quantitative traits. The genetic analysis of quantitative traits does not follow the segregating ratios of qualitative traits, but can be analysed using family means and variances. When the individuals within a family are scored for a quantitative trait, the mean phenotype of the family will be due to the joint action of the genes and interaction with the environment. Due to this interaction, the progeny will exhibit a normal distribution for the trait of interest. Different families will exhibit different means and variances due to the different genotypes of the families. Traits controlled by multiple genes will exhibit additive and/or dominance effects by the genes or alleles of the genes on the expression of the trait.

As the phenotypic variance shown within a population is the result of the genetic variance and environmental factors, when the environmental variations can be reduced to close to zero, then the genotypic variation will approximate the measured phenotypic variation. The environmental variation can be caused by a range of factors, such as seed quality, developmental variations, and

intra-trial micro-environmental variations. Sampling, measurement and statistical errors are also included in the environmental variation parameter (Kearsey and Pooni, 1998).

The proportion of phenotypic variance that is due to the effect of genes is known as the heritability of the trait. Heritability can be expressed in two versions: broad sense heritability and narrow sense heritability. Broad sense heritability (H) represents the ratio of the total genetic variation to the total phenotypic variation. Narrow sense heritability (h^2) measures the proportion of the variation which is due to the additive effects of the genes in a population (Kearsey and Pooni, 1998).

An analysis of progeny means will derive the broad sense heritability of the total parental genetic contribution to the family, while determination of the narrow sense heritability will provide an indication of the additive genetic effect that is passed from the parents to the family. Therefore determination of the narrow sense heritability will provide a real benefit to a breeding program based on additive genetic gain. While determination of the broad sense heritability, will identify the dominant genetic factors that may be transmitted to the progeny.

Narrow sense heritability can also be used to determine the most effective method of selection. Combined family and within-family selection is the most efficient selection method because it optimises use of genetic information, while family selection gives a better response when the narrow sense heritability is low, and within-family selection is more efficient for highly heritable traits (Kearsey and Pooni, 1998).

Animal breeding programs have greatly benefited from the determination of genotypic values using Best Linear Unbiased Prediction (BLUP). BLUP is the standard practice in animal breeding programs, but has been less commonly practiced in plant breeding programs. BLUP, or a similar prediction method, is necessary in animal breeding to predict the genetic merit of a male for female only traits, such as milk yield in dairy cattle (Piepho et al., 2008). Animal programs have benefited from estimating the genetic merit of selection candidates based on phenotypic values of all relatives by using pedigree information. For example, significant gains have been seen in milk yield in Holstein cattle in the USA (Van Vleck et al., 1986). By using the phenotypic values of all relatives, the amount of information is maximised and the most accurate genotypic value will be obtained.

Population development and trials

Breeding populations were developed from selected parents that have a history of superior performance or production of elite progeny. Tubers of these cultivars were grown in pots under glasshouse conditions, and were planted in such a way as to suppress tuber production and to promote plant growth and flower production. This was achieved by planting tubers shallowly in deep pots, and when growing, the mother tuber and stolons are uncovered while leaving the roots undisturbed in the potting mix. This method ensured effective plant growth and diverted maximum resource allocation to flower and fruit production rather than daughter tuber formation.

At flowering, controlled pollinations were conducted by the emasculation of flowers identified for pollination prior to pollen dehiscence, in order to remove the possibility of self-pollination. Then when the stigma was mature, pollen from the male parent was applied to the stigma. The pollinated flowers were labelled and allowed to develop into fruit. At maturity, the fruit was collected and the seed was extracted, washed and stored for use.

Seed from the desired crosses were then germinated and seedlings were established in trays and transplanted to pots in the glasshouse. This stage is known as the G0 generation or the glasshouse seedling generation. Plants are allowed to grow through to senescence. From each pot (plant) a single seedling tuber is collected for planting in the field.

Tubers collected from the glasshouse seedlings were field-planted at wide spacing. Each plant in this field seedling or first field generation (G1) crop was genetically unique. At maturity, plants are individually hand harvested to maintain separation. Tubers from each plant are examined and superior genotypes were visually selected to advance to the next generation. All of the tubers from these selected plants were collected to maximise the size of selection plots in the following generation. A sample of tubers was also collected from the rejected cultivars and was retained in family groups for progeny testing.

Using both cut and whole seed from tubers of the previous season's single plants, selections were planted in short row selection plots (G2) of up to 30 plants. The genotypes were assessed while growing for plant maturity and early blight resistance, and at harvest the tubers are assessed for breeder's visual preference and a reduced set of genotypes were selected and assessed for their cooking performance.

Three populations of cultivars were studied for this work. The '07 series' were germinated in 2006, planted in the G2 trial in 2008, and contained 1132 genotypes in 57 families. The '08 series' were germinated in 2007, planted in the G2 trial in 2009, and contained 1137 genotypes in 42 families. The '09 series' were germinated in 2008, planted in the G2 trial in 2010, and contained 952 genotypes in 61 families. The series numbers relate to the year they were first planted in the field or G1 generation.

Phenotyping methods

Plant maturity

Plant maturity was visually assessed by the inspection of plants towards the end of their life-cycle to determine the longevity of each cultivar, in comparison to cultivars of known maturity. Early cultivars will complete their life cycle in c. 90 days, mid-maturity cultivars in c. 105 days, and late maturity cultivars in c. 135 days, under the environmental conditions at Toolangi, Victoria. Each cultivar was given a maturity rating, which was given a numerical rating for analysis and ranged from 0 for very, very late to 17 for very early.

Early blight resistance

Early blight resistance was also visually assessed by inspecting plants towards the end of their life to determine the level of early blight infection on the foliage. This is assessed under natural infection conditions, which occur each year under the environmental conditions at Toolangi, Victoria. Each cultivar was given an early blight infection rating, which was given a numerical rating for analysis, and ranged from 1 for severe symptoms to 9 for nil symptoms.

Breeder's visual preference

At harvest the tubers were laid on the ground for visual assessment. Each cultivar was assessed on the basis of visual characteristics of the tubers, for example positive characters such as the required tuber size, shape, topography and uniformity, desired yield, good tuber numbers per plant and skin finish, versus negative characters such as tuber deformities, secondary growth, cracking, internal disorders, shooting and chaining. Each cultivar was given a score for breeder's visual preference (BVP) that reflected their performance, ranging from 1 for very poor to 9 for very good.

Cooking performance

Selected genotypes were assessed for their specific gravity (SG) and cooking performance. Specific gravity was assessed by the comparison of the weight of tubers in air compared to their weight in

water. Comparison of these weights provided a density for the tubers, which reflected the starch content. Slices are then taken from tubers and cooked in oil at 180^{o}C for 2 minutes to determine how well they will cook when fried. The resulting potato crisps are then scored on their colour, from 1 for very light to 10 very dark. Whole tubers are peeled and boiled until cooked through. The cooked tubers were then assessed on their boiled flesh colour, any resultant sloughing of the flesh, and any after cooking darkening of the flesh that develops within 24 hours. The cooked flesh colour was scored from 1 for white to 5 for dark yellow. After cooking darkening was scored, from 1 for nil to 5 for very dark. Sloughing following boiling was scored on a scale from 1 for nil to 5 for total breakdown of the tuber.

Yield

Yield potential of the genotypes was assessed in the G3 comparative replicated trial by the comparison of their production against standard cultivars for the relevant market use. Tuber numbers were counted and weighed from a range of size categories to in order to determine total yield, marketable yield and the number of tubers per plant. As this was performed in the G3 trial, it was calculated on a reduced set of genotypes when compared to the G2 trials.

Data analysis

Progeny means

The phenotypic variance (V_P) of a family is a sum of the genetic variance (V_G) and environmental factors (V_E), as expressed in the following equation.

$$V_P = V_G + V_E$$

When environmental variation can be reduced to close to zero, then the genotypic variation will approximate the phenotypic variation. Therefore the analysis of family means will provide an estimation of the genotypic value of the parents of the family. This will in turn enable the identification of better parents and superior families.

To calculate the progeny means for the traits of interest across the three breeding populations a one-way ANOVA was conducted on each set of data using Genstat (VSN International Ltd).

BLUP analysis of heritability and calculation of EBVs

BLUP is a standard method for estimating random effects of a mixed model, and was originally developed in animal breeding for the development of estimated breeding values (EBVs). EBVs are calculated using the phenotype of all relatives, as covariances, not just the full sibs as in the calculation of progeny means. By using the genetic effects from all relatives, the genotypic value is broken down into the additive genetic value and the non-additive (dominance, epistatic) components, as reflected in the following equation.

$$V_P = V_A + V_{NA} + V_E$$

From a breeding perspective, the additive effect is important, as it is these alleles that will be passed on to the progeny without any loss of effect. The BLUP analysis was conducted using ASReml3 (NSW Dept. of Primary Industries and VSN International Ltd), to develop EBVs, estimations of trait heritability and V_P values.

In order to utilise information from all relatives, a pedigree file was developed using the Potato Pedigree Database (van Berloo et al., 2007), various relevant publications and in-house pedigree records.

Estimation of expected genetic gain

Improved genetic gain is the objective of all breeding programs in order to develop superior cultivars. Genetic gain was calculated based on the following equation:

$$\Delta_G = h^2 \times \Delta_S \ (\Delta_S = \text{differential of selection})$$
$$\text{or } \Delta_G = (h^2 \times V_P \times i) / L$$

(h^2 = heritability, V_p = phenotype variation, i = selection intensity, L = length of breeding cycle)

Cross generation prediction

Mid-parent values, individual values, progeny means and EBVs were used to compare the predictability of cross generation prediction. This was conducted for a highly heritable trait, SG, and also for a lowly heritable trait, BVP. This analysis was conducted for two breeding populations, the 08 and 09 series.

As a result of the analysis of the 08 and 09 series G1 and G2 data, showing low cross generation prediction for BVP, the 10 series G1 population was subjected to an altered selection regime to determine if a milder selection practice should be employed. Typically we have obtained a 10-20% selection rate in the G1 generation, based solely on BVP. For the 10 series we conducted our typical selection method and obtained c. 20% from our first round of selection. We then conducted a second round of selection on the population, aiming to select another 20% of individuals showing some promise. We then followed up with a third round of selections in a few families to collect individuals with a slight promise. The individuals in the G2 generation were then selected without reference to which round they were selected from, and the results compared.

Development of a selection tool

Potato breeding uses recurrent selection of superior cultivars to reduce the number of genotypes after screening for a number of desirable traits. As the calculation of EBVs provides a value for each genotype, these values can be ranked for each genotype for the important traits for the French fry, crisping and fresh markets. When the desirable trait has a target value within the current phenotypic variation, the genotypes were ranked by how close they were to the target value. The genotypes were ranked in descending order, and the values were added to provide a total score.

Results

1. Review of the literature and publicly available resources

Potato genetics

The cultivated potato has four copies of each chromosome and is therefore genetically a tetraploid (2n = 4x = 48) (Bradshaw, 2007a). As it is considered to have arisen from a single species, *S. brevicaule* (Spooner et al., 2005), it is also considered to be an autotetraploid.

At any given locus, 5 distinct genotypes (aaaa [nulliplex]; Aaaa [simplex]; AAaa [duplex]; AAAa [triplex] or AAAA [quadruplex]) may be observed, contributing to high levels of genetic heterogeneity (Howard, 1970, Ross, 1986, Hawkes, 1990, Bradshaw, 2007a, Jansky, 2009). Cytological studies have revealed that homologous chromosomes in autopolyploids may exhibit a mixture of bivalent and quadrivalent pairing during meiosis (Swaminathan and Howard, 1953, Wallace and Callow, 1995, Stein et al., 2004). Genes of interest in bivalent pairs will show chromosomal segregation, but quadrivalents may exhibit both chromosomal and chromatid segregation. The relative prevalence of the two different segregation methods is dependent on the distance between the gene of interest and the centromere. If close, an intervening recombination event is unlikely and chromosomal segregation will result. If distant, a cross-over is likely to occur, and chromatid segregation needs to be considered.

Given these genetic complexities, conventional breeding for selection and fixation of key characters can be highly demanding. Genetic variance may be non-additive for several desirable traits, which requires the breeder to maximise allele diversity at any given locus in order to optimise heterozygosity and expected heterosis.

Despite the added complexity that autotetraploidy provides, the inheritance of traits still follows the principles of Mendelian genetics for traits under single gene control or quantitative genetics for traits under multiple gene control.

Qualitative genetics and traits

In 1865 Gregor Mendel described the principles of inheritance for simple or qualitative traits, which are those that are controlled by single genes. In this description, he proposed that genes have two variants or alleles due to their inheritance ratios in the progeny.

In potato there are a number of traits that are regulated by major genes, or are qualitative traits. These include a number of disease resistance genes: *H1* for *Globodera rostochiensis* Ro1 and Ro4, *Nx* or Rx_{adg} for *Potato Virus X* (PVX), *Ny* or Ry_{sto} for *Potato virus Y* (PVY), and there are several *R* genes for race specific *Phytophthora infestans* (late blight pathogen). Major genes have also been mapped for the control of flesh, skin and flower colour, for tuber shape and for eye depth (Bradshaw, 2007b).

Progeny tests can be used to determine the allele dosage for single dominant gene traits in prospective parents, as the progeny ratios will be in proportion to the allele dosage of the parent. This property has implications for screening within a breeding program. If both parents are susceptible, there is no requirement to screen, as all the progeny will be susceptible. With simplex and duplex parents, the progeny require screening to determine how many, and which, progeny are resistant. If triplex parents are used, the vast majority of the progeny will contain the gene, but screening will still be necessary, due to the low probability of double reduction that will result in the

absence of the relevant allele from a cultivar. In the instance of a parent being quadruplex, there is no requirement for screening, as all the progeny will contain the relevant allele.

Quantitative genetics and traits

Mendel proposed his theory of genetic inheritance based on the effect of major genes. Unfortunately, most of the traits of interest in plant breeding are under the control of multiple genes, and are quantitative traits.

The genetic analysis of quantitative traits does not follow the segregating ratios of qualitative traits, but can be analysed using family means and variances. When the individuals within a family are scored for a quantitative trait, the mean phenotype of the family will be due to the joint action of the genes and interaction with the environment. Due to this interaction, the progeny will exhibit a normal distribution for the trait of interest. Different families will exhibit different means and variances due to the different genotypes of the families. Traits controlled by multiple genes will exhibit additive and/or dominance effects of the genes or alleles of the genes on the expression of the trait.

The phenotypic variance displayed within a population is the result of both genetic variance and environmental factors. When the environmental variations can be reduced by trial design and replications to be close to zero, then the genotypic variation will approximate the measured phenotypic variation (Kearsey and Pooni, 1998).

There are a large number of potato traits that are regulated by multiple genes, or are quantitative traits. Major quantitative trait loci (QTLs) have been identified for a number of quality, agronomic and disease resistance characters. These include plant maturity and resistances to late blight, *Verticillium* wilt, potato cyst nematodes and *Potato leaf roll virus*. Other traits under the control of complex polygenic interactions include dormancy, dry matter, starch content, fry colour, *Erwinia* resistance, tuberisation and yield (Bradshaw, 2007b).

Progeny tests to identify superior progeny sets based on phenotypic evaluation will determine the value of the parent for these traits, without identifying the location or number of genes that regulate the expression of the trait. The analysis of quantitative traits is important for population improvement, method of selection and genetic gain (Moose and Mumm, 2008). Animal breeding programs have greatly benefited from estimating the genetic merit of selection candidates based on phenotypic and pedigree information. For example, significant gains have been seen in milk yield in Holstein cattle in the USA (Van Vleck et al., 1986).

The potential for marker-assisted selection in potato

Genetic markers based on variations in DNA, derived from research in molecular genetics, provide great potential to assist plant breeders in the identification of genes of interest for the development of new cultivars.

A number of factors need to be considered for the use of DNA-based markers in marker-assisted selection (MAS), and application in plant molecular breeding. The markers must be reliable and closely linked, preferably less than 5 centimorgans (cM) from the gene of interest (Collard et al., 2005, Collard and Mackill, 2008). Close proximity of the marker locus to the target gene is preferable, as this will reduce the probability of recombination occurring between the marker and the gene. Markers positioned in flanking locations on each side of the gene will also improve the reliability of selection, as the presence of both markers in genotypes will indicate the absence of recombination.

The technical procedure for the marker assay needs to be simple and straight-forward, so that results can be obtained in a timely manner. Some techniques require large quantities of high quality genomic DNA, which may be difficult to obtain in practice. High-throughput methods are also desirable in order to rapidly analyse a large number of samples. The marker locus needs to be polymorphic and capable of discrimination between genotypes in the breeding population, and the use of the marker must be cost-effective (Collard and Mackill, 2008). Co-dominant markers will detect the presence of recessive alleles and are also able to distinguish between homozygous and heterozygous states (Collard et al., 2005).

There are a number of different types of genetic marker systems that have been used historically, including restriction fragment length polymorphism (RFLP), amplified fragment length polymorphism (AFLP), rapid amplification of polymorphic DNA (RAPD) and simple sequence repeats (SSRs) which have also been known as microsatellites. These systems each have advantages and disadvantages (Collard et al., 2005). Other marker systems that are useful for MAS include sequence tagged site (STS), sequence characterised amplified region (SCAR) or single nucleotide polymorphism (SNP) markers that are derived from a specific DNA sequence (Shan et al., 1999, Sanchez et al., 2000, Sharp et al., 2001).

The most widely used markers in current plant molecular breeding practice are SSRs and SNPs. SSR markers are generally highly polymorphic, reliable, and relatively simple and cheap to use. SSRs can also be multiplexed (Collard and Mackill, 2008), and more modern methods use capillary based electrophoresis platforms to detect the amplified products.

The relative abundance of SNPs across the genome makes them the most attractive molecular marker system that has yet been developed. There are estimates of a SNP occurring every 100-300 base pairs in any genome, while in plant genomes they appear to be more frequent (Gupta et al., 2001). Based on such large numbers of markers, it is possible to scan the entire genome at extremely high densities for association of individual markers with traits, which is known as whole-genome scanning (WGS), genome-wide association studies (GWAS), or association genetics (McCarthy et al., 2008, Ganal et al., 2009).

SNPs can be identified in both coding and non-coding sequences, and when present in coding sequences can show 100% association with the trait variation, and be very useful for MAS (Gupta et al., 2001). Amplicon resequencing for the identification of SNPs in autopolyploids would also be very useful as it is sensitive enough to detect SNPs in a dosage sensitive manner (Ganal et al., 2009), permitting the identification of genotypes with multiple copies of desirable genes.

DNA markers that are closely linked to a gene of interest can be used for MAS. MAS uses the presence or absence of a marker to replace or assist in phenotypic selection in a way that makes it more efficient, reliable and cost effective compared to the more conventional phenotypic methodology (Collard et al., 2005).

Qualitative or quantitative traits can both be identified using MAS, but the effectiveness will be greater for qualitative traits as only a single gene needs to be identified for the trait to be expressed in the individual. As well as a requirement for multiple markers for quantitative traits, there are problems in accurate QTL identification, as the genotype is not unambiguously associated with the phenotype. A given phenotype can result from different genotypes, while the same genotype can provide different phenotypes when affected by other genes or environmental effects (Kearsey and Pooni, 1998). Consequently, selection of QTL-containing regions using MAS is not as straight forward as using MAS for qualitative traits.

MAS may be easier than phenotypic selection, or may be conducted much earlier than conventional methods due to the limited availability of plant material for screening. MAS can be done at, or soon after, the seedling stage. The expression of a number of quantitative traits can be affected by the environment or the maturity of the individual. Reliable data can also be collected from single plants, when phenotypic evaluation would require many more plants, more trials or multiple years or environments. MAS can also be used when quarantine or biosecurity restrictions would not allow phenotypic screening to be carried out due to contamination risks.

Publicly Available Resources

A broad variety of on-line resources are available to support genomics-assisted breeding of potato, an indicative (but by no means fully comprehensive) selection of which are summarised in Table 1.2.1.

Table 1.2.1. On-line resources available to support genomics-assisted breeding of potato

Title and Web Site Address (URL)	Purpose
European Cultivated Potato Database http://www.europotato.org/menu.php	Characteristics of cultivars, including level of disease resistance and other specific characters, along with breeding history and pedigree data.
Potato Pedigree Database http://www.plantbreeding.wur.nl/potatopedigree	Breeding history and pedigree data.
The British Potato Variety Database http://varieties.potato.org.uk/menu.php	Characteristics of cultivars, including level of disease resistance and other specific characters.
North Carolina State Potato Variety Database www.potatoes.wsu.edu/varieties/	Characteristics of cultivars, including level of disease resistance and other specific characters.
Potato genome sequencing consortium (PGSC) http://www.potatogenome.net/index.php/Main_Page http://potatogenomics.plantbiology.msu.edu/index.html	Collaboration between 16 international research groups, providing links to data from the genome sequencing outcomes from the target genotypes RH89-039-16, and DM1-3 516R44, including BLAST application, download and genome browser.
PoMaMo www.gabipd.org/projects/Pomamo	Repository of molecular genetic marker information, related sequences, genetic maps and function maps for pathogen resistance.
GCP Molecular marker kit http://s2.generationcp.org/gcp-tmm/web/	Molecular marker information for a number of crops, including potatoes. The GCP MM Toolkit provides information on currently available and validated markers to support the adoption of modern agricultural technologies.
SOL Genomics Network http://solgenomics.net/	Contains genomic, genetic, and phenotypic information for members of the Solanaceae family
Solanaceae Co-ordinated Agricultural	Support for comparison of sequence data from

Title and Web Site Address (URL)	Purpose
Project (SolCAP) http://solcap.msu.edu/potato.html	tomato and potato to understand sequence homology with phenotypic variation for crop improvement. Contains data on genotypes subjected to phenotypic analysis and details on SNPs identified from transcriptome comparisons to the genome sequence and the initial design of the Illumina Infinium potato SNP genotyping chip.
J. Craig Venter Institute (JCVI) TIGR Solanaceae Genomics Resource www.jcvi.org/potato/	Stores previous data sets from initial genome studies using cDNA based microarrays on potato. Remains hosted through the JCVI.

Potato marker-assisted selection

Marker-assisted breeding in potato has not yet been applied with the vigour observed in other crops, such as the cereals (Collard and Mackill, 2008). This is presumably because of the complexities of autotetraploid genetics, the absence of detailed functional maps and lack of markers linked with the numerous qualitative and quantitative traits of interest. The majority of studies to identify markers have also been performed on diploid cultivars and populations, which do not always prove useful in tetraploid breeding populations. For example, Moloney et al. (2010) have recently developed a marker to identify the *GpaIV*$^{s}_{adg}$ locus for *Globodera pallida* resistance, but when this was used on tetraploid breeding populations, recombination events were seen in the progeny.

Increasingly since 1988, detailed genetic maps have been developed for potato (Bonierbale et al., 1988, Gebhardt et al., 1989, Gebhardt et al., 1991, Tanksley et al., 1992, Van Os et al., 2006). Functional maps for genetic factors controlling traits of interest have also been developed (Leister et al., 1996, Grube et al., 2000, Gebhardt and Valkonen, 2001, Chen et al., 2001, Bradshaw et al., 2008). There have also been a significant number of studies aimed at locating genes or QTLs for traits of interest, although they have mainly been performed on diploid populations, and the feasibility of data application to tetraploid populations remains a question. Van Eck (2007) provided a review of studies on morphological and tuber traits, while Simko et al. (2007) provided a review of studies on pest and disease resistance.

The Consultative Group on International Agricultural Research (CGIAR) has developed the Generation Challenge program's Molecular Marker Toolkit to allow rapid access to currently available and validated markers, to support the adoption of modern agricultural technologies for the benefit of the poor in developing countries. This toolkit is a compilation of information available via internet sources, public databases and papers that has been verified against plant breeders' experiences. The toolkit lists only seventeen markers for potato, for seven traits of interest to potato breeders. These seven traits are golden cyst nematode resistance (*Globodera rostochiensis*), pale cyst nematode resistance (*Globodera pallida*), root-knot nematode resistance (*Meloidogyne chitwoodi*), potato late blight resistance, *Potato virus Y* resistance, *Verticillium* wilt disease resistance and anthocyanin-pigmented potato tuber flesh (http://s2.generationcp.org/gcp-tmm/web/).

MAS could be utilised in a potato breeding program in Australia, when more cost-effective than conventional screening, or in order to provide an earlier or more effective screening methodology. The initial traits targeted for the development of MAS should be relevant to Australian potato

breeding priorities, and applicable to Australian germplasm. Markers currently available for traits of interest to Australian potato breeding are listed in Table 1.2.2.

Table 1.2.2. Markers currently available for traits of interest to Australian potato breeding.

Trait	Gene of interest	Marker code	Source of resistance	Reference
Potato cyst nematode resistance (*Globodera rostochiensis* Ro1)	*H1*	TG689	*S. tuberosum* ssp *andigena*	(De Koeyer et al., 2010)
Potato cyst nematode resistance (*Globodera rostochiensis* Ro1)	*H1*	57R	*S. tuberosum* ssp *andigena*	(Schultz et al., 2012)
Potato virus Y resistance	Ry_{adg}	M45	*S. tuberosum* ssp *andigena*	(Brigneti et al., 1997)
Potato virus Y resistance	Ry_{adg}	RYSC3	*S. tuberosum* ssp *andigena*	(Whitworth et al., 2009)
Potato virus Y resistance	Ry_{sto}	STM0003	*S. stoloniferum*	(Song et al., 2005)
Potato virus X resistance	Rx_{adg}	GP34	*S. tuberosum* ssp andigena cv Cara, Atlantic	(Bendahmane et al., 1997)
Potato virus S resistance	*Ns*	CP16, $SC811_{454}$	*S. tuberosum* ssp *andigena*	(Marczewski et al., 2002, Witek et al., 2006)
Late blight resistance	numerous		numerous	(Simko et al., 2007)
Root-knot nematode resistance (*Meloidogyne chitwoodi*)	$R_{Mc1(blb)}$	193I9	*S. bulbocastanum*	(Zhang et al., 2007)
Root-knot nematode resistance (*Meloidogyne chitwoodi*)	$R_{Mc1(blb)}$	39E18	*S. bulbocastanum*	(Zhang et al., 2007)
Root-knot nematode resistance (*Meloidogyne chitwoodi*)	$R_{Mc1(blb)}$	524K16	*S. bulbocastanum*	(Zhang et al., 2007)
Root-knot nematode resistance (*Meloidogyne chitwoodi*)	$R_{Mc1(blb)}$	56F6	*S. bulbocastanum*	(Zhang et al., 2007)
Root-knot nematode resistance (*Meloidogyne chitwoodi*)	$R_{Mc1(blb)}$	406L19	*S. bulbocastanum*	(Zhang et al., 2007)
Verticillium wilt resistance	*StVe1*	STM1051-193	*S. tuberosum*	(Simko et al., 2004b)
Verticillium wilt resistance	*StVe1*	C287F1/ C287R2	*Solanum* spp.	(Bae et al., 2008)

2. International linkages

Scotland
Scotland is recognised as having, at the James Hutton Institute (JHI), one of the leading potato genetics and genomics groups. They have a strong pre-breeding focus, as well as conducting commercially funded cultivar breeding. We have developed a working relationship with colleagues in Scotland. Tony Slater has visited SCRI/JHI three times during the past five years, and DPI has been visited by Drs. Glenn Bryan and Finlay Dale. We have also had regular email and phone contact, and both Dr. Bryan and Ms. Karen McLean helped us with the PCN marker protocols. This has resulted in a co-authored article on original research, and a review article as a draft manuscript. We have also had advanced discussions regarding an internationally funded VC application for the next round of funding.

United States of America
The USDA runs federal and tri-state breeding programs, which focus on cultivar breeding. This program is supported by various research groups. We have developed a strong relationship with Drs. Rich Novy and Shelly Jansky from the USDA. Tony Slater has had long discussions with both regarding an internationally funded VC application for the next project. This followed from the visit of Dr. Lee Schultz to their research programs in 2011. Rich Novy and Jonathan Whitworth also assisted us with implementation of the PVY marker protocol.

The Netherlands
The Netherlands is recognised as a leading potato research hub, with a number of research laboratories at the University of Wageningen. We have held good discussions with Drs. Anna-Marie Wolters, Erin Bakker and Anna Finkers-Tomczak regarding PCN and TSWV markers. We have also been invited by Dr. Herman van Eck to develop a review article on MAS.

Ireland
Ireland has an effective genomics program at the Crops Research Centre, Teagasc, with good linkages with the Scottish program, as the leader of the genomics program, Dr. Dan Milbourne, was trained at SCRI. We have had a number of discussions with Dr. Milbourne on their genetic marker program, and it was proposed that they would also be co-authors on the review proposed by Dr. Herman van Eck.

Germany
Germany has a well recognised potato genomics research group at the Max-Planck Institute for Plant Breeding Research at Cologne. This group is run by Dr. Christiane Gebhardt, and a number of productive discussions have been held on MAS.

Poland
We have had discussions with Prof. Waldermar Marczewski regarding MAS, and he has sent us DNA in order to validate a marker for PVS resistance.

France
We have had a series of discussions with Dr. Eric Bonnel and Ms. Gisele Lairy-Joly of Germicopa on their breeding program and selection methods.

New Zealand
We have had discussions with Drs. Jeanne Jacobs and Tony Conner from Plant & Food Research, New Zealand and exchanged information on the activities within the respective programs.

Canada
We have discussed our respective programs with Dr. David De Koeyer and Ms. Agnes Murphy of Agriculture and Agri-Food, Canada.

We have also had good and open discussions with colleagues from Denmark, Peru and Japan.

3. Potato Genotype Identity Kit

The application of molecular markers to develop a potato genotype identity (PGI) kit, relevant to the Australian potato industry has been undertaken within the project. A collection of 12 highly polymorphic molecular markers that generate a total of 88 specific fragment sizes were identified and experimentally validated over all the parent material used in the Australian breeding program. The amplified product sizes that each marker produces are presented in Table 3.2.1. This collection of markers was finalised as a tool to distinguish between the relevant cultivar material for the Australian potato industry.

Table 3.2.1. Amplified marker alleles produced by each marker

	Marker	A	B	C	D	E	F	G	H	I	J
1	STM0006	102	106	108	113	117	121	123	125		
2	STM2005	168	174	180	186	199	211				
3	STM2022	193	196	199	208	251					
4	STM5127	256	257	258	260	266	269	287	290		
5	STG0016	141	144	147	151	154	157	162	174		
6	STM3012	181	209	211	213	215	225				
7	STM3023	195	203	205	213						
8	SSR1	222	225	227	229	233	235	237	242	246	
9	STI0004	93	96	99	105	111	113	116	119	122	
10	STM3009	159	163	166	169	175	181	187			
11	STM0019	180	189	202	208	212	214	216	220	225	251
12	STM5136	250	257	260	263	269	272	277	289		

A total of 323 samples were assessed using the described collection of markers. This collection of samples included 304 unique samples, with 6 cultivars that had multiple entries. The cultivars with multiple entries were; Atlantic, Kennebec, Russet Burbank, Sebago, Trent and Wontscab. All of the unique plant genotypes that were assessed could be individually identified as having a unique marker profile. The most industry relevant lines that were assessed have been presented as a subset of data, in the form of a dendogram (Figure 3.2.1). The complete data set is presented as a table in the SOP, with all marker alleles produced per marker listed against each cultivar. The relationships of the cultivars seen in the dendogram are based only on the limited data set generated through the use of the PGI kit. As the marker number used in the PGI kit has specifically been reduced to a minimum in order to permit identification of the cultivars under assessment, the output and data generated should not be over interpreted or viewed as absolutely accurate in comparison to the genetic relationships between the cultivars. However the data has been presented as an indication of reliability and discriminatory power.

The outcome of this test is that cultivar protection and disputes over germplasm integrity can now be resolved using molecular marker techniques that can assist all parties involved with the Australian potato industry.

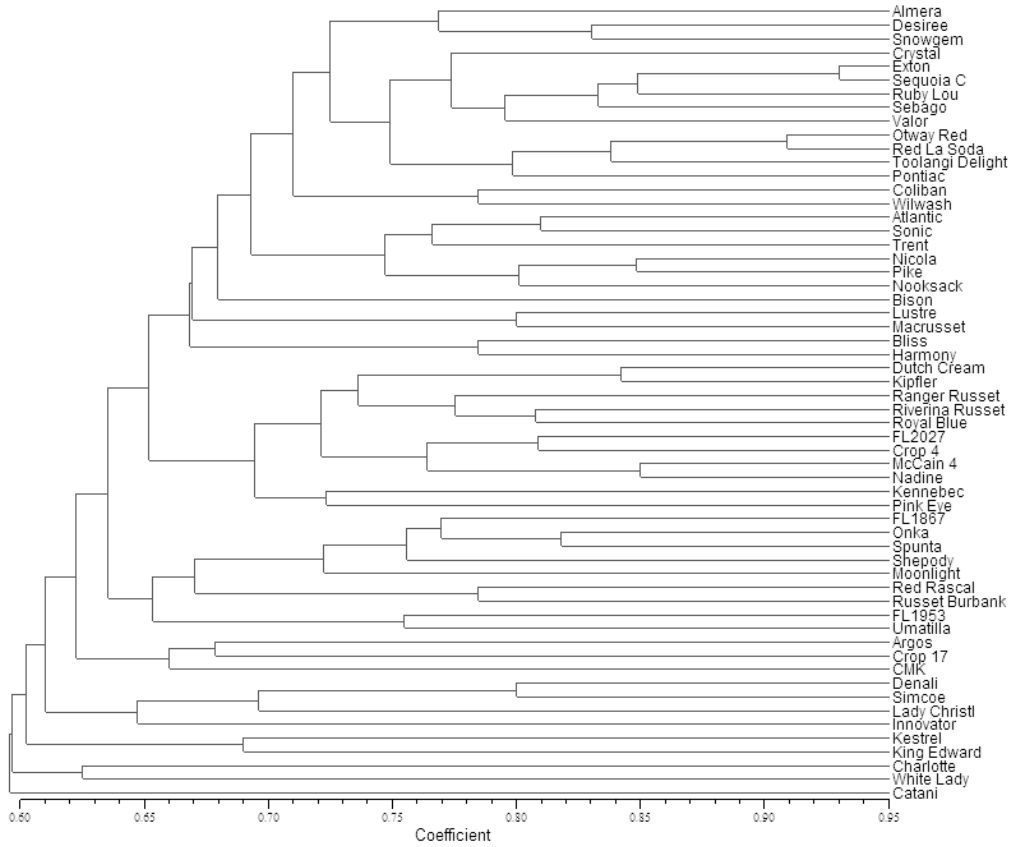

Figure 3.2.1. Dendogram obtained from the DICE similarity coefficient and UPGMA cluster method for the main 58 cultivars used for the Australian breeding program.

4. Prioritising traits for MAS

To develop MAS for Australian potato breeding, target traits were prioritised for their importance to Australian potato breeding, their ease or difficulty of phenotyping and genotyping, as well as a cost analysis of the marker assay application in comparison to phenotypic screening.

Tuber shape, eye depth, cold sweetening resistance and after cooking darkening were in the highest priority group for both phenotyping and genotyping priority. Heel depth, maturity, yield, plant size and dormancy were also a high priority from a phenotyping perspective, but not as easily genotyped. All of these traits are reasonably easily phenotyped. Alternatively, PCN, PVY, PVS, PVX and TSWV disease resistance traits were a high priority from a genotyping perspective, but are not as easily phenotyped and are therefore prime candidates for the development of MAS for potato breeding in Australia (Figure 4.2.1).

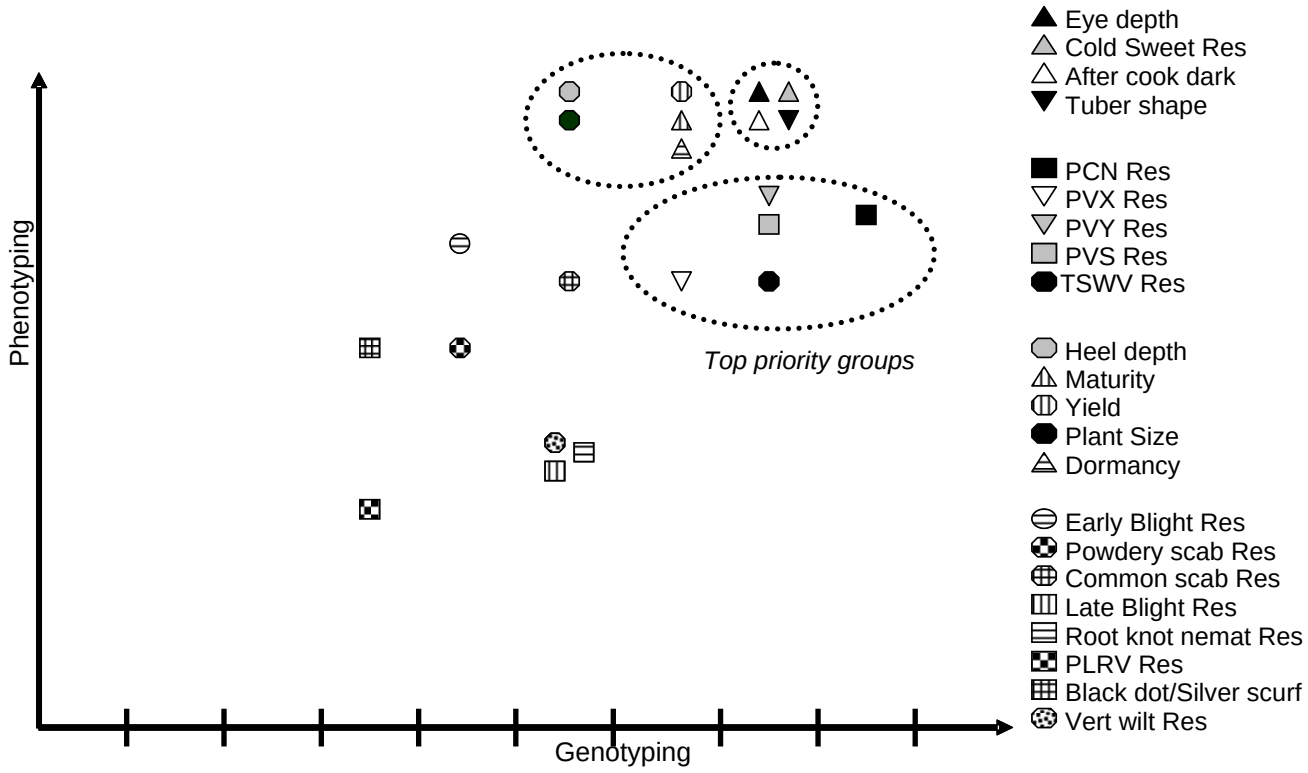

Figure 4.2.1. Priority plot of traits for marker-assisted selection for Australian potato breeding

5. PCN marker evaluation

Of the 308 cultivars that had been phenotyped, 90 were found to be resistant and 218 were susceptible to PCN (Appendix 1). When the main Australian commercial cultivars were examined, 16 were found to be resistant, and of these 11 were fresh market cultivars and 5 were used for crisp processing, while none were used for French fry style processing (Table 5.2.1). When the resistance phenotype observed here was compared to the ratings described on the European Cultivated Potato Database, the results were consistent with the results for resistance to *G. rostochiensis* Ro1 or Ro4, but not the other pathotypes (Appendix 2).

A total of 281 cultivars that have been phenotyped were also genotyped with the TG689 marker assay, leading to the identification of 7 cultivars for which results between the phenotyping study and the TG689 marker disagreed (Table 5.2.1. and Appendix 3). Four cultivars that were resistant when phenotyped failed to amplify the TG689 139 bp amplicon. These cultivars were Agria, Brora, Granola and Harborough Harvest (Table 5.2.1.), and this result could indicate a loss of association with the TG689 marker or the presence of a different source of resistance. Alternatively, three cultivars that were susceptible when phenotyped produced a positive response to the 139 bp amplicon, which should predict resistance. These cultivars were Candy Cane, Desiree and Onka (Table 5.2.1.), and this false positive response correctly indicates a loss of association.

The 57R SCAR marker assay was combined in duplex ratio with the STM1051 SSR marker as an internal control for the presence of DNA, and all reported cultivars exhibited the STM1051 marker. The 281 cultivars that have been phenotyped were also genotyped with the combined 57R and STM1051 assay. A total of 89 of 90 resistant cultivars generated the 452 bp resistance-associated allele. None of the 191 PCN-susceptible cultivars generated the resistance-predictive allele (Table 5.2.1, appendix 3). With the exception of 'Brora', the 6 other cultivars which displayed anomalous results for the TG689 genotype, all displayed complete concordance between the predictive allele and *H1*-mediated PCN resistance/susceptibility (Table 5.2.1). The cultivar Brora failed to exhibit the resistance allele, suggesting the presence of a source of resistance other than the *H1* gene.

Table 5.2.1. A comparison of genotypes derived from the TG689 and 57R assays with PCN phenotypes for the top Australian commercial cultivars, and the cultivars which lost association between TG689 and the PCN phenotype.

Cultivar	PCN phenotype	$TG689_{140}$	$57R_{452}$
Agria	Resistant	no	yes
All Red	Susceptible	no	no
Almera	Resistance	yes	yes
Atlantic	Resistance	yes	yes
Brora	Resistance	no	no
Candy Cane	Susceptible	yes	no
Catani	Susceptible	no	no
Coliban	Susceptible	no	no
Desiree	Susceptible	yes	no
Driver	Susceptible	no	no
Exton	Susceptible	no	no
FL1867	Resistance	yes	yes
Granola	Resistance	no	yes
Harborough Harvest	Resistance	no	yes
Innovator	Susceptible	no	no
Kennebec	Susceptible	no	no
Lady Christl	Resistance	yes	yes

Cultivar	PCN phenotype	$TG689_{140}$	$57R_{452}$
Lustre	Susceptible	no	no
Moonlight	Resistance	yes	yes
Nadine	Resistance	yes	yes
Nicola	Resistance	yes	yes
Nooksack	Susceptible	no	no
Onka	Susceptible	yes	no
Pike	Resistance	yes	yes
Ranger Russet	Susceptible	no	no
Red Rascal	Susceptible	no	no
Royal Blue	Resistance	yes	yes
Ruby Lou	Susceptible	no	no
Russet Burbank	Susceptible	no	no
Sebago	Susceptible	no	no
Sequoia	Susceptible	no	no
Shepody	Susceptible	no	no
Simcoe	Resistance	yes	yes
Spunta	Susceptible	no	no
Trent	Susceptible	no	no
Valor	Resistance	yes	yes
White Lady	Resistance	yes	yes
Wilwash	Susceptible	no	no

Note: All cultivars produced internal control amplicons for both the TG689 and 57R markers. Shading indicates disagreement between phenotype and genotype results

Due to the highly predictive nature of the 57R marker assay for the H1 conferred PCN resistance, a selection of previously unphenotyped parental cultivars, were screened using the automated 57R assay. Of 103 cultivars, 30 amplified the 57R resistance-predictive allele (Appendix 4).

6. PVY and PVX marker evaluation

PVY sequencing

With the exception of 4 isolates (Gatton 2007, Thorpdale 2009, PVY C and PVY 55), over 80% of the virus genome was sequenced for each PVY isolate (Table 6.2.1). The main gaps in the genome sequences are located near the 5´-terminus of the genome, around the P1 and P3 region.

Table 6.2.1. The percentage of each PVY isolate genome that has been sequenced.

Isolate	PVY isolate	Strain	% of genome sequenced	Used in phylogenetic analysis
1	Gatton 2007	N	71.9%	no
2	Thorpdale 2008	N	80.7%	no
3	Warragul 2009	N	94.0%	yes
4	Ballarat 2008	N	99.4%	yes
5	Ballarat 2009	N	95.1%	yes
6	Ellinbank 2009	N	89.4%	yes
7	Thorpdale 2009	N	68.5%	yes
8	SA 2010	N	92.7%	yes
9	PVY^{C}	C	78.9%	yes
10	PVY^{O}	O	84.8%	yes
11	PVY^{N} 2005	N	97.7%	yes
12	Nicola tuber	NTN	97.8%	yes
13	PVY 55	n/a	78.6%	no
14	PVY 72	O	92.7%	yes
15	PVY 1473	NTN	88.9%	no
16	PVY 1622	NTN	92.7%	yes

To analyse the relationships between the different PVY isolates, a phylogenetic tree was constructed using the sequences from the NIb, CP and 3´-UTR regions of the PVY genomes. This region of 2184 bp in size, starting from position 7500 to position 9683 of the PVY genome (in reference to genome AB331516.1) was chosen for comparison because it was sequenced for 12 out of the 16 isolates used in this study. The PVY^{N} isolates from Nicola tuber, Victoria 2005, Ballarat 2008, Ballarat 2009, Warragul 2009 and South Australia 2010 and the PVY^{NTN} strain 1622 from Queensland clustered closely together on the same branch of the phylogenetic tree, indicating high similarity based on their genetic composition (Figure 6.2.1). The lowest sequence homology between these 7 isolates is 99.5%, whilst the highest is 99.9% (Table 6.2.2). Their closest relatives are the American PVY^{NTN} strains EF026075.1 and FJ204165.1 (Figure 6.2.1). The Thorpdale 2009 PVY isolate is 100% identical to the PVY^{O} strain based on their NIb-CP-3´-UTR sequences (Figure 6.2.1, Table 6.2.2) and both are placed on the same branch as the Ellinbank 2009 PVY strain, all of which are most closely related to the Canadian PVY^{O} strain (Accession U09509.1; Figure 6.2.1). PVY isolate 72 is 99.8% similar to the PVY^{C} strain and was placed in the same cluster of the phylogenetic tree (Figure 6.2.1, Table 6.2.2).

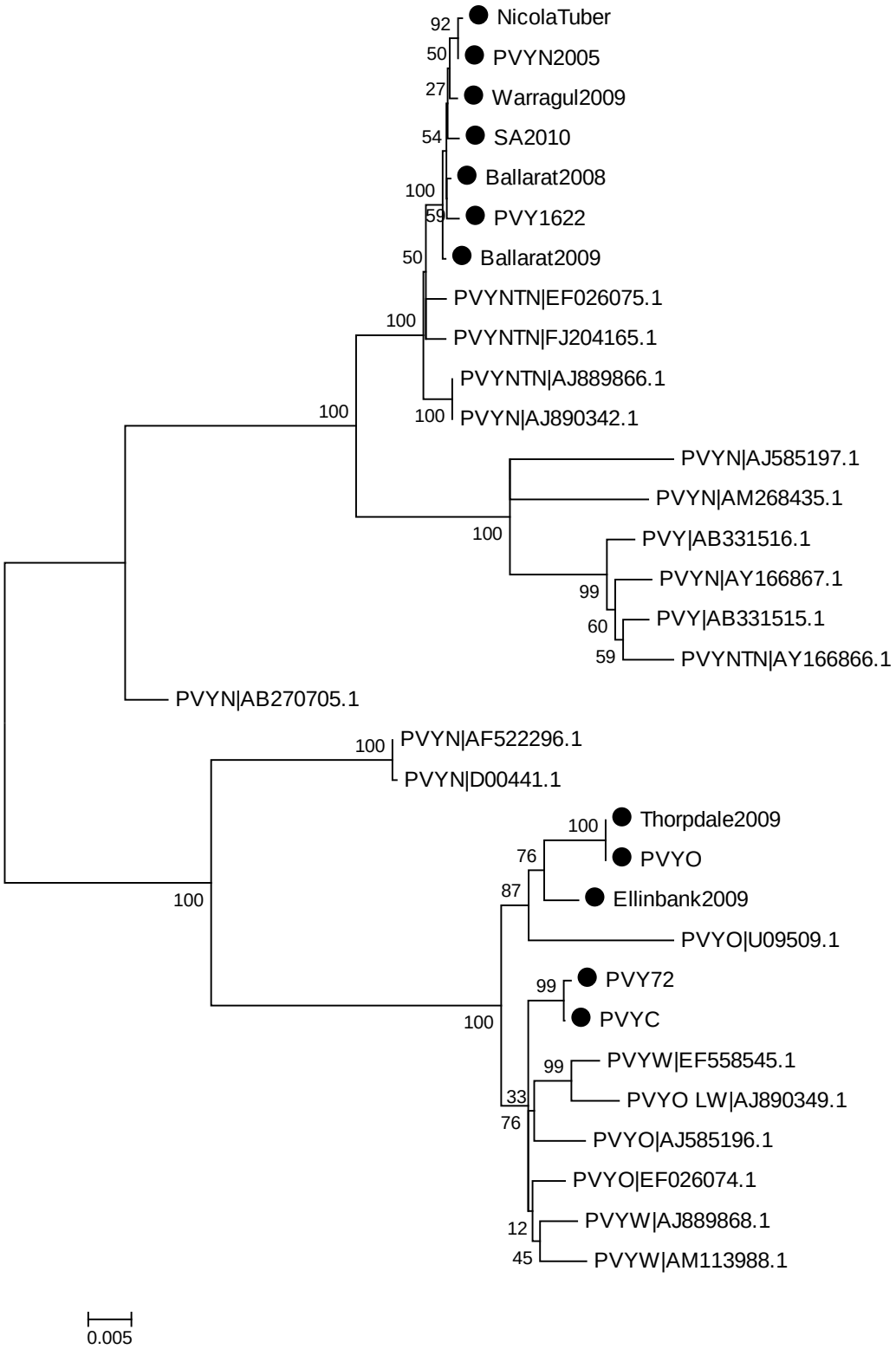

Figure 6.2.1. Evolutionary relationships of 12 PVY isolates from this study compared to published PVY genomes representing different strains around the world

The evolutionary history was inferred using the Neighbour-Joining method with bootstrap consensus. The tree is drawn to scale and branches corresponding to partitions reproduced in less than 50% bootstrap replicates are collapsed (MEGA 4.0).

Table 6.2.2. Percentage nucleotide sequence match for the 12 PVY isolates used in the phylogenetic analysis

	Nicola Tuber	PVY^{N} 2005	Ballarat 2008	Ballarat 2009	Warragul 2009	SA 2010	PVY 1622	PVY^{O}	Thorpdale 2009	Ellinbank 2009	PVY^{C}	PVY 72
Nicola Tuber	100	99.9	99.7	99.7	99.7	99.6	99.5	88.9	88.9	89.0	89.4	89.3
PVY^{N} 2005	99.9	100	99.8	99.7	99.8	99.7	99.6	88.9	88.9	89.1	89.5	89.4
Ballarat 2008	99.7	99.8	100	99.8	99.8	99.8	99.7	89.0	89.0	89.2	89.5	89.5
Ballarat 2009	99.7	99.7	99.8	100	99.7	99.8	99.6	89.0	89.0	89.2	89.6	89.5
Warragul 2009	99.7	99.8	99.8	99.7	100	99.8	99.6	88.9	88.9	89.1	89.5	89.4
SA 2010	99.6	99.7	99.8	99.8	99.8	100	99.6	88.9	88.9	89.1	89.5	89.4
PVY 1622	99.5	99.6	99.7	99.6	99.6	99.6	100	88.8	88.8	89.0	89.4	89.3
PVY^{O}	88.9	88.9	89.0	89.0	88.9	88.9	88.8	100	100	98.8	97.7	97.7
Thorpdale 2009	88.9	88.9	89.0	89.0	88.9	88.9	88.8	100	100	98.8	97.7	97.7
Ellinbank 2009	89.0	89.1	89.2	89.2	89.1	89.1	89.0	98.8	98.8	100	98.8	98.8
PVY^{C}	89.4	89.5	89.5	89.6	89.5	89.5	89.4	97.7	97.7	98.8	100	99.8
PVY 72	89.3	89.4	89.5	89.5	89.4	89.4	89.3	97.7	97.7	98.8	99.8	100

Grey shaded areas indicate where the isolate was compared to itself; underlined numbers indicate the PVY^{NTN} group and the double underline indicate the non PVY^{NTN} group

PVY marker evaluation

Of the 69 cultivars that have been phenotyped for PVY^{O} resistance, 28 were shown to be susceptible, while 6 tested negative on three or more occasions and were therefore considered resistant. Sixteen others tested negative twice and may also be resistant, although a third trial would be desirable to provide confidence in their status. Of the 74 cultivars that have been phenotyped for PVY^{NTN} resistance, 61 were shown to be susceptible, while only 3 tested negative on three occasions and were considered resistant, while two others tested negative twice and may also be resistant (Table 6.2.3.)

The 74 cultivars that were phenotyped for PVY resistance were also genotyped with the markers RYSC3, M45 and STM0003. Only three cultivars amplified the RYSC3 marker, while the M45 marker was amplified in the same three cultivars and eight additional cultivars (Table 6.2.3). The three cultivars that amplified both markers were Emma, Eva and PO3, while the M45 marker was also amplified in BC0894-2, Carlingford, Friar, Galil, KT3, Lady Christl, Melody and Royal Blue. These eleven cultivars all tested negative for the virus, when phenotyped for resistance to both strains of PVY, except for Emma, which was identified as susceptible to PVY^{NTN} (Table 6.2.3). The

STM0003 marker was identified in two different cultivars, namely Rioja and White Lady, which have both phenotyped as resistant cultivars. All other cultivars that did not amplify any of the markers, displayed a susceptible phenotype to at least the PVY^{NTN} strain (Table 6.2.3). The cultivars that were resistant to PVY^{O} and susceptible to PVY^{NTN} did not display the marker for either extreme resistance gene, and therefore may contain a hypersensitive resistance gene.

Table 6.2.3. A comparison of phenotypes and genotypes indicated by the RYSC3, M45 and STM0003 assays with PVY phenotypes for the top Australian commercial cultivars, and relevant cultivars.

Cultivar	No. of PVY^{O} trials	PVY^{O} resistance phenotype	No. of PVY^{NTN} trials	PVY^{NTN} resistance phenotype	RYSC3	M45	STM0003
813/28			1	susceptible	no	no	no
Almera	1		2	susceptible	no	no	no
Amerosa			2	susceptible	no	no	no
Argos			2	susceptible	no	no	no
Atlantic	1	susceptible	4	susceptible	no	no	no
BC0894-2	1		1		no	yes	no
Bison	1		3	susceptible	no	no	no
Bliss	1	susceptible	1	susceptible	no	no	no
Carlingford	1		1		no	yes	no
Catani	1	susceptible	2	susceptible	no	no	no
Charlotte	1	susceptible	2	susceptible	no	no	no
CMK			1	susceptible	no	no	no
Coliban	2	resistant?	2	susceptible	no	no	no
Crop 17	1		2	susceptible	no	no	no
Crop 8	1		3	susceptible	no	no	no
Crystal	2	resistant?	1	susceptible	no	no	no
Denali	2	resistant?	1	susceptible	no	no	no
Desiree	2	resistant?	2	susceptible	no	no	no
Dutch Cream	1	susceptible	1	susceptible	no	no	no
Emma	2	resistant?	2	susceptible	yes	yes	no
Eos	1		1		no	yes	no
Eva	2	resistant?	3	resistant	yes	yes	no
Exton	2	resistant?	2	susceptible	no	no	no
FL1867	1	susceptible	2	susceptible	no	no	no
FL1953	2	resistant?	2	susceptible	no	no	no
FL2027	1	susceptible	2	susceptible	no	no	no
Friar	1		1		no	yes	no
Galil	1		1		no	yes	no
Harmony	1	susceptible	2	susceptible	no	no	no
Kennebec	1	susceptible	1	susceptible	no	no	no
Kestrel	1		1	susceptible	no	no	no
King Edward	1	susceptible	1	susceptible	no	no	no
Kipfler	1	susceptible	1	susceptible	no	no	no
KT3	1		1		no	yes	no
Lady Christl	2	resistant?	2	resistant?	no	yes	no
Lustre	1	susceptible	1	susceptible	no	no	no
Macrusset	1	susceptible	1	susceptible	no	no	no
McCain 1	1	susceptible	1	susceptible	no	no	no
McCain 4	1	susceptible	1	susceptible	no	no	no

Cultivar	No. of PVY^{O} trials	PVY^{O} resistance phenotype	No. of PVY^{NTN} trials	PVY^{NTN} resistance phenotype	RYSC3	M45	STM0003
Melody	1		1		no	yes	no
Mirridong	1	susceptible	1	susceptible	no	no	no
Moonlight (Crop 13)	1		3	susceptible	no	no	no
Nadine	3	resistant	1	susceptible	no	no	no
Nicola	1	susceptible	1	susceptible	no	no	no
Nooksack	1	susceptible	1	susceptible	no	no	no
Onka	1	susceptible	1	susceptible	no	no	no
Otway Red	2	resistant?	1	susceptible	no	no	no
Pike	1	susceptible	1	susceptible	no	no	no
Pink Eye	1	susceptible	1	susceptible	no	no	no
PO3	2	resistant?	3	resistant	yes	yes	no
Pontiac	2	susceptible	1	susceptible	no	no	no
Ranger Russet	1		1	susceptible	no	no	no
Red La Soda	1	susceptible	1	susceptible	no	no	no
Red Rascal	4	resistant	1	susceptible	no	no	no
Rioja	2	resistant?	3	resistant	no	no	yes
Riverina Russet	1		1	susceptible	no	no	no
Royal Blue	3	resistant	2	resistant?	no	yes	no
Ruby Lou	1	susceptible	1	susceptible	no	no	no
Russet Burbank	2	susceptible	2	susceptible	no	no	no
Sebago	2	resistant?	2	susceptible	no	no	no
Sequoia	4	resistant	1	susceptible	no	no	no
Shepody	1	susceptible	1	susceptible	no	no	no
Simcoe	3	resistant	1	susceptible	no	no	no
Snowgem	2	resistant?	1	susceptible	no	no	no
Sonic	1		1	susceptible	no	no	no
Spunta	1		1	susceptible	no	no	no
Toolangi Delight	2	resistant?	1	susceptible	no	no	no
Trent	2	susceptible	1	susceptible	no	no	no
Umatilla	1		1	susceptible	no	no	no
Valor	1	susceptible	1	susceptible	no	no	no
White Delight (Crop 4)	1		2	susceptible	no	no	no
White Lady	3	resistant	2	resistant?	no	no	yes
Wilwash	1		1	susceptible	no	no	no
Wontscab	2	resistant?	2	susceptible	no	no	no

Note: shading represents disagreement between phenotype and marker presence.

PVX marker evaluation

Of the 49 cultivars that have been phenotyped for PVX resistance, 27 were shown to be susceptible, while 5 tested negative on the two occasions that they were tested. (Table 6.2.4.). Of these 49 cultivars, 14 displayed positive results for the RXSC1 marker. To date, all cultivars displaying the RXSC1 marker have shown a resistance phenotype. Six other cultivars tested negative, but five of those have only been tested once. Umatilla tested negative twice, and may be resistant. When the other main commercial cultivars were tested with the RXSC1 marker, another 8 cultivars displayed a positive result, although they are yet to be phenotyped (Table 6.2.4.).

Table 6.2.4. A comparison of phenotypes and genotypes indicated by the Rx1 gene marker assay with PVX phenotypes for the top Australian commercial cultivars, and relevant cultivars.

Cultivar	PVX ELISA trial 1	PVX ELISA trial 2	No. of trials	Resistance result	RXSC1 gene marker
813/28					yes
Almera					no
Andover	pos		1	susceptible	no
Argos	neg	neg	2		yes
Atlantic	neg		1		yes
Bison					no
Bliss		neg	1		no
Catani		neg	1		no
Charlotte		pos	1	susceptible	no
CMK					yes
Coliban	pos		1	susceptible	no
Crop 17		neg	1		yes
Crystal		neg	1		yes
Delaware	pos		1	susceptible	no
Denali					yes
Desiree	pos		1	susceptible	no
Driver (Crop 8)					no
Dutch Cream					yes
Exton					no
FL1867	pos		1	susceptible	no
FL1953		pos	1	susceptible	no
FL2027		neg	1		yes
Granola	neg		1		no
Harmony	mid		1		no
Innovator	pos		1	susceptible	no
Kennebec	pos		1	susceptible	no
Kestrel	pos		1	susceptible	no
King Edward					no
Kipfler		pos	1	susceptible	no
Lady Christl	neg		1		no
Lustre		neg	1		yes
Macrusset					no
Malin	neg	neg	2		yes
McCain 4		pos	1	susceptible	no
Moonlight (Crop 13)		neg	1		no
Nadine	pos		1	susceptible	no
Nectar	neg		1		yes
Nicola	neg	neg	2		yes
Nooksack		pos	1	susceptible	no
Onka					no
Otway Red					yes
Pike	neg	neg	2		yes
Pink Eye		pos	1	susceptible	no
Pontiac		pos	1	susceptible	no
Ranger Russet	pos		1	susceptible	no

Cultivar	PVX ELISA trial 1	PVX ELISA trial 2	No. of trials	Resistance result	RXSC1 gene marker
Red La Soda		pos	1	susceptible	no
Red Rascal		neg	1		no
Riverina Russet					yes
Royal Blue					no
Ruby Lou					no
Russet Burbank	pos		1	susceptible	no
Sebago	pos		1	susceptible	no
Sequoia	pos		1	susceptible	no
Shepody	pos		1	susceptible	no
Simcoe					no
Snowgem		pos	1	susceptible	no
Sonic		pos	1	susceptible	no
Spey	neg	neg	2		yes
Spunta	pos		1	susceptible	no
Toolangi Delight					yes
Trent	pos		1	susceptible	no
Umatilla	neg	neg	2		no
Valor	neg	neg	2		yes
White Delight (Crop 4)		neg	1		yes
White Lady					yes
Whitu	mid	neg	2		yes
Wilwash	pos		1	susceptible	no

Note shaded results currently disagree with the RXSC1 predicted phenotype.

7. TSWV

The TSWV trials did not provide reliable results. In the spray inoculation trial of 1 to 4 spray inoculations, a few symptoms were detected on plants that were sprayed 3 or 4 times, although the symptoms were not consistent. In the four spray inoculation trial, when the plants were monitored for symptom expression, there were no or very minor symptoms, which could have also been attributed to early blight. ELISA testing of these leaves for TSWV obtained a negative result. Hand inoculations over three seasons also produced inconsistent results with inconsistent symptoms and negative ELISA results. Until a reliable phenotyping protocol can be developed, marker evaluation cannot be conducted.

8. Verticillium wilt

The STM1051 marker was screened across all the parental germplasm, demonstrating that the $STM1051_{193}$ amplicon is reasonably common in the germplasm collection, being found in over 60% of the collection.

9. Multiplexed marker assay

To deliver molecular marker technology with optimal performance at the lowest cost, the validated diagnostic molecular markers were combined in multiplex ratio into a single tube marker assay. In addition, multiple combinations were considered for multiple breeding objectives and applications. Before experimental activities were initiated, all markers were comprehensively validated as single (simplex) markers. Initial combinations of 57R and STM1051 (duplex), and 57R, RYSC3 and STM1051 (triplex) were highly successful, with marker profiles being delivered reliably from all tested assays (Table 9.2.1). Addition of the STM0003 and RXSC1 markers was individually validated in a quadruplex assay. The marker M45 generates a product of 493 bp as a positive diagnostic result, and as such is marginal for integration into the multiplexed assay using capillary electrophoresis, for which the typical range of product resolution is between 100-400 bp in size. Integration of this marker into a multiplexed panel as a result was problematic, and efforts to redesign or optimise the assay would be required for delivery in a multiplexed manner in the future. Attached to this final report is a comprehensive SOP that includes all of the required information to repeat the laboratory work and analysis undertaken in the application of the multiplexed assay.

Table 9.2.1. Validated combinations of molecular markers for routine application in potato germplasm diagnostics.

Pest and gene	Marker	Simplex	Duplex	Triplex	Quadruplex	Quadruplex
PCN – *H1*	**57R**	✓	✓	✓	✓	✓
PVY – Ry_{adg}	**M45**	✓				
PVY – Ry_{adg}	**RYSC3**	✓		✓	✓	✓
PVX – *Rx1*	**RXSC1**	✓				✓
PVY – Ry_{sto}	**STM0003**	✓		✓	✓	
DNA control & Verticillium wilt	**STM1051**	✓	✓		✓	✓

10. Cost comparison of marker screening

For MAS to be adopted into a breeding program, the marker system used must be reliable, technically simple, be suitable for high-throughput analysis and, above all, be cost effective. Several studies have analysed the cost of genotyping (Yu et al., 2000, Van Sanford et al., 2001, Dreher et al., 2003, Kuchel et al., 2005, Collard and Mackill, 2008), but not for potato. In order for adoption, a comparison of costs is required between commonly used phenotyping methods, and that of MAS.

A potato breeding program uses conventional screening methods by running field trials, glasshouse trials or laboratory tests. A field trial is used to evaluate the genotypes performance under normal commercial cultivation in order to quantify plant characters, tuber characteristics and yield, followed by postharvest performance following harvest. Field trials can also be used for screening of disease resistance, or they can be conducted as glasshouse pot trials. To screen quarantine pests such as PCN, this work must be conducted under strict quarantine conditions.

The overall cost of running a field trial to evaluate genotype performance is relatively expensive at $54,000 per hectare (Table 10.2.1). However, when this is broken down into cost per cultivar and trait assessed, the cost is reasonable at $5 per trait per cultivar. On the other hand the cost of running specific disease screening trials is much more expensive, at between $135 to $222 per cultivar for each pest or disease (Tables 10.2.2, 10.2.3, 10.2.4). When a comparison is made to the cost of genotyping, screening for a marker for a disease resistance gene is $7.74 per cultivar (Table 10.2.5). This cost is further reduced when multiple markers are combined into a 5 or 10 marker assay, as the cost of a multiplexed assay is not significantly higher than for a single marker assay (Table 10.2.5). This property makes the use of MAS highly cost effective, once the markers are validated.

Table 10.2.1. Cost of a conventional field trial to screen for plant, tuber and postharvest characteristics.

Activity	Description	Cost
Trial set up	Field & seed preparation	10,132
Conduct trial	Planting & trial maintenance	11,606
Assess trial	Harvest and assess trial, data analysis	29,590
Trial clean up	Storage, site clean up and waste disposal	2,924
Total cost		**$54,252**
Cost per cultivar		**$54.25**
Cost per cultivar & trait		**$4.93**

Trial design – 1 hectare of 1000 plots, with on average 24 plants per plot.
Screen for 11 traits: Plant size, plant maturity, early blight resistance, tuber shape and morphology, internal defects, breeder's visual preference, specific gravity, crisp score, boil score, after cooking darkening and dormancy.

Table 10.2.2. Cost of a field trial to screen for powdery scab resistance.

Activity	Description	Cost
Trial set up	Includes field, seed & inoculum preparation	1,261
Conduct trial	Includes planting, maintenance & inoculation	1,365
Assess trial	Harvest, assess trial and data analysis	3,861
Trial clean up	Clean up and waste disposal	250
Total cost		**$6,737**
Cost per cultivar		**$134.74**

Trial design: Randomised complete block, 3 replicates, 50 cultivars, 0.25 ha

Table 10.2.3. Cost of a glasshouse trial to screen for PCN resistance.

Activity	Description	Cost
Trial set up	Includes glasshouse, seed & inoculum preparation	1,755
Conduct trial	Includes planting, maintenance & hygiene	3,041
Assess trial	Assess trial and data analysis	1,650
Trial clean up	Clean up and waste disposal	130
Total cost		**$6,576**
Cost per cultivar		**$219.20**

Trial design: Randomised complete block, 3 replicates, 30 cultivars, 2 benches

Table 10.2.4. Cost of a glasshouse trial to screen for virus resistance.

Activity	Description	Cost
Trial set up	Includes glasshouse, seed & inoculum preparation	755
Conduct trial	Includes planting, maintenance & inoculation	4,551
Assess trial	Assess trial and data analysis	1,245
Trial clean up	Clean up and waste disposal	130
Total cost		**$6,681**
Cost per cultivar		**$222.70**

Trial design: 4 replicates, 30 cultivars, 4 benches

Table 10.2.5 Genotyping costs, per complete 96 well plate of samples

Activity	Cost for 1 marker assay	Cost for 5 marker assays[1]	Cost for 10 marker assays[2]
Sample harvesting and DNA extraction	361.10	361.10	361.10
PCR amplification	141.73	142.26	237.14
Product detection and analysis	232.60	232.60	232.60
Total cost[3,4]	**735.43**	**735.96**	**830.84**
Per sample cost[5]	7.74	7.75	8.75
Cost per data point per cultivar	7.74	1.55	0.88

[1] Based on an assumption that the 5 marker assays could be amplified in a single tube reaction and resolved through a single capillary separation.

[2] Based on an assumption that the 10 marker assays could be resolved through a single capillary separation, but the PCR amplification has been processed as 2 sets of 5 multiplexed assays.

[3] Additional assumption that on each 96 well plate there will be a standard sample for QA/QC of the process, so per 96 well extractions there will be 95 experimental test samples

[4] Cost also includes depreciation of the ABi3730xl equipment and liquid handling robotic platforms. This includes instrument purchase price / expected lifetime of the instrument, semi-consumable elements such as capillaries in the ABi3730xl platform and annual service contracts for both instruments.

[5] The slight increase in the per sample cost for 5 and 10 marker assays compared to 1 marker assay is due to the cost of purchasing the additional primer pairs to deliver the assay.

11. Progeny testing and the development of estimated breeding values

Computing / databases

During the lifetime of the project, several database and analysis software packages were evaluated for their utility and ease of use.

- BASC: At the start of the program, it was proposed to use this database, which DPI had developed for use in other breeding programs, and develop it for potatoes. Due to advances in database construction and computational ability, this database architecture became archaic, and so development and use was halted.
- Germinate: This database architecture was developed by the Plant Bioinformatics Group at JHI Dundee, Scotland, and was also investigated, but again found to be redundant.
- Agrobase: This is the current breeding database that is being investigated for use. This database has the advantage that it will import and export Microsoft Excel spreadsheets, as well as linking different types of genomic and phenomic information.
- Potato pedigree database: This database is available on the internet, and provides pedigree and progeny information of a large number of international potato cultivars.
- Potato peditree: This database was developed by this program using the software package "Peditree: Pedigree tree drawing and analysis", developed by R. van Berloo, Wageningen University, the Netherlands. This database was locally implemented during this project and incorporates the information from the Potato pedigree database and the pedigree information held by the Australian potato breeding program.
- Genstat: This is a statistical package commonly used to analyse trial data.
- ASReml: This is a statistical package developed specifically to analyse large datasets with covariates in the analysis. This package was developed specifically for livestock breeding and the analysis of genotype performance using pedigree as a covariate in the analysis. It can also be used to analyse genotype-by-environment (G x E) interactions.
- We have also used all the internet databases listed in Table 1.2.1.

Progeny testing for family means

Analysis of the mean value of the progeny from a given family will enable the determination of the genetic make up and value of the parents. This analysis has been conducted over three successive breeding populations for eight traits. These traits were plant maturity, early blight resistance, breeder's visual preference (BVP), and a reduced set of genotypes were assessed for specific gravity (SG) of tubers and their cooking performance when fried and boiled. This included their crisp colour following frying, and their flesh colour, after cooking darkening (ACD) and sloughing following boiling.

The progeny means of the 09 series families are listed in Table 11.2.1, where they are ranked for BVP. The better families for French fry processing were 09-02, 09-36 and 09-52, while for crisp processing they were 09-45 and 09-20, and for fresh use the families 09-25 and 09-41 scored highest. Interestingly, four of the five lowest ranked families did not contain any progeny suitable for collection, and their cooking performance was consequently not assessed. The superior families for mean BVP need to be assessed for their performance against the other traits that are important or relevant for their end use. Once this is done, individuals can be identified for use as parents in the next round of crossing.

Table 11.2.1. Progeny means for eight traits from the 09 series.

Family	Use	Maturity	Early blight resistance	BVP	SG	Average crisp score	Boil colour	ACD	Sloughing after boiling
09-02	french fry	6.67	5.00	6.23	1.081	6.60	2.33	1.67	4.33
09-36	french fry	6.00	4.00	5.78	1.084	8.40	2.00	3.33	3.67
09-52	french fry	11.50	2.00	5.75	1.089	6.30	4.00	1.50	4.50
09-45	crisp	10.00	2.70	5.70	1.090	6.51	2.44	1.75	2.88
09-12	french fry	6.00	5.50	5.60	1.090	7.35	2.50	2.00	4.00
09-30	french fry	8.07	2.96	5.54	1.075	8.85	3.77	1.64	2.00
09-32	french fry	7.00	2.00	5.50	1.077	7.40	3.50	3.00	1.00
09-60	french fry	7.65	3.55	5.45	1.080	7.26	4.23	3.07	2.73
09-09	french fry	5.50	6.20	5.39	1.082	9.15	2.50	3.00	2.83
09-51	french fry	8.86	3.35	5.39	1.078	8.22	4.58	1.69	1.62
09-53	french fry	10.73	2.75	5.30	1.078	7.41	4.33	2.42	1.58
09-25	fresh	6.98	5.39	5.24	1.073	9.24	4.70	2.07	1.44
09-03	french fry	8.00	4.17	5.20	1.079	8.46	4.18	2.63	1.95
09-41	fresh	6.15	6.70	5.20	1.084	7.54	4.08	1.92	2.15
09-50	french fry	8.87	3.07	5.13	1.091	8.03	1.64	2.36	3.18
09-61	french fry	7.33	3.06	5.11	1.087	8.13	3.38	3.54	4.31
09-10	french fry	9.44	3.67	5.07	1.074	8.80	4.27	2.73	1.27
09-16	fresh	6.94	6.12	5.06	1.083	7.60	3.85	1.90	2.70
09-37	french fry	10.00	3.20	5.05	1.085	8.03	3.17	3.00	1.67
09-20	crisp	8.92	4.83	5.05	1.097	5.92	2.88	1.63	3.63
09-59	french fry	8.22	2.67	5.03	1.091	8.24	1.40	2.80	4.20
09-19	crisp	9.95	3.26	5.03	1.093	4.63	2.83	1.50	2.96
09-56	fresh	6.00	5.00	5.00	1.086	8.40	4.00	1.00	3.00
09-28	fresh	7.65	4.68	4.98	1.074	8.22	3.20	1.45	2.10
09-48	fresh	8.80	3.46	4.93	1.069	8.85	4.16	1.92	1.32
09-57	fresh	6.38	5.71	4.84	1.079	8.54	4.42	2.62	2.46
09-07	fresh	7.52	4.17	4.80	1.077	7.61	4.27	2.31	1.46
09-24	fresh	7.09	5.50	4.71	1.076	7.54	4.91	1.59	1.29
09-05	crisp	6.88	5.81	4.66	1.088	6.26	3.29	2.07	3.29
09-22	crisp	9.18	3.45	4.54	1.088	5.86	3.30	1.60	3.40
09-29	fresh	4.00	6.00	4.50	1.074	6.20	4.00	4.00	1.00
09-47	fresh	5.69	5.19	4.49	1.080	8.30	2.88	1.25	2.50
09-38	french fry	8.18	4.84	4.48	1.077	7.58	4.31	1.94	1.83
09-31	fresh	7.43	4.95	4.44	1.068	9.74	4.21	2.43	1.14
09-06	crisp	9.11	3.72	4.44	1.097	5.50	2.81	2.19	3.38
09-43	fresh	8.39	3.61	4.39	1.070	7.87	3.67	1.11	1.56
09-17	fresh	6.96	4.67	4.37	1.071	9.18	4.80	1.60	1.00
09-14	fresh	9.83	3.17	4.35	1.071	8.63	3.34	1.89	1.32
09-08	fresh	8.82	5.40	4.34	1.067	8.38	3.09	1.86	1.27
09-13	fresh	8.13	4.50	4.29	1.072	8.10	1.00	1.50	2.00
09-27	fresh	9.09	4.17	4.26	1.071	9.22	3.11	1.83	1.28
09-44	crisp	9.47	4.06	4.25	1.095	6.84	3.14	1.36	3.86
09-04	fresh	5.21	6.00	4.02	1.084	8.62	2.95	3.40	3.50
09-58	fresh	8.90	3.85	4.02	1.076	8.69	3.21	3.14	1.29
09-01	french fry	7.50	4.00	4.00	1.096	5.40	1.00	3.00	3.00
09-21	fresh	6.79	6.14	3.91	1.077	7.80	3.50	1.25	2.50
09-33	fresh	10.44	2.78	3.84	1.063	8.39	3.88	1.13	1.00

Family	Use	Maturity	Early blight resistance	BVP	SG	Average crisp score	Boil colour	ACD	Sloughing after boiling
09-23	french fry	10.14	3.14	3.75	1.083	7.90	3.25	1.25	3.25
09-11	french fry	9.25	3.75	3.69	1.096	7.06	2.86	2.14	4.14
09-39	french fry	8.71	2.86	3.57	1.087	7.03	3.00	3.33	3.67
09-34	fresh	6.44	6.78	3.56					
09-46	french fry	10.00	3.33	3.28	1.083	7.40	2.38	1.75	2.50
09-40	fresh	4.00	8.00	3.00					
09-18	fresh	10.00	4.00	2.00					
09-35	fresh	6.00	6.00	2.00					

Calculation of estimated breeding values

The same progeny data can be used for the calculation of estimated breeding values (EBVs). The calculation of EBVs provided an overall mean value for the data set and individual EBVs, with their standard error (SE), which deviate positively or negatively from the overall mean. As well as obtaining EBVs for each family, they are also obtained for individual genotypes, and any standard cultivar or parental cultivar that is listed in the pedigree of the families. This analysis provided a very large data set for each trait, so an example of the 09 series BVP EBVs, ranked for BVP, is provided for the families in Table 11.2.2, for the top 10 cultivars used in the pedigree or as standards in Table 11.2.3, and for the top 20 progeny that were assessed in Table 11.2.4. This data will not only identify the better performing families, but also the better performing individuals from within those families. Close examination of the top 20 listed progeny identified 12 individuals from the top listed family and 15 from the top three, while the remaining five were obtained from the top 11 families.

Table 11.2.2. Estimated breeding values for BVP for the 09 series families.

Family	Estimated breeding value	SE	Rank	overall mean	Family value
09-30	0.6660	0.2545	1	4.777	5.4430
09-02	0.5661	0.4332	2	4.777	5.3431
09-60	0.5317	0.2826	3	4.777	5.3087
09-52	0.5107	0.4834	4	4.777	5.2877
09-51	0.4927	0.2767	5	4.777	5.2697
09-36	0.4879	0.4139	6	4.777	5.2649
09-09	0.4638	0.3372	7	4.777	5.2408
09-25	0.4270	0.2217	8	4.777	5.2040
09-45	0.4118	0.3407	9	4.777	5.1888
09-53	0.3931	0.3034	10	4.777	5.1701
09-03	0.3701	0.2536	11	4.777	5.1471
09-59	0.3419	0.3487	12	4.777	5.1189
09-50	0.3130	0.3028	13	4.777	5.0900
09-41	0.3047	0.2815	14	4.777	5.0817
09-12	0.2991	0.4712	15	4.777	5.0761
09-37	0.2543	0.4109	16	4.777	5.0313
09-61	0.2360	0.2898	17	4.777	5.0130
09-10	0.2140	0.2886	18	4.777	4.9910
09-19	0.1502	0.2831	19	4.777	4.9272
09-16	0.1185	0.2979	20	4.777	4.8955
09-28	0.1117	0.2427	21	4.777	4.8887

Family	Estimated breeding value	SE	Rank	overall mean	Family value
09-20	0.0866	0.3291	22	4.777	4.8636
09-48	0.0836	0.2313	23	4.777	4.8606
09-01	0.0689	0.4585	24	4.777	4.8459
09-56	0.0645	0.4622	25	4.777	4.8415
09-32	0.0101	0.4951	26	4.777	4.7871
09-55	-0.0105	0.5757	27	4.777	4.7665
09-57	-0.0132	0.2781	28	4.777	4.7638
09-07	-0.0212	0.2696	29	4.777	4.7558
09-24	-0.0523	0.2459	30	4.777	4.7247
09-29	-0.0927	0.4843	31	4.777	4.6843
09-26	-0.1045	0.5525	32	4.777	4.6725
09-05	-0.1052	0.2982	33	4.777	4.6718
09-49	-0.1283	0.5855	34	4.777	4.6487
09-54	-0.1608	0.5997	35	4.777	4.6162
09-15	-0.1649	0.5540	36	4.777	4.6121
09-22	-0.1995	0.3163	37	4.777	4.5775
09-47	-0.2152	0.2999	38	4.777	4.5618
09-38	-0.2480	0.2375	39	4.777	4.5290
09-06	-0.2547	0.2919	40	4.777	4.5223
09-42	-0.2687	0.5158	41	4.777	4.5083
09-31	-0.2772	0.2771	42	4.777	4.4998
09-13	-0.3333	0.3573	43	4.777	4.4437
09-17	-0.3369	0.2650	44	4.777	4.4401
09-43	-0.3550	0.2575	45	4.777	4.4220
09-14	-0.4056	0.2288	46	4.777	4.3714
09-44	-0.4123	0.2929	47	4.777	4.3647
09-08	-0.4144	0.2190	48	4.777	4.3626
09-27	-0.4746	0.2411	49	4.777	4.3024
09-18	-0.5545	0.5277	50	4.777	4.2225
09-35	-0.5610	0.5180	51	4.777	4.2160
09-04	-0.5625	0.2658	52	4.777	4.2145
09-21	-0.6254	0.3089	53	4.777	4.1516
09-40	-0.6338	0.4980	54	4.777	4.1432
09-58	-0.6481	0.2796	55	4.777	4.1289
09-39	-0.6526	0.3744	56	4.777	4.1244
09-23	-0.6759	0.3612	57	4.777	4.1011
09-33	-0.6849	0.2519	58	4.777	4.0921
09-11	-0.8213	0.2807	59	4.777	3.9557
09-34	-0.8769	0.3507	60	4.777	3.9001
09-46	-1.1090	0.2980	61	4.777	3.6680

Table 11.2.3. Estimated breeding values for BVP for the top 10 cultivars involved in the 09 series trial as parents, grandparents or standards.

Cultivar	Estimated breeding value	SE	Rank	overall mean	Family value
Innovator	0.6384	0.4532	1	4.777	5.4154
Nadine	0.5140	0.3781	2	4.777	5.2910
Windsor	0.4406	0.4423	3	4.777	5.2176
MacRusset	0.4358	0.5050	4	4.777	5.2128
Stampede	0.4348	0.4335	5	4.777	5.2118
Mirridong	0.4142	0.4126	6	4.777	5.1912
Lemhi Russet	0.3857	0.5552	7	4.777	5.1627
Penguin	0.3758	0.5402	8	4.777	5.1528
Summerside	0.3562	0.5182	9	4.777	5.1332
RZ-84-2580	0.3192	0.5744	10	4.777	5.0962

Table 11.2.4 Estimated breeding values for BVP for the top 20 cultivars in the 09 series trial.

Cultivar	Estimated breeding value	SE	Rank	overall mean	Family value
09-30-20	0.9833	0.4660	1	4.777	5.7603
09-30-05	0.8592	0.4660	2	4.777	5.6362
09-30-21	0.8592	0.4660	3	4.777	5.6362
09-60-04	0.8036	0.4786	4	4.777	5.5806
09-30-13	0.7971	0.4660	5	4.777	5.5741
09-30-22	0.7723	0.4660	6	4.777	5.5493
09-36-05	0.7652	0.5466	7	4.777	5.5422
09-30-10	0.7599	0.4660	8	4.777	5.5369
09-30-28	0.7599	0.4660	9	4.777	5.5369
09-60-14	0.7416	0.4786	10	4.777	5.5186
09-30-06	0.7351	0.4660	11	4.777	5.5121
09-30-12	0.7351	0.4660	12	4.777	5.5121
09-30-17	0.7351	0.4660	13	4.777	5.5121
09-03-22	0.7242	0.4659	14	4.777	5.5012
09-25-14	0.7119	0.4530	15	4.777	5.4889
09-30-01	0.7102	0.4660	16	4.777	5.4872
09-02-01	0.7096	0.5579	17	4.777	5.4866
09-51-02	0.7074	0.4757	18	4.777	5.4844
09-51-05	0.7074	0.4757	19	4.777	5.4844
09-30-03	0.6978	0.4660	20	4.777	5.4748

A comparison of the 09 series family BVP progeny means with their corresponding EBVs, revealed a high degree of similarity, although differences were observed between the progeny means and the EBV family values (Table 11.2.5). These differences were easily visualised when the values are plotted in Figure 11.2.1. There was also a difference when the families were ranked for their respective values (Table 11.2.5, Figure 11.2.2), although the relationship was much closer. When data that deviate from the slope were investigated, these families were found to contain a low number of siblings in each family (1-3 individuals), illustrating the advantage of using EBVs over progeny means when families are small, as the EBVs are calculated using all information from half-siblings and other relatives.

Table 11.2.5. Comparison of the family means, the estimated breeding value for the families and their rankings for BVP in the 09 series.

Family	Family mean BVP	Rank	Estimated breeding value	SE	Family value	Rank
09-02	6.2333	55	0.5661	0.4332	5.3431	54
09-36	5.7750	54	0.4879	0.4139	5.2649	50
09-52	5.7500	53	0.5107	0.4834	5.2877	52
09-45	5.7000	52	0.4118	0.3407	5.1888	47
09-12	5.6000	51	0.2991	0.4712	5.0761	41
09-30	5.5393	50	0.6660	0.2545	5.4430	55
09-32	5.5000	49	0.0101	0.4951	4.7871	30
09-60	5.4450	48	0.5317	0.2826	5.3087	53
09-09	5.3900	47	0.4638	0.3372	5.2408	49
09-51	5.3857	46	0.4927	0.2767	5.2697	51
09-53	5.3000	45	0.3931	0.3034	5.1701	46
09-25	5.2435	44	0.4270	0.2217	5.2040	48
09-03	5.2000	43	0.3701	0.2536	5.1471	45
09-41	5.1950	42	0.3047	0.2815	5.0817	42
09-50	5.1267	41	0.3130	0.3028	5.0900	43
09-61	5.1056	40	0.2360	0.2898	5.0130	39
09-10	5.0667	39	0.2140	0.2886	4.9910	38
09-16	5.0625	38	0.1185	0.2979	4.8955	36
09-37	5.0500	37	0.2543	0.4109	5.0313	40
09-20	5.0455	36	0.0866	0.3291	4.8636	34
09-59	5.0333	35	0.3419	0.3487	5.1189	44
09-19	5.0263	34	0.1502	0.2831	4.9272	37
09-56	5.0000	33	0.0645	0.4622	4.8415	31
09-28	4.9794	32	0.1117	0.2427	4.8887	35
09-48	4.9268	31	0.0836	0.2313	4.8606	33
09-57	4.8429	30	-0.0132	0.2781	4.7638	29
09-07	4.7957	29	-0.0212	0.2696	4.7558	28
09-24	4.7125	28	-0.0523	0.2459	4.7247	27
09-05	4.6625	27	-0.1052	0.2982	4.6718	25
09-22	4.5385	26	-0.1995	0.3163	4.5775	24
09-29	4.5000	25	-0.0927	0.4843	4.6843	26
09-47	4.4875	24	-0.2152	0.2999	4.5618	23
09-38	4.4763	23	-0.2480	0.2375	4.5290	22
09-31	4.4429	22	-0.2772	0.2771	4.4998	20
09-06	4.4412	21	-0.2547	0.2919	4.5223	21
09-43	4.3857	20	-0.3550	0.2575	4.4220	17
09-17	4.3708	19	-0.3369	0.2650	4.4401	18
09-14	4.3548	18	-0.4056	0.2288	4.3714	16
09-08	4.3420	17	-0.4144	0.2190	4.3626	14
09-13	4.2875	16	-0.3333	0.3573	4.4437	19
09-27	4.2600	15	-0.4746	0.2411	4.3024	13
09-44	4.2529	14	-0.4123	0.2929	4.3647	15
09-04	4.0208	13	-0.5625	0.2658	4.2145	10
09-58	4.0200	12	-0.6481	0.2796	4.1289	7
09-01	4.0000	11	0.0689	0.4585	4.8459	32
09-21	3.9143	10	-0.6254	0.3089	4.1516	9
09-33	3.8379	9	-0.6849	0.2519	4.0921	4

Family	Family mean BVP	Rank	Estimated breeding value	SE	Family value	Rank
09-23	3.7500	8	-0.6759	0.3612	4.1011	5
09-11	3.6900	7	-0.8213	0.2807	3.9557	3
09-39	3.5714	6	-0.6526	0.3744	4.1244	6
09-34	3.5556	5	-0.8769	0.3507	3.9001	2
09-46	3.2813	4	-1.1090	0.2980	3.6680	1
09-40	3.0000	3	-0.6338	0.4980	4.1432	8
09-35	2.0000	2	-0.5610	0.5180	4.2160	11
09-18	2.0000	1	-0.5545	0.5277	4.2225	12

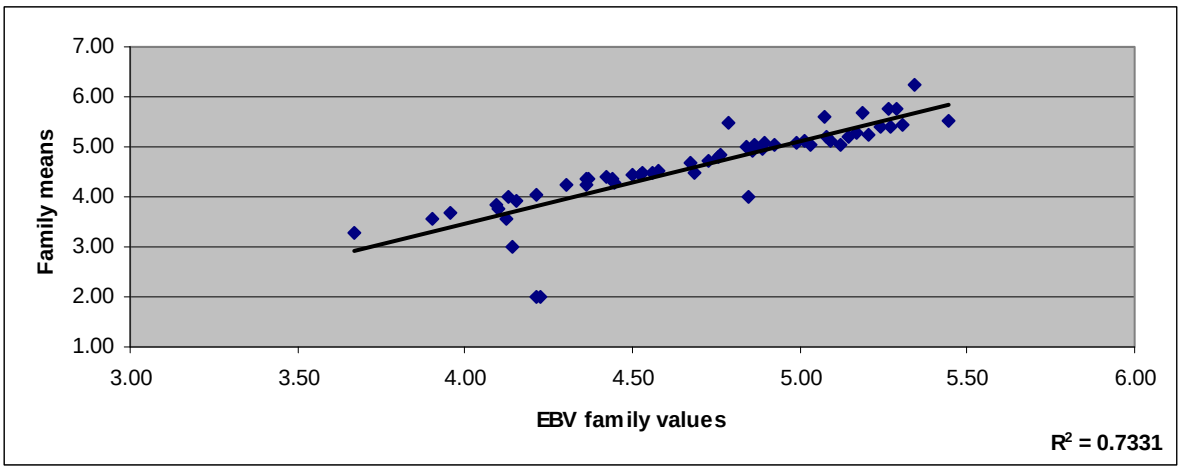

Figure 11.2.1. Comparison of the 09 series BVP progeny means with the EBV family values.

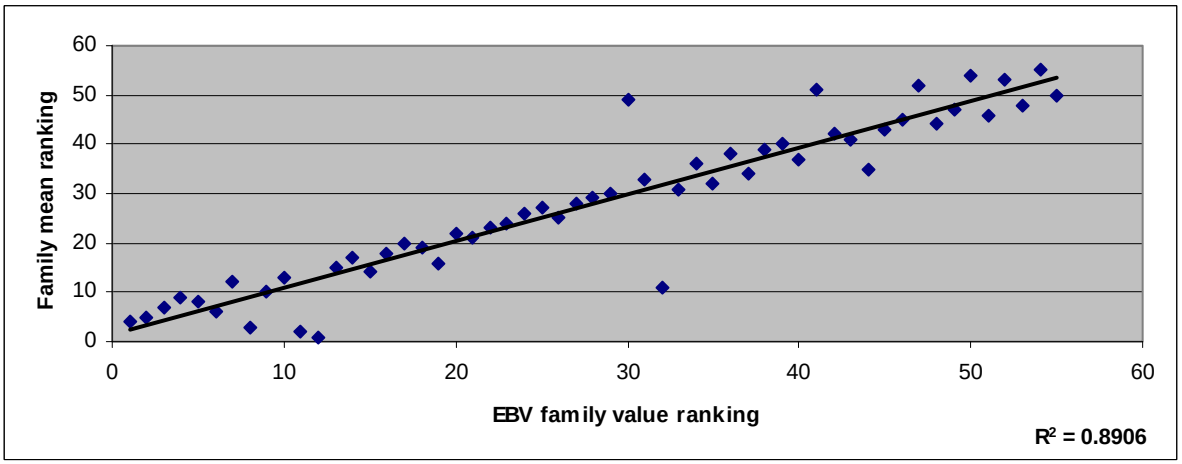

Figure 11.2.2. Comparison of the 09 series BVP progeny means with the EBV family value rankings.

A similar good relationship was observed when the 08 series family BVP progeny means were compared to their corresponding EBVs. Although differences were still seen between the progeny means and the family EBVs, as well as when families were ranked for these values, the relationship was stronger in both cases (Table 11.2.6).

Table 11.2.6. Relationships between the BVP progeny means and their EBVs and the family rankings for BVP for two breeding populations.

Comparison	09 series	08 series
BVP progeny means v EBV family values	0.73	0.91
BVP progeny mean rank v EBV family value rank	0.89	0.93

Estimation of heritability

As the BLUP analysis for calculating EBVs considers pedigree in the analysis, it also provides an estimation of heritability for each of the traits investigated. Flesh colour, tuber SG and average crisp score showed the highest heritability values, followed by early blight resistance, sloughing and after cooking darkening following boiling, while the lowest heritability values were obtained for plant maturity and breeder's visual preference (Table 11.2.7).

The increased power of analysis that is a result of using information from all relatives for the calculation of EBVs has also enabled the analysis of a much smaller data set for yield from the third generation trials. This data set only contained 141, 138 and 112 genotypes in the 07, 08 and 09 series respectively, but the analysis indicated a moderate heritability (Table 11.2.7).

The heritability estimations also make sense when the expression of the traits is considered. BVP heritability is likely to be low due to the inclusion of numerous traits, and would be effected by environment and seed size. Plant maturity can also be affected by environmental conditions, such as weather, water and fertiliser availability, and disease pressure from blight infection. The early blight resistance ratings would be expected to be affected by the seasonal inoculum levels. Total yield would be affected by the growing season. Boil after cooking darkening would be affected by environment and population composition. Boil sloughing would be affected by the extent of cooking and population composition. On the other hand tuber specific gravity displayed generally high heritability, but can be affected by nutrition. The average crisp score exhibited generally high heritability, but can be affected by nutrition and cold. Boil colour should show high heritability, as it is not known to be affected by the growing environment.

Table 11.2.7 Potato trait heritability over 3 breeding populations.

Trait	07 series	08 series	09 series	Combined
Plant maturity	0.13 ± 0.04	0.21 ± 0.06	0.27 ± 0.06	0.22 ± 0.03
Early blight resistance	0.18 ± 0.05	0.22 ± 0.06	0.36 ± 0.07	0.47 ± 0.05
Breeder's visual preference	0.23 ± 0.05	0.07 ± 0.03	0.22 ± 0.06	0.19 ± 0.03
Total yield	0.36 ± 0.11	0.45 ± 0.11	0.42 ± 0.11	0.41 ± 0.08
Tuber specific gravity	0.69 ± 0.07	0.75 ± 0.06	0.88 ± 0.04	0.58 ± 0.05
Average crisp score	0.22 ± 0.06	0.80 ± 0.06	0.84 ± 0.05	0.58 ± 0.05
Boil colour	0.41 ± 0.08	0.84 ± 0.05	0.85 ± 0.05	0.61 ± 0.05
Boil after cooking darkening	0.19 ± 0.06	0.29 ± 0.07	0.47 ± 0.10	0.35 ± 0.05
Boil sloughing	0.14 ± 0.05	0.51 ± 0.10	0.62 ± 0.10	0.35 ± 0.04

Estimation of genetic gain

Accelerated genetic gain is the aim of all plant and animal improvement programs, in order to develop progeny that are more productive than their parents. Genetic gain is a function of both the heritability of the trait and the amount of phenotypic variation available in the target population.

Table 11.2.8. Estimation of the rate of genetic gain following phenotypic confirmation of potato traits.

Trait	Heritability (h^2)	Phenotypic variance (V_p)	Selection intensity (i)*	Length of breeding cycle	Expected genetic gain (units/year)
Early blight resistance	0.47	3.72	1.755	12	0.255
Breeder's visual preference	0.19	1.65	1.755	12	0.047
Total yield	0.41	80.67	1.755	12	4.89
Boil after cooking darkening	0.35	0.65	1.755	12	0.033

* Selection intensity is in standard deviation units under a normal distribution (Falconer and Mackay, 1996).

Table 11.2.9. Estimation of the rate of genetic gain using EBVs of potato traits

Trait	Heritability (h^2)	Phenotypic variance (V_p)	Selection intensity (i)*	Length of breeding cycle	Expected genetic gain (units/year)
Early blight resistance	0.47	3.72	1.4	5	0.489
Breeder's visual preference	0.19	1.65	1.4	5	0.089
Total yield	0.41	80.67	1.4	5	9.35
Boil after cooking darkening	0.35	0.65	1.4	5	0.062

* Selection intensity is in standard deviation units under a normal distribution (Falconer and Mackay, 1996).

At the start of this project, genetic gain was not being measured, but following the adoption of progeny testing and calculation of EBVs, this can be achieved. The program has been using a selection intensity of 10%, and a breeding cycle of 12 years, which would provide an expected genetic gain for total yield of 4.9 tonnes per hectare per year (Table 11.2.8). By using EBVs, these predicted values are increased significantly to 9.4 tonnes per hectare per year (Table 11.2.9), despite reducing the selection intensity to 20%, because it has reduced the breeding cycle to 5 years.

Cross generation prediction

As potato breeding uses recurrent selection across a series of generations and screening trials to determine superior cultivars, it is important to understand how reliable parental and early generation phenotypes can be used to predict the final phenotype of individuals. Mid-parent values, progeny tests and EBVs can be used to determine the better predictive model to use to determine the reliable expression in subsequent generations. This has been performed for a strongly heritable trait, SG, and an important trait with lower heritability, BVP.

Collection of the data to calculate progeny means and EBVs allows for the comparison of data for individuals and families across successive field generations, in order to determine the best selection strategy.

SG – a high heritability trait

SG has a high level of heritability (0.58), as shown in Table 11.2.7, but this value can be affected by nutritional status. SG was found to show a good relationship across generations, including the glasshouse and field seedling generations, although these showed the weakest relationship. The best relationship was shown from the mid-parent values to the G2 family means, which was improved when the mid-parent EBVs were used. The mid-parent EBVs showed a very strong relationship (over 0.8), when compared to the G2 family means (Table 11.2.10). These improving relationships can be seen in Figures 11.2.3 to 11.2.6. This trend was consistent for both the 08 and 09 series (Table 11.2.10).

Table 11.2.10. Relationship between mid-parent and cross generation values for specific gravity.

Comparison	09 series	08 series
G0 family means v G2 family means	0.35	0.65
G1 family means v G2 family means	0.60	0.59
Mid-parent value v G2 family means	0.75	0.75
Mid-parent value v G2 family EBVs	0.78	0.76
Mid-parent EBVs v G2 family means	0.83	0.86
Mid-parent EBVs v G2 family EBVs	0.86	0.88

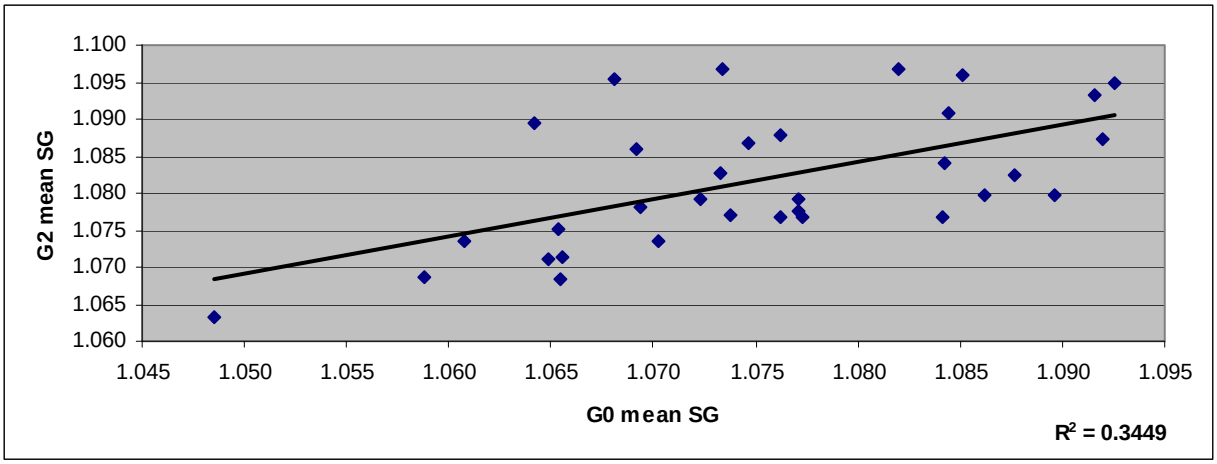

Figure 11.2.3. Comparison of the 09 series G0 and G2 family means for SG

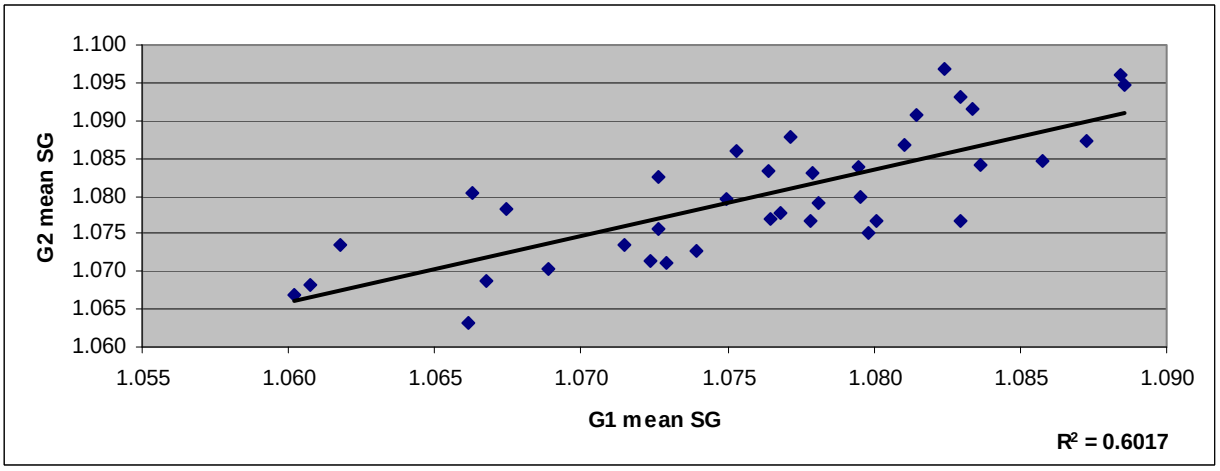

Figure 11.2.4. Comparison of the 09 series G1 and G2 family means for SG

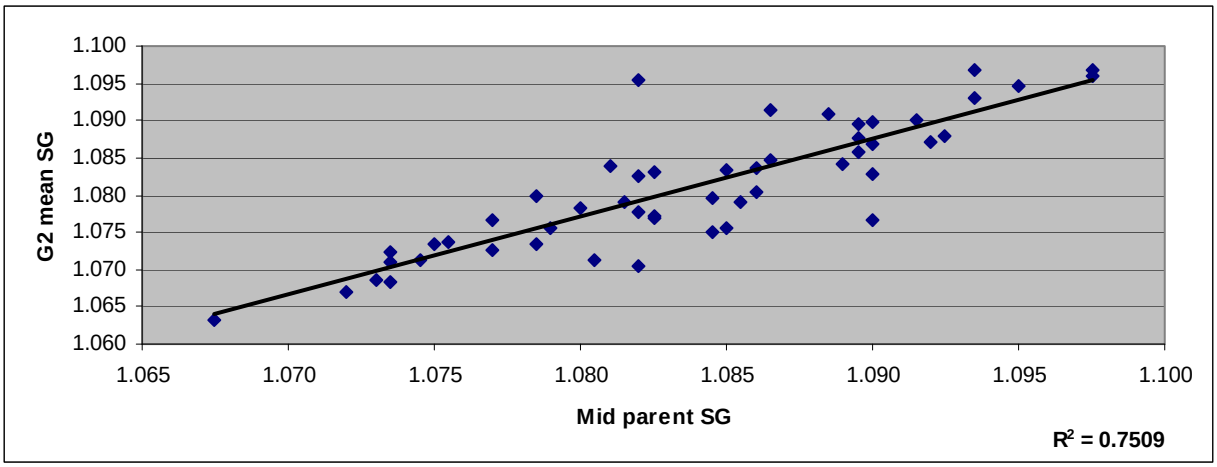

Figure 11.2.5 Comparison of the 09 series mid parent values and G2 family means for SG

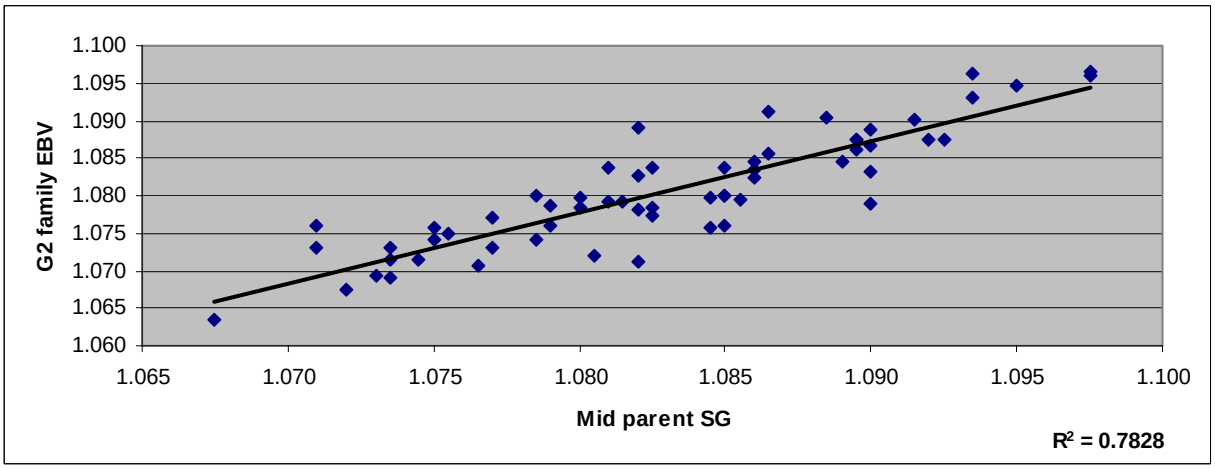

Figure 11.2.6. Comparison of the 09 series mid parent values and G2 family EBVs for SG

BVP – a low heritability trait

BVP has a low heritability of 0.19, as shown in Table 11.2.7, as it is based on the expression of a number of traits. It is also likely to be effected by the growing environment and seed size. When the G1 and G2 BVP scores were compared for individual cultivars across two breeding populations (09 and 08 series), the relationship between the scores was very low, although positive (Table 11.2.11). In both populations there are individuals that score low or high in both generations. But there are also individuals that score low in the G1 but high in the G2, and others that scored low in the G1 but high in the G2 generation (Figure 11.2.7). There was also a poor relationship when the mid-parent values were compared to the mean G2 family means. This outcome indicated that neither the mid-parent values nor early generation scores are reliable.

Table 11.2.11. Relationship between cross generation values for BVP

Comparison	09 series	08 series
G1 v G2 genotype comparison	0.03	0.04
G1 family means v G2 family means	0.14	0.17
G1 family EBVs v G2 family EBVs	0.20	0.14
Mid-parent value v G2 family means	0.00	0.02
Mid-parent value v G2 family EBVs	0.00	0.05
Mid-parent EBVs v G2 family means	0.28	0.36
Mid-parent EBVs v G2 family EBVs	0.29	0.43

The relationship improves when family means are compared across the same two data sets. It improves further when the mid-parent EBVs are used (Table 11.2.11). These improving relationships can be seen in Figures 11.2.7 to 11.2.9. This trend was consistent for both the 08 and 09 series (Table 11.2.11).

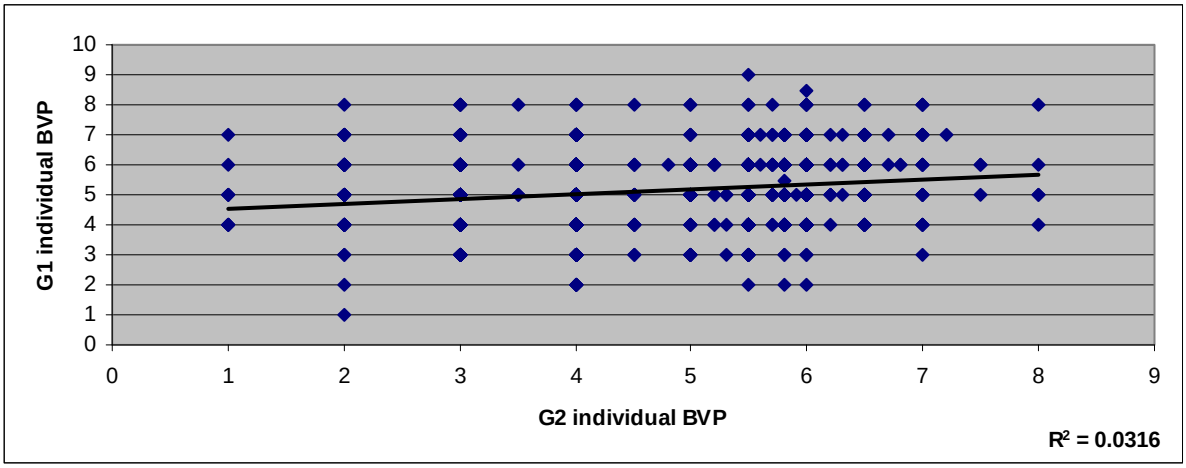

Figure 11.2.7. Comparison of the 09 series G1 and G2 BVP scores for individual cultivars.

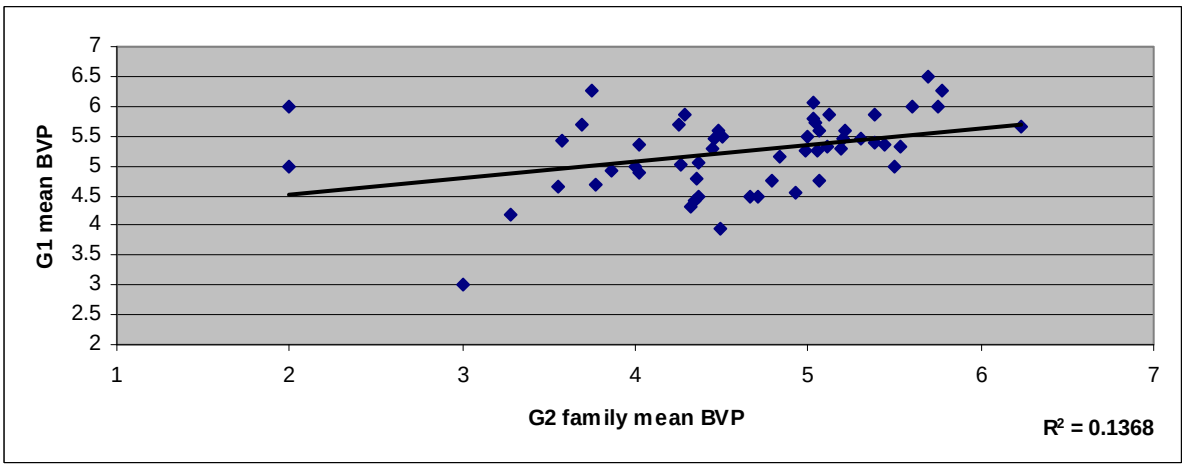

Figure 11.2.8. Comparison of the 09 series BVP G1 and G2 family mean

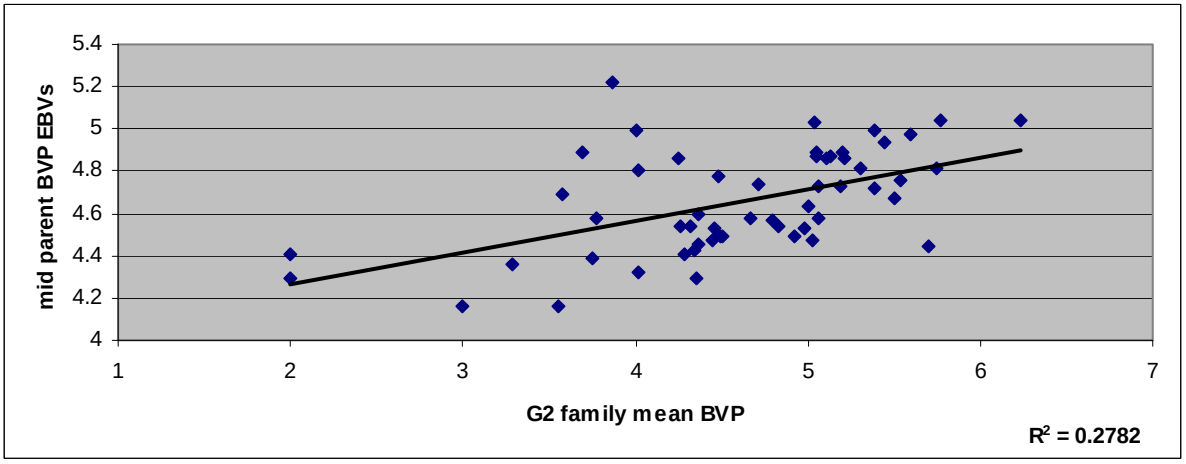

Figure 11.2.9. Comparison of the 09 series BVP mid-parent EBVs and G2 family mean.

Modified BVP selection rates for the 10 series

The results of the modified selection practice on the 10 series G1 generation are presented in Table 11.2.12. This data shows that 25% of the G1 population was selected in the first round, another 23% in the second round and a further 7% in the second round, providing a very mild selection rate of over 50% of the G1 population selected to be grown in the G2 generation. The results from the selection of the G2 generation show that the majority of individuals selected were from the first round of selection as expected, but a high percentage of 34% were also from the second round, and a significant number at 9% were even from the third round of selections. These results indicate that a very mild selection pressure should be placed on the breeding populations if it is based on BVP alone, which is the case in the G1 generation. When a more intense selection pressure is used, a significant number of individuals that could have been selected in the G2 generation would have already been rejected at the G1 generation.

Table 11.2.12. Comparison of the selection pressure on the G1 population to the G2 individuals selected.

	% G1 selected	% of G2 trial	% selected from G2
Bag 1	25.7	46.2	57.4
Bag 2	22.5	40.4	34.0
Bag 3	7.4	13.4	8.7
Total	55.5	100	

Using EBVs as a selection tool to advance superior cultivars

After the 09 series G2 data had been analysed and the individual cultivars had been identified to advance to the G3 trial, the EBVs of the progeny were ranked for the most important characters to retrospectively determine whether EBVs could be used to determine which cultivars should advance. As an example, the top 30 progeny are listed in Table 11.2.12, after their EBV values were ranked and then these rankings combined for a total score for the French fry characters of BVP, SG and average crisp score. The higher ranking genotypes should then be assessed for other important traits before inclusion in further trials. All the French fry genotypes listed had been either placed into the G3 trial, or returned to the G2.

Table 11.2.12. 09 series top 30 progeny ranked by their total score for the French fry characteristics of BVP, SG and average crisp score.

Cultivar	Use	BVP	SG	Ave Crisp	Total	Rank	French fry comment
09-45-03	crisp	952	891	942	2785	1	
09-60-12	f fry	942	990	852	2784	2	French fry G3 trial
09-60-06	f fry	886	957	925	2768	3	Not enough for G3 trial, back to G2
09-02-01	f fry	978	874	865	2717	4	French fry G3 trial
09-19-19	crisp	920	813	955	2688	5	
09-53-02	f fry	951	994	735	2680	6	French fry G3 trial
09-45-09	crisp	928	850	886	2664	7	
09-45-08	crisp	902	856	897	2655	8	
09-19-17	crisp	769	868	994	2631	9	
09-45-11	crisp	901	756	971	2628	10	
09-41-07	fresh	850	827	946	2623	11	
09-60-20	f fry	969	759	887	2615	12	French fry G3 trial
09-60-14	f fry	985	879	749	2613	13	French fry G3 trial
09-52-02	f fry	960	679	972	2611	14	French fry G3 trial

Cultivar	Use	BVP	SG	Ave Crisp	Total	Rank	French fry comment
09-41-06	fresh	874	915	818	2607	15	
09-45-07	crisp	844	857	898	2599	16	
09-20-08	crisp	721	933	943	2597	17	
09-53-05	f fry	893	909	785	2587	18	French fry G3 trial
09-45-10	crisp	826	855	896	2577	19	
09-02-02	f fry	962	651	953	2566	20	Rejected just, back to G2
09-53-01	f fry	894	932	736	2562	21	French fry G3 trial
09-16-04	fresh	788	889	881	2558	22	
09-50-11	f fry	851	782	922	2555	23	French fry G3 trial
09-05-10	crisp	630	970	954	2554	24	
09-19-15	crisp	660	907	976	2543	25	
09-52-01	f fry	867	882	789	2538	26	French fry G3 trial
09-19-04	crisp	771	773	989	2533	27	
09-30-17	f fry	982	794	752	2528	28	Rejected just, back to G2
09-12-02	f fry	927	775	824	2526	29	French fry G3 trial
09-19-12	crisp	805	739	977	2521	30	

Discussion

Understanding potato genetics is not just about potato breeding, it has much broader applications.

Over the last five years, the present project has obtained a much greater understanding of potato genetics, leading to a much improved understanding of the relevant biology and methods for control of two major pest and disease problems the Australian industry has experienced over recent years, PCN and PVY. Through an understanding of the resistance mechanisms controlling PCN and PVY, answers can be provided for all parties in the Australian potato industry.

A genetic analysis technique used in livestock breeding has been adapted and could be applied to the majority of potato research and produce significant advances. This technique could also be more widely adapted to other target crops for horticultural research in Australia, as we expect global adoption following publication of our findings.

Breeding programs can operate in the absence of application of molecular techniques and quantitative genetic analysis. However, in order to deliver results and products with greater assurance, rates of genetic gain, and crop improvement these tools are essential. The rate of adoption and integration of these tools and techniques will determine and dictate the most viable and successful programs internationally.

Understanding potato genetics

During the course of the current project, the team members have undertaken a detailed review of the extensive literature on potato genetics and molecular genetics, as well as investigating the publicly available on-line resources for the development of a DNA marker-assisted selection program as recommended by the Brennan review of potato breeding in Australia.

This investigation has also entailed attendance by Tony Slater and Lee Schultz at five international conferences, visiting leading research laboratories and understanding the developments in this cutting-edge research globally. During this time, the program team has become a contributor to this research and have developed significant international relationships. This has placed this Australian research program at the forefront of world's best practice and enabled the Australian breeding program to be an early adopter of technology, becoming one of the most advanced potato breeding programs internationally. This outcome will result in significant benefits and advances for the Australian industry.

Results from the program will be published in well respected scientific journals over the coming years, which will continue to contribute to a better understanding of the biology and genetics of traits that are important to the Australian industry, as well as enabling the global potato industry to adopt the developments and techniques that the program has generated.

Understanding PCN

PCN was detected in 2004 in the Koo Wee Rup district, and then in 2008 in the Thorpdale district of Victoria. On both occasions, these detections resulted in a series of biosecurity control measures to be adopted to maintain market access. The Koo Wee Rup district was a supply area for crisp processing, and restrictions were placed on tuber, machinery and vehicle movement. The Thorpdale detection could have had more profound repercussions, as this was a seed supply district for most of mainland Australia, except Western Australia, and the pest may have been widely spread across

Australian growing areas as a consequence. Therefore, understanding the biology of PCN, which pathotype is present and how to manage this pest was vitally important.

This project has phenotyped and genotyped a large number of potato cultivars that are available to Australian growers. This has enabled the project to determine which pathotype of PCN is in Australia, and therefore to predict which cultivars developed in any breeding program will be resistant to the Australian pathotype. The phenotyping work has identified that the Koo Wee Rup detection was of the *G. rostochiensis* Ro1 pathotype. This supports the earlier identification of the detections in Western Australia and Gembrook of *G. rostochiensis* Ro1 (Hinch et al., 1998).

As a result of this phenotyping work, we now know that growers should be growing cultivars for their market that are resistant to the *G. rostochiensis* Ro1 pathotype. Breeding of cultivars resistant to this PCN pathotype has been an aim of a number of breeding programs across a number of countries. In fact there are now countries, such as Poland, that will not allow a cultivar onto the market unless it is resistant to this PCN pathotype.

By understanding the genetics of resistance to the PCN pathotype, a number of resistance genes that can be used to confer resistance can be identified. The gene known as *H1* is one such gene, which confers near absolute resistance, and has been used in breeding programs since the 1950s without loss of effectiveness. This project has validated a genetic marker to adopt MAS for this gene, and Australia now has the ability to rapidly develop new cultivars that are resistant to this PCN pathotype and suitable for a range of markets and Australian growing environments. Australian growers or companies can now also identify cultivars developed elsewhere that are resistant to the Australian PCN pathotype.

Although this significant step has been achieved, the Australian industry must still be vigilant and monitor for PCN, as it can have a significant impact. If PCN is again detected, especially if it is detected in a resistant crop, it could mean that another PCN pathotype has been found, and that this *H1* resistance gene would not work.

Understanding PVY

PVY has been recorded in all Australian States, although it is currently absent or is successfully controlled in Western Australia. Reported yield losses due to the PVY infections are 10-80%, depending on cultivar, virus strain characteristics and the field and tuber storage conditions. Some potato varieties express no, or only mild, symptoms after the infection, but their marketable yield can be reduced by up to 65%, which is similar to the yield losses recorded from symptomatic varieties.

Victorian seed surveys, which account for 50% of Australian seed, have shown negligible levels of PVY from 1970 to 2000, while recent surveys have shown 0.03% in 2005/06, less than 0.02% in 2006/07 and just above 0.02% in 2007/08. However, this 2007/08 level saw 45 hectares of seed potato rejected due to PVY infection, and in 2008/09 the incidence of PVY in seed crops had increased tenfold to 0.27%, and has continued to escalate since that time. This upward trend in PVY incidence is of concern, and highlights the requirement to develop effective management strategies for potato growers to better control this disease.

PVY can be transmitted by aphid feeding and mechanical damage due to machinery, tools and by brushing plants while walking through the field. Multiple strategies can be used to control PVY in potato production. Reduction of PVY inoculum and vector control techniques are two

complementary methods for PVY management, as well as the use of resistant cultivars. Understanding of the biology of this virus, the identity of the strains responsible for the infection, and how to manage this virus are important for its control.

There are several strains of PVY and the main groups are: common (PVY^O), tobacco veinal necrosis (PVY^N), and stipple streak (PVY^C). PVY^{NTN} was first detected as an N strain that caused Tuber Necrosis (NTN) in Europe (Eu-PVY^{NTN}) in the late 1980's, and has since spread to most continents including North America and was first detected in Australia in 2003.

There are two main types of resistance mechanisms to PVY, being hypersensitive response and extreme resistance. The hypersensitive response causes cell death which inhibits viral spread, while extreme resistance causes a reduction in virus replication in infected cells (Solomon-Blackburn and Barker, 2001b, Song and Schwarzfischer, 2008). The different PVY strains are identified by their infection of differential potato cultivars that possess different genes controlling the described hypersensitive responses. While the extreme resistance genes, will confer resistance against all known strains of PVY.

This project has sequenced the genome of 16 isolates of PVY detected in Australia, including 10 obtained since PVY^{NTN} was first identified in 2003. Two strains, PVY^O and PVY^{NTN}, were identified and isolates of each strain are nearly identical, and likely to be multiple detections of the same strain.

This project has also screened the main commercial cultivars that are available to Australian growers against both of these strains. Very few cultivars were found to be resistant to PVY^{NTN}, but more were resistant to PVY^O. From screening in this program, only Eva, PO3 and Rioja were resistant to PVY^{NTN} on three occasions, although Lady Christl, Royal Blue and White Lady are also suspected to be resistant, as they were not infected in two trials. From the PVY^O trials, Nadine, Red Rascal, Royal Blue, Sequoia, Simcoe and White Lady were resistant, on three occasions, while 16 others are also suspected to be resistant as they were not infected in two trials. These were Coliban, Crystal, Denali, Emma, FL1953, Desiree, Eva, Exton, Lady Christl, Otway Red, PO3, Rioja, Sebago, Snowgen, Toolangi Delight and Wontscab, which include some significant commercial cultivars.

Based on an understanding of the genetics of resistance to the various PVY strains, markers may be identified for extreme resistance genes that will confer resistance to both strains. This has been achieved for two genes, leading to the identification of 14 cultivars that have these markers, although 1 cultivar (Emma) has lost association between the marker and the resistance gene. It was also concluded that the cultivars that are resistant to PVY^O and not to PVY^{NTN} are likely to contain a hypersensitive resistance gene for PVY^O.

The significant number of commercial cultivars that contained the hypersensitivity gene for PVY^O and are now susceptible to PVY^{NTN}, may explain why the new strain has been seen more frequently since its first detection in 2003. For the majority of the main Australian cultivars used today, there is no inherent genetic resistance to the PVY^{NTN} strain. Control of the virus must be obtained through management, while resistant cultivars are developed to mitigate epidemics and significant production issues.

Adoption of MAS for potato

Genetic markers based on variations in DNA, provide great potential to assist plant breeders in the identification of genes of interest for the development of new cultivars. A number of factors need to

be considered for the use of DNA markers in MAS before they can be adopted in plant breeding. The marker must be reliable and closely linked to the gene of interest, to prevent obtaining false data that can occur from an intervening recombination event along the strand of DNA. Alternatively markers could flank the gene to improve the reliability, or with the reduction in genotyping costs that is currently being experienced due to technological advances, whole genome profiling is a realistic proposition in the near future. Such technology advances will improve this area again and deliver even greater assurance of plant performance to breeders and industry.

To adopt MAS, the sample collection and assay must be cost-effective and straight-forward, with the results available in a timely manner. A number of methods have been evaluated in this study and the type of markers that can be analysed rapidly over large populations have been identified. The cost of using this marker system compared to various conventional screening methods has also been determined, revealing significant cost savings when compared to conventional screening for traits such as disease resistance. This marker system is also suitable for combining assays for a number of markers, which will make MAS very cost-effective by reducing the number of assays that need to be conducted.

For MAS to be adopted, training is also needed for the breeders and the various participants in the program to understand the techniques and possibilities. Application of advanced molecular breeding techniques and tools is a very sophisticated and cutting-edge technology that could rapidly bring benefits to the Australian industry.

During this project, markers have been investigated for desirable traits that are typically only screened after a number generations, when selections have already been made for other traits. The markers allow these characters to be given a similar priority to other traits. The markers investigated were for PCN, PVY, PVX and Verticillium wilt resistance, which can now be used in an early generation alongside conventional screening for agronomic traits, permitting screening for these traits for the first time.

Adoption of MAS into the breeding of commercial cultivars will enable the program to rapidly combine desirable traits. Identification of markers that are relevant for the Australian market and environment is important, and will allow cultivars to be selected that are suitable for our local conditions, whether the markers or cultivars are used or bred in Australia or elsewhere.

Understanding complex traits for genetic gain

Most of the traits of interest in plant breeding are under the control of multiple genes, which makes improving them a complex task. Analysis of these traits is important to obtain genetic gain or improvement, and can be achieved by an analysis of the entire family to determine the better families. When the individuals within a family are scored for a complex trait, the mean score for the family will reflect the genetic value of the parents, but this will also be affected by the environment. These environmental effects can be significant, and come from factors such as seed size and quality, plant developmental variations or within trial variations. For complex traits with low heritability, these environmental influences can be very significant, which will delay or prevent genetic gain. Important traits under the control of multiple genes include yield and cooking performance.

For traits with low heritability, selection of individuals displaying better characters may take a number of years and trials to allow the identification of a superior genotype. For those traits for which the environment has a significant effect, it has also been shown that potential superior genotypes could have been rejected early in the process.

During this project, progeny tests have been conducted on the breeding populations to permit genetic analyses on the families, in order to identify superior families and parents. This strategy followed discussions with staff of breeding programs in Scotland and France, and is very different to the intense selection regime used in very large breeding programs to manage population sizes. The intense selection used in many of these programs was found to be inefficient, in that they rejected a high proportion of superior cultivars, as the environment was having a larger influence than the genetic potential of the individual. The better breeding programs are now growing much smaller populations, but in-depth analysis of the populations leads to the identification of superior families and individual genotypes, leading to genetic gain for these characteristics.

During this project, a further advance has been to compare how these traits are analysed in livestock breeding, where gains have been made in milk yield for dairy cattle, and meat production for beef, poultry and salmon. Significant gains have been obtained over the last twenty years by using the genetic information of all relatives, not just the full-siblings within a family.

These analytical methods have been adapted and assessed on our potato breeding populations and focussed on eight complex traits, including yield and cooking performance, to estimate breeding values (EBVs) for all cultivars. From these EBVs, heritability and the expected genetic gain for the trait were also estimated, which should see significant improvements in the average values for these traits in future breeding populations. EBVs were also demonstrated to be valuable as a selection tool for the identification of which cultivars should be selected for further evaluation. We can also use them to combine the most superior parents in the most informed and directed way for the greatest impact, at a very early generation.

These analyses have also shown that the heritability of a trait will affect the future selection strategy. For example, for highly heritable traits such as specific gravity and crisping score, where the environment has a low effect, mid-parent values are highly predictive of progeny mean performance, and individual performance is predictable across generations. Conversely, for lowly heritable trait such as BVP, the environmental conditions have a much larger effect than the individual's genetic make up. A minimal relationship was detected between the mid-parent values and the G2 individual values and the individual values across the G1 and G2 generations. A moderate relationship was seen between the G1 and the G2 family means, and a stronger relationship was seen between the mid-parent estimated breeding values and the G2 family means. It is these latter relationships that should be used in the future.

As the environmental conditions have a much larger effect than individual genetic make up, they should be reduced as much as possible, before significantly reducing the diverse breeding population. This will change the future selection rate based on BVP alone that is practiced in advancing individual genotypes from the G1 to the G2 generation, which has been shown to not be as effective as the G1 family means or the mid-parent estimated breeding values.

This last outcome will lead to a change in the selection rates in the breeding program in Australia. It is now possible to use EBVs to design populations, and continual genetic gain is expected as a consequence for important traits such as yield, visual preference and cooking performance. These analyses could also be extended to other complex traits including complex disease resistance to diseases such as common scab and powdery scab.

A reduced selection rate in the G1 generation will also enable this generation to be grown anywhere in Australia, enabling a potato company to run a program under the guidance and analysis of the central program for the families' performance, and the provision of EBVs for continued genetic

gain. Critically, this will enable populations to be grown in biosecure areas such as Tasmania and Western Australia.

Understanding the potato genome and future markers

The sequence of the potato genome was publically released in July 2011 (Potato Genome Sequencing Consortium, 2011), and will provide major opportunities for the rapid development of markers for a range of traits. The technology supporting genomic research has continued to improve during the lifetime of this project. Significant improvements through next-generation DNA sequencing technology and high-throughput genotyping are now available to the program team.

The potato genome was 86% sequenced and assembled, and revealed a great deal of detail. Over 39,000 protein-coding genes were identified, including genes for tuber formation and disease resistance. Over 700 sequences were identified that correspond to the type that are typically found in disease resistance genes (Bakker et al., 2011), and when these are assessed they should identify which sequences confer desirable disease resistances. Once these genes are identified, it will be possible to develop markers from within the gene itself.

The genome sequence also identified when there were single nucleotide changes to the DNA sequence, which are known as single nucleotide polymorphisms (SNPs). The sequence identified that there was a frequency of 1 SNP every 40 nucleotides, distributed across the entire genome. This means that a marker will be able to be developed in almost any gene as required, and through the application of highly multiplexed SNP marker assays, many thousands of genes controlling important traits can be selected for at minimal costs. By assessing potato genetics in this manner, it will soon be possible to develop genomic EBVs which will maximise genetic gain in a breeding population at even earlier generations, allowing better cultivars to be generated more rapidly than before.

Developing technologies and methods for future improvements

This project was developed as a direct result of the Brennan review of the program. It was designed to develop technologies and methods for future improvements. It has successfully developed and implemented MAS for four traits, and adapted EBVs for potato improvement, which is a world first outcome. These methods have been adopted into the commercial potato breeding project (PT07017) as they were developed, for ‘proof-of-concept’ and to directly implement the technologies into a commercial early adopter program. One marker that was developed within the program (for PCN) has already been adopted in two other international breeding programs. It is anticipated that as all of the outputs of this program are released publicly, there will be a significant uptake internationally of the tools and techniques that will benefit potato breeding internationally, which will have flow-on impact for the Australian potato industry.

The use of markers and EBVs should also be looked at in broader terms for application in other potato, as well as other horticultural crop, research programs.

Technology Transfer

The program has actively promoted the work that is occurring in various ways. The program has been visited by colleagues from New Zealand, France, and Scotland.

The program has presented talks at meetings and field days, so that the work in the program can be presented to a wide audience within the industry. This has also included presentations at the 2010 Potato Industry Conference in Geelong.

Tony Slater attended four international conferences and Lee Schultz attended one international conference. The information that was learned from this travelwas transferred to the breeding team, and to industry through an article in Potatoes Australia, and the investing companies through the annual meetings. These conferences included attending two European Association for Potato Research Trienniel conferences in 2008 and 2011, which are the most important potato scientific conferences held.

The program has actively published articles in industry magazines, newsletters and conference proceedings. The list of articles published from the Potato Breeding Program during projects PT07017 and PT08033 follows:

1. Slater, T., Wilson, G. and Lauder, S. (2007). National Potato Breeding Program trials 2006/2007. Department of Primary Industries. Toolangi.
2. Slater, T., Wilson, G., Lauder, S. and Verstraten, M. (2008). National Potato Breeding Program trials 2007/2008. Department of Primary Industries. Toolangi.
3. Slater, T. (2008). New Potato Varieties – Three new fresh potato cultivars for potential commercial release. *Potatoes Australia*. August. p 28-29.
4. Slater, T., Milinkovic, M., Brown, P. and Kirkham, J. (2008). The European Potato Conference. *Potatoes Australia*. October. p 30-31.
5. Slater, T. (2009). Program breeds new cultivars for industry. *Potatoes Australia*. February. p 32-33.
6. Slater, T. (2009). Resistance is essential. *Potatoes Australia*. June. p 30-33.
7. Slater, T., Wilson, G., Lauder, S. and Verstraten, M. (2009). National Potato Breeding Program trials 2008/2009. Department of Primary Industries. Toolangi.
8. Slater, T. (2009). Moving to private funding for breeding. Potato industry report 08 – 09. p7.
9. Slater, T. (2009). Better screening for desirable traits. Potato industry report 08 – 09. p15.
10. Milinkovic, M., T. Slater and B. Rodoni. (2009). Screening for Tomato spotted wilt virus resistance in potatoes. Proceedings of the 9th International symposium on Thysanoptera and Tospoviruses.
11. Slater, T. (2010). Breeding Better Potatoes. *Potatoes Australia*. June/July. p 20-21.
12. Slater, A.T., Schultz, L., Cogan, N.O.I., Forster, J.W., Rodoni, B. and Milinkovic, M. (2010). Developing molecular genetic marker technology capability to enhance Australian potato breeding. Proceeding of "Potato breeding after the completion of the DNA sequence of the potato genome". p 43. Wageningen. The Netherlands.
13. Slater, T., Wilson, G., Schultz, L., Verstraten, M., Cogan, N., Rodoni, B., Milinkovic, M. and Forster, J. (2010). Australian Potato Breeding Program – Outcomes for Industry. Proceedings of "2010 Potato Industry Conference". pp 23-25. Geelong.
14. Slater, T. (2010). Molecular tools improve breeding and identify cultivars. *Potatoes Australia*. August/September. p 12-13.
15. Slater, T., Wilson, G., Schultz, L., Cogan, N., Forster, J. and Verstraten, M. (2010). National Potato Breeding Program trials 2009/2010. Department of Primary Industries. Knoxfield.

16. Slater, A.T., Schultz, L., Cogan, N.O.I., Forster, J.W., Rodoni, B. and Milinkovic, M. (2010). Developing molecular genetic marker technology capability to enhance Australian potato breeding. *Potato Research.* **53:** 216.
17. Schultz, L., Milinkovic, M., Rodoni, B., Cogan, N.O.I., Forster, J.W. and Slater, A.T. (2010). Development of a robust screening method for Tomato Spotted Wilt Virus infection in Potato. *Potato Research.* **53:** 246.
18. Schultz, L., Cogan, N.O.I., Forster, J.W. and Slater, A.T. (2010). Development and optimization of a genetic identity kit for Australian potato germplasm. *Potato Research.* **53:** 246-7.
19. Schultz, L., Cogan, N.O.I., Forster, J.W. and Slater, A.T. (2010). Evaluation and optimization of the TG689 marker linked to PCN resistance. *Potato Research.* **53:** 247-8.
20. Slater, T. (2010). Developing molecular tools to enhance breeding. Potato Industry Annual Report 2009/10. p 6.
21. Slater, T. (2010). New Australian-bred fresh potato cultivars for commercial release. *Potatoes Australia*. December. p 27.
22. Slater, T. (2011). Cold sweetening resistance – the holy grail for a storage cultivar. *Potatoes Australia*. February. pp 26-27.
23. Slater, T., Wilson, G., Verstraten, M., Schultz, L., Cogan, N. and Forster, J. (2011). National Potato Breeding Program trials 2010/2011. Department of Primary Industries. Knoxfield.

The program has also published two papers in peer reviewed scientific journals:

1. Faggian R, Powell A, Slater A.T. (2011) Screening for resistance to potato cyst nematode in Australian potato cultivars and alternative solanaceous hosts. Australasian Plant Pathology:1-9.
2. Schultz L, Cogan N.O.I, McLean K, Dale M.F.B, Bryan G.J, Forster J.W, Slater A.T (2012) Evaluation and implementation of a potential diagnostic molecular marker for *H1*-conferred potato cyst nematode resistance in potato (*Solanum tuberosum* L.). Plant Breeding 131:315-321.

Recommendations

This project was undertaken following the Brennan review (2004) recommendation that the potato breeding program investigate adopting marker-assisted selection (MAS). This has led to a greater understanding of the genetics and biology of important traits, molecular genetics and quantitative genetics. We have undertaken a review of the publicly available resources, attended important international scientific conferences and established significant international links with the leading potato research groups. We have also published our results in peer-reviewed journals. These efforts have enabled this Australian research program to become internationally recognised.

This project has benefited from a direct linkage to the potato cultivar development project, as that project with its links to potato companies provided direction into the priorities of this project. As a consequence we have worked on a number of important disease resistance and agronomic traits for the Australian industry. This linkage has also enabled the rapid uptake of the methods developed in this project by the cultivar development project. This shows the importance of linking research projects to companies for industry adoption, and should continue formally or informally.

As Tony Slater and Lee Schultz attended and presented papers at international conferences, they visited potato breeding programs and research laboratories in Europe and the USA. We discussed various relevant techniques and have adopted the most advanced. We have also investigated selection strategies used in livestock breeding in order to improve selection strategies for complex traits, such as breeder's visual preference, yield and cooking performance. These trips were extremely informative and further trips should not only be supported in the future, they should be promoted as a way of informing and keeping Australian researchers abreast of the most progressive research.

This work has phenotyped Australian cultivars for a number of important disease issues. This has identified resistant cultivars that growers can cultivate now, and also that the potato breeding program can use as parents to develop superior resistant cultivars for the future. Understanding the genetics of resistance for these diseases, has also enabled the future identification of resistant cultivars developed globally that could also be introduced into Australia. This work on phenotyping for important disease resistance and investigating the genetics of the traits should continue to be tightly linked as it provides a holistic approach to understanding the biology of the trait, as well as identifying resistant cultivars for the Australian industry.

We have validated molecular markers for resistance against four disease issues for Australia. These markers have been deployed into the cultivar development project over the last two years, and this has seen the identification of PCN and PVY resistant cultivars within the breeding populations. This use of MAS should continue to be promoted for the development of resistant cultivars for important traits. New markers should also be developed for the next list of priority traits. These will include *Potato virus S* (PVS), and Root knot nematode where markers have already been developed. It should also include zebra chip, common scab and powdery scab, where some resistance has been identified, but research is needed to identify the resistance genes and develop the markers.

During this work we have explored analysis techniques used for quantitative traits in livestock breeding programs, and compared them to analysis techniques currently used in various potato breeding programs. By visiting some of these breeding programs we have also seen the various techniques in practice. By conducting progeny tests, and using the livestock breeding analysis we have developed estimated breeding values for complex quantitative genetic traits, including yield. We now understand their heritability and can predict the expected rate of genetic gain for these traits. The breeding program will now use these calculations to identify which parents to combine

and to alter selection rates between generations to maximise genetic gain for these traits. With these changes we have shown that the expected rate of genetic gain will double, and that the gain for an important trait such as yield could be as much as 9 tonnes per hectare per year. This analysis is world best practice and once published, should be adopted by the progressive potato breeding programs internationally.

A combination of the molecular techniques with the quantitative genetic analysis could also be applied to understanding other research issues, and managing problems such as common scab or powdery scab resistance, as well as abiotic tolerances such as water use efficiency or drought tolerance. The application of these techniques could rapidly see solutions to these issues for the Australian potato industry and should be applied in any future research program. These techniques are not just applicable to the potato industry, but will be applicable to other horticultural industries, and it is recommended that HAL explores the opportunities that they present.

These findings could also change the necessity of having a breeding program at a single central site, to a model where companies could undertake the early field generation trials locally in their own districts, but under the guidance and analysis of the central program with good science support. This would see the future development of cultivars suitable to anywhere in Australia, and the development of cultivars that would still be productive if the predicted changes to the climate occur. It would also enable programs to be run in states such as Tasmania and Western Australia, without the current biosecurity constraints.

Finally, it is recommended that these advances and opportunities be communicated to the Australian potato industry, through a series of articles in Potatoes Australia, through the HAL extension program, and by presentations at various potato industry events.

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