Designer Pears

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Perfect Pears – The National Pear Breeding Program

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Perfect Pears – The National Pear Breeding Program

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The National Pear Breeding Program commenced in 1993. This report details progress from June 2006 to September 2008.

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Media Summary

Perfect Pears is a national pear breeding program funded by the Victorian Department of Primary Industries, HAL and APAL. The Australian pear industry needs a quantum step change in the quality of new pear varieties to expand the pear market in both production and market sales. New varieties need to re-invigorate consumer interest in eating pears, to attract new pear consumers and increase per capita consumption. The varieties need to attract a price premium to facilitate expansion of pear orchard production under more intensive production systems.

The key breeding objectives are to develop new pear varieties with a combination of attractive fruit appearance, superior flesh eating quality, good storability and shelf life. New varieties ripening across the range of the pear harvest season and pear scab resistance to reduce fungicide use are also desirable.

The breeding program, based at DPI Tatura, has been breeding and assessing new pear cultivars over the last 15 years. The program has produced up to 66,000 seedling trees in that time from over 200 different pear crosses. Of the crosses that have been made, the greatest proportion of selections has come from Guyot x Corella and Guyot x Rogue Red crosses. There are now 257 new pear selections under evaluation in replicated trials.

There are 2 selections to progress to advanced large-scale evaluation trials on fruit grower properties in 2008. One selection ripens in early January (Photo 1) and has a pinkish-red blush on a yellow background with an attractive pyriform shape. It maintains a crisp juicy texture and can be tree ripened with a short storage life. The mid-season selection (Photo 2) has strong red blush on a green background with a smooth reasonably symmetrical shape. It develops a soft, juicy, texture with aromatic flavours similar to the variety Comice. This selection has the potential to handle and store long term similar to the well known pear variety Packham.

The current status of genetic diversity amongst *Pyrus* cultivars grown in Australia is largely unknown. A study of 95 cultivars/species and hybrid selections from the breeding program found a wealth of genetic diversity between them. This diversity can be utilised in cross breeding to produce a greater genetic gain in important economic traits such as disease resistance and eating quality.

Photo 1. An early season European pear selection with pinkish-red blush.

Photo 2. A mid season European pear selection with attractive green/red skin.

Technical summary

The National Pear Breeding Program aims to develop new high-quality pears with good fruit appearance and eating quality, good storage and shelf potential, diversified maturity, better scab resistance and grower-friendly tree characteristics. This report summarises the progress of the program from June 2006 to September 2008.

Over 66,000 seedling hybrids from 212 controlled crosses have been planted at the DPI Victoria Tatura site since the commencement of the program in 1993. Over 44 existing pear varieties and 18 elite selections from the program itself were used for crossing. Currently 30,000 seedling trees, varying in age from 1 to 10 years, remain in seedling orchards. Each cropping season about 600 seedling trees were visually selected and their fruit harvested at the mid-point maturity date of the parents and 2 weeks either side of the mid-point. They were cool stored for 2 to 3 months and then the fruit quality assessed by a sensory panel after one week post fruit ripening. The greatest proportion of selections over the last two years has come from Guyot x Corella and Guyot x Rogue Red crosses.

Over 250 selections are now under evaluation as orchard trees principally on one rootstock, D6, trained to an open Tatura Trellis system. In 2007 season, the 01, 06 and 07 selections were assessed on a 1-7 likeness scale for fruit shape, colour and overall appearance, and fruit texture, flavour and overall eating experience. In 2008 all selections were assessed but the later selections had to be sourced from the original tree in the primary block as these selections are yet to be established or fruiting in replicated trials. Storage attributes: neck shrivel, scald, limb rub, internal browning and mealiness were also rated. The top 50 selections were identified for collection of more detailed tree and fruit characteristics over the next few years to fast track their commercial release.

Seven distinct series of promising pears have been identified in the program that ripen across the harvest season. The bi-coloured series have a strong red blush on a dark green background colour and will be distinct from other pear varieties currently in the market but still display a typical pear shape. The Corella series of red-blushed pears are principally from the Guyot x Corella family. The small pears in this series have the potential to appeal to children and have a niche in the lunchbox/snack market as they are small and firm and have a crisp juicy texture and very mild pear flavour that would be appealing to the majority of consumers. The Rogue Red series of pears consist of small full red pears and medium-large red blushed pears. The majority have a fine buttery texture and aromatic pear flavour with varying levels of grit. There is a light pink-blushed series of pears with high eating quality. The late season green pears have a fine buttery texture. Their appearance is not unique but they represent the traditional green pear with an improved eating quality. Only a few inter-specific crosses were made, as breeding inter-specific hybrids is only a minor component of the program. However the inter-specific series of pears currently has two promising selections. The disease resistance series of pears encompass selections with potential high level of fire-blight and scab resistance.

There are 2 selections to progress to advanced large-scale evaluation trials on fruit grower properties in 2008. One ripens in early January and has an attractive appearance of a uniform pyriform shape and pinkish-red blush on a yellow background. It would come onto the market before William's Bon Chretien and potentially open the pear season for consumers with a new appealing pear variety. The mid-season has the potential to handle and store long term and can offer consumers an alternative to the traditional variety Packham's Triumph but with more attractive appearance and improved eating quality.

The current status of genetic diversity amongst *Pyrus* cultivars grown in Australia is largely unknown. In a study, 95 cultivars/species and hybrid selections from the breeding program were genotyped across a suite of 13 codominant SSR markers in order to assess the inherent genetic diversity and delineate the underlying relationships. Statistical analysis supports the separation of the European pear (*P. communis)* in a discrete cluster from the Japanese *(P. pyrifolia),* the Chinese pear (*P. bretschneideri*) and 5 other Asian species (*P. calleryana, P. salicifolia, P. pashia, P. x. complexa, P. fauriei*). A wealth of diversity between species and cultivars is apparent, whilst relationships generally conform to the geographic proximity of cultivars or their parents.

Introduction

1.1 Germplasm enhancement

Pear is one of the major fruit crops in Australia. The industry produced 139,000 t fruit with a farm gate value of A$ 85.7 million in 2006 (ABS Source, 2006). European pear is the mainstream product (96%), and Japanese pear, namely Nashi, has been grown for fresh and export market since 1980's but remains small (3 500 tons, 2%).

Pear orchards are mainly located around the cool regions of the continent away from the coastal line. When splitting the production according to the state based on 2006 statistics, Victoria contributed to 87% of the national production, followed by West Australia (WA) and South Australia (SA), which together took up 10% and a minor contribution from the other three states: New South Wales (NSW), Queensland and Tasmania.

In the varietal scene, old varieties, William's Bon Chretien (WBC) and Packham's Triumph still play a dominant role and respectively contribute to 48% and 39% of the total production, followed by Buerre Bosc (7%) (ABS Source, 2006). Although interest in growing Corella, a blushed pear variety, has increased during the past decade, it only attributed to 0.4% of the national production today, which is similar to the other marginally important varieties, such as Red Sensation, Red d'Anjou. Nashi production relies on one major variety, Nijisseiki (20th Century) (http://www.nashiaustralia.com.au/index.html).

Pears are mainly consumed as a canning fruit after processing or as a fresh fruit, which are sold in the wholesale fruit markets, supermarkets and retail fruit outlets across the nation. Being located in the Southern Hemisphere, Australia can produce and supply fresh fruit during the off-season in the Northern Hemisphere (Little and Holmes, 2000). Europe and many South Asian nations were once the destination of Australian pear products. At their peak production, a record 22% production was exported annually (Mitchelmore, 1995). In the last decade, however, the amount of exports has decreased significantly to around 6% of pear production (http://www.apal.org.au/marketing-export-stats.cfm). Increased competition of pear exports from South Africa, Argentine and Chile and the lack of distinctive products have both contributed to the decline in export of Australian pears.

Breeding efforts in European pears in the 20th century has resulted in the release of more than 150 different cultivars. Most of these originated from European and North American breeding programs. Among those cultivars, there are some significant genetic improvements in relation to fire blight resistance, skin colour and adaptability to different climatic conditions. However, with the exception of a few new cultivars derived from bud mutations, such as Taylor's Gold in NZ, and early season Conference in Europe, becoming important in limited areas, none has grown to be as popular as those traditional cultivars mentioned above. One explanation is the lack of quality and appearance, which surpasses that of traditional cultivars. The lack of notorious biotic constraints in Australia, such as pear fire blight and psylla, allows appearance and fruit quality to become a primary breeding objective. The Australian National Pear Breeding Program based at DPI Tatura, has been breeding and assessing new pear cultivars over the last 15 years. The program has produced up to 66,000 seedling trees in that time from over 200 different pear crosses. More than 250 selections have been identified which now require further assessment on their harvest indices and storage and potential consumer acceptance before they are recommended for release.

During the initial phase (prior to 2001) the program focused on developing and establishing new seedling breeding populations through interstate collaboration and crossing between the major known European pear varieties. Details of the inception and development of this project are given in the previous final reports of project AP310 (1996) and AP96032 (1999). From 2003 to 2008 the program initiated a new phase of intercrossing using the most improved pear selections identified so far from the first breeding phase. It also undertook genetic studies to understand the inheritance of fruit quality attributes and pear scab resistance (refer final report AP99007 (2005), AP04019 (2006)).

1.2 Genetic Diversity Study

Pears (*Pyrus spp)* have been cultivated for thousands of years, and consist of over 22 primary species and nine interspecific hybrids (Bell et al, 1996). As a predominately outcrossing species, pears possess the potential for extensive genetic diversity (Ghosh *et al.,* 2006). However, analysis of the genetic diversity of pear germplasm in comparison to other fruit species is very limited (Oliveira *et al.,* 1999; Monte-Corvo *et al*., 2001; Ghosh *et al*., 2006, Volt *et al*. 2006). The pear market is dominated by only a few major cultivars. For example, whilst there are over 5000 cultivars of European pear, "Conference", "Williams Bon Chretien", "Abaté Fétel" and "Rocha" alone dominate 64% of current pear production in Europe and "Packhams Triumph" and "Williams Bon Chretien" constitute 86% of pear cultivars grown in Australia (WAPA press release 12 August 2008). In addition, it appears that the major commercial pear cultivars can be sourced from a pivotal period of pear improvement (1750 – 1850) conducted in France and Belgium; potentially generating a genetic bottleneck (Bell *et al*., 1996). The loss of local cultivars highlights the continual risk of elimination of beneficial alleles from pear breeding, whilst pedigree data on most commercial cultivars is still unknown (Oliveira *et al.,* 1999).

In addition to genetic diversity analysis, the need also exists for accurate and reliable cultivar identification; fundamental to cultivar protection, selection and improvement in breeding programs. This necessity is heightened by the fact pears are generally cultivated by vegetative propagation; such asexual reproduction can make individual clones difficult to resolve (Oliveira *et al.,* 1999). Contemporary molecular techniques can, however, provide the resolution to distinguish between cultivar genotypes and assess the genetic diversity amongst germplasms.

Molecular markers constitute specific loci throughout the genome of an individual, which exhibit variation in DNA sequence relative to other members of the population (Hine and Martin, 2004). Numerous markers exist and have been adopted for cultivar identification and genetic analysis. Whilst phenotypic variation and isozyme studies were utilised initially (c. 1960's) for *Pyrus* analysis, these markers had limited polymorphism and could be confounded by environmental and physiological factors (Ghosh *et al.,* 2006).

It is only recently that the application of hypervariable molecular markers has enabled multi-locus fingerprinting and acquired the sensitivity and robustness to explore genetic relationships (Jones and Arden, 2003). Simple sequence repeat (SSR), or microsatellite, markers are currently the most efficient DNA marker for cultivar identification, diversity analysis and source tracing. These markers consist of short sequences of DNA repeated in tandem, located terminally (SSR) or internally (ISSR) which produce species or cultivar-specific profiles for use as a diagnostic tool (Monte-Corvo *et al*., 2001). They are codominantly inherited, highly polymorphic, abundantly distributed throughout the genome and readily automated by PCR (Yamamoto *et al*., 2002a).

Numerous genetic markers have been successfully exploited across many commercial fruits to address cultivar identification and assess genetic diversity; including grapevine, *Vitis*, (Gaspero *et al.,* 2000), kiwi fruit, *Actinidia* (Huang *et al.,* 1998), apple, *Malus* (Guildford *et al*., 1997; Hokanson *et al*., 1998) and peach, *Prunus* (Testolin *et al*., 2000; Dirlewanger *et al*., 2002; Yoon *et al*., 2006). Both pears and apples are in the same sub-family, Pomoideae within Rosaceae (Bell *et al*., 1996); previous studies indicate the potential to use genetic markers derived from other species within Pomoideae to assess the genetic diversity within pear, by optimising PCR conditions (Hemmat *et al*. 2003; Pierantoni *et al.,* 2004). The use of SSR's derived from other species to assess genetic diversity in the *Pyrus* genome is made possible by the fact that adjacent, promoter regions of the markers may be well conserved in related taxa*;* for example, approximately 50% of marker flanking regions are conserved between apples and pears (Monte-Corvo *et al*., 2001). It is also possible to target these mutations occurring *within* the regions flanking SSR loci as a source of discriminating polymorphism and study of evolutionary change (Huang *et al.,* 1998).

In terms of population structure and intra-cultivar variation amongst *Pyrus* from previous genetic relationships studies, there is clear distinction between *P. pyrifolia* and both European and North America variations of *P. communis* (Oliveira *et al.,* 1999, Yamamoto *et al.* 2002b, Ghosh *et al.* 2006). This is consistent with the distinct morphological and phenological profiles of *P. communis* (European) and *P. pyrifolia* (Japanese). Further structure may also exist amongst the cultivars of *P. communis;* clusters conformed to those originating from South Canada, Western Europe and interspecific hybrids and rootstocks of similar parentage (Ghosh *et al.* 2006). The French cultivars also form a distinct sub – cluster, as do the Portuguese cultivars within *P. communis* (Oliveira *et al*., 1999).

Overall, it seems apparent that the genetic relationships between *Pyrus* cultivars generally conform to geographic proximity, phenotypic and phenological similarity of individual genotypes (Oliveira *et al.*, 1999). However, given the limited studies done, molecular ambiguities and the inability of some cultivars to conform as expected, there is much scope for further resolution.

The current state of genetic diversity amongst *Pyrus* species and cultivars grown in Australia is unknown. Therefore, to address this issue and continue progress in pear breeding and improvement, this study aims to assess the genetic diversity of 96 pear accessions including 36 European cultivars (P. *communis*), 17 Asian cultivars (*P. pyrifolia;* P. x *bretschneideri*; P. *ussuariensis*), 6 interspecific cultivars, 6 naturally occurring species and 31 hybrids developed within the Australian Pear Breeding Program, in order to expose any underlying population structure and degree of genetic diversity available for germplasm improvement.

Materials and Methods

Hybridisation and seedling establishment

Since 2003, selections from the program with improved quality characters have been extensively used as parents in crosses. They were crossed with each other and with major varieties. In 2006 and 2007 seven cultivars were chosen as parental genotypes and were inter-crossed with one to another according to a half diallel mating design. These cultivars were Williams Bon Chretien (WBC), Packham's Triumph, Josephine de Malines, Doyenne du Comice, Corella, Dr Jules Guyot and Harrow Delight. The objective of the cross pollinations were to develop a diversified maker population using well-known pear germplasm accessions with distinctive phenotypic merits to facilitate pear genetic and genomic research.

Controlled pollinations were carried out in spring at the DPI Tatura site. Flowers of the female parents were first hand-emasculated at the 'balloon' stage, followed by hand pollination and then bagging of the inflorescence. Pollen was collected and used in the same season when crossing was being conducted or was collected during the previous season and stored in a freezer until use. Hand pollination was conducted immediately after emasculation for the first time and was then repeated after 5 days. The covering bags were removed seven days after the last pollination.

Cross pollinated fruit was harvested at physiological maturity and stored in a cool room (1°C) for 6 to 8 weeks. Seeds were extracted, cleaned and surface sterilized with 2.5% common bleach. The seeds were then planted in propagation tubes with Debco® Seed Raising mixture and placed into a cool room for stratification for about 6 weeks. The seeded tubes were then placed in a heated glasshouse for germination and seedling establishment. Four weeks after seedling establishment, they were progressively hardened, first by moving them into an unheated glasshouse and then outdoors.

Seedlings were planted in orchard blocks at a row x tree spacing of 3.5 m x 0.5 m in early spring. The orchard blocks are managed similar to commercial orchards (i.e. irrigation, fertilisation) except that timing of some management practises is based on the earliest flowering progeny and earliest harvest date (ie chemical applications) as the evaluation blocks contain pear trees with a range of flowering and maturity dates. Seedling trees are not pruned regularly, but branches along the leader trunk up to 1 m above ground are removed during the first three seasons to ensure rapid growth of the leader trunk and subsequent ease of access to mature orchards for regular maintenance.

Identification of new selections

A three step strategy developed in 2003/04 is used to identify desirable hybrids in seedling families that have begun to set fruit. The strategy includes visual selection in the orchard, followed by cold storage and then comprehensive quality assessment after the fruit has fully ripened. This process is described in detail in Liu et al (2005).

Based on predefined 1-5 and 1-7 point scales, sensory scores are given for fruit appearance, including attributes such as: fruit symmetry, bumpiness, smoothness, shape, and skin and lenticel colour. The following quality attributes are also determined during the assessment: flesh firmness, juiciness, fineness, grittiness, sweetness, acidity, sugar/acid balance and aromatic flavour. Seedling trees with

desirable fruit quality compared to the commercial varieties, WBC and Buerre Bosc, are identified. Selected trees are then propagated on commercial rootstock D6 to produce orchard trees. The originally selected seedling trees are also maintained in the breeding orchard for one or two seasons more, to allow repeated assessment of their fruit quality.

Propagation and evaluation of selections

Bud wood of selected trees is collected in late winter (August) and then grafted onto rootstock D6. Rootstocks are supplied by AusBuds Pty Ltd and are certified virus free. To propagate the trees, bench grafting using the whip-tongue technique is conducted in early spring (September). Trees are immediately planted in the tree nursery after grafting. The nursery is regularly watered and kept weed free by hand weeding and/or application of selective herbicides.

Orchard trees derived from seedling selections are initially evaluated at DPI Tatura. Each season a minimum of four trees per selection are planted at the DPI Tatura site in replicated trials. Propagated trees are grown for one year in a nursery and then transplanted into double staggered rows on an open Tatura trellis in early spring with a row x tree spacing of 3.5 m x 1.5 m. The trials are a randomised factorial design with selection and tree position (east or west) as treatment factors.

One to two of the most promising selections are provided to the Australian Pome Fruit Improvement Program (APFIP) for regional evaluation in different production regions. Trees for APFIP sites are planted in different regions in spring. The trees are trained to a central leader system across all sites. One to 4 trees per selection are planted at each APFIP site. APFIP manages and evaluates selections independently, and the outcomes are then given to the breeding program.

Large scale commercial evaluation is conducted through APFIP. Two elite selections were recommended for commercial evaluation in 2008 and up to 2000 trees of each will be established on grower properties in winter 2008 and 2009.

Based on a 1-7 likeness scale, scores were given for fruit shape, colour and overall appearance, and fruit texture, flavour and overall eating experience. Only the 2001, 2006 and 2007 selections were assessed on this scale in 2007. In 2008 all selections were assessed but the later selections had to be sourced from the original tree in the primary block as these selections are yet to be established or fruiting in replicated trials. Storage attributes: neck shrivel, scald, limb rub, internal browning and mealiness were rated on a 1-5 scale. Parent varieties were assessed along with selections to act as controls. Harvest indices of fruit weight(g), height(mm), width(mm), firmness (kg), sugar (^{o}brix), seed colour and starch level were measured on 1 to 5 fruit after 10 weeks cool storage at 1^{o}C prior to ripening in 2007. Only a small number of selections were assessed for harvest indices prior to cool storage.

Genetic diversity study

Sample Population

The specific and generic name of each pear accession is listed in *Table 1 (Appendix 2).* Fifteen SSR markers were amplified in the pear genome in order to identify and discriminate between 96 accessions of *Pyrus* within the Australian Pear Breeding Program. Markers were derived from apple and pear studies, both contained within the subfamily *Pomoideae.*

DNA Extraction

DNA was extracted from newly emerged young leaves that were preserved under liquid nitrogen prior to extraction.

PCR Protocol

Fifteen 96 – well plates were prepared containing DNA extracted from each of the 96 unique accessions, for PCR under each of the SSR primer pairs. DNA amplification was performed on *Applied Biosystem Perkin Elmer* models. Each 12µl reaction volume subject to PCR consisted of 2 µl of 5 ng µl^{-1} gDNA, 1.2 µl 10 x buffer, 1.2 µl 2mM dNTPs, 0.05 µl each primer, 0.05 µl Immolase and made up with 7.45 µl dH2O.

Two PCR programs were used for the 15 primers in monoplex PCR; touch – down 1 and touch – down 2. These programs were tailored according to optimal annealing temperatures for primers. The touch –

down 1 program constitutes the following thermal profile; initial denaturation at 95°C for 10 minutes; 1 minute at 95°C; 10 cycles of 30 seconds at 65°C (-1°C per cycle) and 1 min at 72°C; 20 cycles of 95°C for 30 seconds, 55°C for 30 seconds and 72°C for 1 minute; preceded by the final extension at 72°C for 10 minutes. The thermal profile of the touch – down 2 is identical, except that the first 10 cycles begin at 60°C, rather than 65°C and the annealing temperature in the 20 cycle series at 50°C, rather than 55°C. Information regarding each of the 15 primer pairs used in this analysis is listed in *Table 2 (Appendix 2).*

Prior to washing and sequencing, samples were pooled according to their associated fluorochrome marker, enabling 3 primers to be run *simultaneously* on the ABI – 3730 DNA Analyser. The set up was such that each pooled plate contained PCR product from three separate markers, each assigned specifically to one of three different fluorochrome dyes; FAM (blue), HEX (green), TAMRA (yellow). Markers were associated with their fluorochrome dye by approximate estimation of the size of their products; to minimise overlap on the ABI. 3 µl of PCR product was aliquotted into 96 – well plates for markers associated with the FAM fluorochrome dye, whilst 3.5 µl was used for those labelled with HEX and TAMRA. Plates were consistently vortexed and centrifuged between each step.

PCR "Clean up"

Subsequently, pooled PCR products (10 µl) were washed and concentrated by precipitation. 1µl of 7.5M ammonium acetate was added to each well of the plate, followed by 27.5µl of 100% ethanol (EtOH). Mixing and centrifugation encourages the PCR products to precipitate at the bottom of each well. The supernatant was removed and a further wash was performed using approximately 150µl of 70% EtOH. The PCR precipitate was subsequently re-suspended in 30 µl of dH2O.

ABI – 3730 Protocol

Following this, PCR products were subject to the ABI – 3730 DNA Analyser; designed to separate and sequence amplicons by capillary electrophoresis on a vertical, polyarcylamide gel. This involved aliquotting 1 µl from each well of pooled solution into prepared 96 – well (Liz) plates with 8.95 µl of Hi – di farmamide; an amide substance which helps degrade the DNA.

Plate *Pool 1* was then initially loaded onto the ABI – 3730 DNA Analyser to check the intensity of signals were appropriate. The resultant signals were far too weak. Therefore, an additional 2.5 µl of re-suspended DNA solution was added to the pooled plates containing farmamide; such that 3.5 µl re-suspended PCR product was subject to further analysis for all markers. Plates *Pool 1 – 5* were subsequently run on the ABI sequencer.

Data Analysis

A range of software was adopted in this study, in order to provide a comprehensive and reliable statistical analysis of both the codominant and binary datasets. The software used were: *GeneMapper v3.7* to annotate the genomes, producing high – quality images of individual allele signals that can be measured and scored; *Structure* to incorporate genotype data across a suite of unlinked markers and identify any underlying, putative population structure ((Pritchard *et al.*2000); *Arlequin* program to provide a comprehensive set of statistical tests for use on genotype data, in order to infer particular demographic features amongst populations (Excoffier *et al.*2005); *GeneAlex* is a program nested within Excel which enables datasets to be managed, formatted and subjected to many of the basic statistical tests (Peakall and Smouse, 2006); and *NTSYS* to infer population structure within genotypic datasets (Rohlf, F. J. 1994).

Results

Hybridisations

The main objective of hybridisations for 2006 and 2007 were to develop a diversified marker population using well-known pear germplasm accessions with distinctive phenotypic merits to facilitate pear genetic and genomic research in future years of the project. The number of seedlings derived from crosses in 2006 and 2007 for a marker population and for germplasm enhancement are listed in table 1.

Table 1: Cross pollinated seedlings produced in 2006 and 2007.

Crosses for marker population	**Number of seedlings**		**Crosses for germplasm enhancement**	**Number of seedlings**	
	2006	**2007**		**2006**	**2007**
Comice/Guyot	36		Comice/A-4-29	79	
Comice/Josephine	30		Comice/D-6-33	28	
Comice/Packham	48		Comice/F-10-58	32	
Comice/Corella		146	Comice/F-49-98	72	
Comice/Harrow Delight		179	Comice/G32-17		274
Comice/WBC		145	Howell/BMP	23	
Corella/Guyot		45	Howell/C31-42	144	
Corella/Harrow Delight		34	Packham/A-1-42	20	
Harrow Delight/Guyot		23	Packham/C31-42	23	
Josephine/Corella		169	Packham/F-10-58	27	
Josephine/Guyot		35	Packham/L'Inconne	21	
Josephine/Harrow Delight		84	WBC/C31-42	111	
Packham/Josephine	24		Winter cole/C31-42	420	
Packham/Corella		293			
Packham/Guyot		280			
WBC/Corella	87				
WBC/Guyot	72				
WBC/Josephine	103				
WBC/Packham	76				
WBC/Harrow Delight		226			
Total	**478**	**1659**	**Total**	**1,000**	**274**

The seedling population at DPI Tatura currently consists of 29,500 trees of which 10,000 are second generation crosses that are yet to bare fruit (table 2).

Table 2: Seedling populations at DPI Tatura (Stage 1).

Breeding Phase	**Number crosses**	**Number trees**	**Number trees removed**	**Number trees remaining**
1st generation	131	56,000	36,500	19,500
2nd generation	81	10,000		10,000
Total	212	66,000		29,500

Identification of new selections

About 600 seedling trees were visually selected in 2006 and 2007 from among fruit-bearing seedling populations for post-ripening assessment at three different harvest dates. Fifty one and 27 seedling trees were selected in each season respectively. In 2008 no new selections were made as labour resources were temporarily focused on evaluation of all current selections in the same season. As many recent selections are yet to fruit on in the replicated trials some fruit were sampled from the original tree if it still existed in the primary blocks. The greatest proportion of these selections came from the Guyot x Corella and Guyot x Rogue Red (and reciprocal) crosses.

Since the initiation of the program 257 selections have been identified from seedling populations of which seven are from inter-specific crosses (Table 3). It is expected that up to another 100 selections will be identified over the next 5 seasons as the remaining trees in the primary blocks start to fruit.

Table 3: Total pear selections (Stages 2 and 3).

	Stage 2 selections2 selections DPI Trials			Stage 3 selections APFIP large-scale trials
Crosses	**2001 -2003**	**2004-2008**	**Total**	**2008**
European	94	156	250	2
Inter-specific	2	5	7	
Total	96	161	257	2

Evaluation of current selections

Seven distinct series of promising pears have been identified in the program that ripen across the harvest season (photos 1 to 7). Many of the selections listed in table 4 and appendix 1 have been identified in the last two seasons so there is limited data on their performance as fruit was only available off the originally selected tree for assessment. Some of the selections have been put into two groups (a and b) based on their size in the Corella series (photo 2) and the Rogue Red (photo 3) series. However the size potential of all the selections is yet to be established so the selections currently classified as small pears may have the potential to produce larger fruit. Data was also collected on the starch levels and seed colour but there was little variation and all fruit tested pre-ripening after 10 weeks storage had 90% or more of the cortex free of starch and seed colour was black.

Table 4: Harvest data measured on top 10 selections after 10 weeks cool air storage at 1^{o}C, pre-ripening 2008.

Selection	**Female**	**Male**	**Harvest range**	**Weight (g)**	**Height (mm)**	**Width (mm)**	**Firmness (Kg)**	**Sugar (^{o}Brix)**
C-31-42	BPM	Corella	11-25 Jan	150-200	75-85	60-70	4.5-8	13-15
F-01-88	Comice	BPM	18 Jan-13 Feb	200-300	80	65-75	6.5	14-16
F-11-82	Corella	Comice	30 Jan-21 Mar	150-250	75-85	65-75	5.5-7.5	14-16
C-41-67	Comice	Ya Li	30 Jan-26 Mar	150-250	80-90	60-70	3-4	15-17
I-21-19*	Guyot	Rogue Red	5-14 Feb	50-100	50-60	50-60	3	14-15
G-30-57*	Guyot	Corella	6 Feb-1 Mar	50-100	65	50	6	17
G-32-17*	Guyot	Corella	6 Feb-1 Mar	50-100	60-70	50-60	5-6	14-16
K-19-20*	Rogue Red	Guyot	8 Feb-4 Mar	100-150	60-70	60-70	5-6	16-17
K-19-21*	Rogue Red	Guyot	22 Feb-1 Mar	50-150	55-65	55-65	5.5-9	12-14
E-24-28	Comice	Josephine	15 Mar	NA	NA	NA	NA	NA

* Data taken from original selected tree unpruned and planted at high density in primary assessment block.

Photo 1: Series of medium bi-coloured pears with an intermediate straight shape and flat base.

F-01-88
Comice x BPM
18 Jan – 13 Feb

F-11-82
Corella x Comice
30 Jan – 20 Mar

I-38-76
Rogue Red x Josephine
9 Feb – 4 Mar

Photo 2a: Corella series of small red-blushed pears with crisp juicy texture and slight musk flavour.

C-31-42
BPM x Corella
11 – 21 Jan

G-30-57
Guyot x Corella
6 Feb – 1 Mar

G-32-17
Guyot x Corella
6 Feb – 1 Mar

Photo 2b: Corella series of medium-large red-blushed pears with crisp juicy texture.

H-51-59
Guyot x Corella
15 – 20 Feb

G-24-31
Guyot x Corella
6 Feb – 27 Feb

G-46-45
Guyot x Corella
4 – 11 Mar

Photo 3: Rogue Red series of small red pears with fine buttery texture and aromatic pear flavour.

I-21-19
Guyot x Rogue Red
4 – 15 Feb

K-19-20
Rogue Red x Guyot
8 Feb – 4 Mar

K-19-21
Rogue Red x Guyot
22 Feb – 1 Mar

Photo 3b: Rogue Red series of medium-large red-blushed pears with fine buttery texture.

K-23-55
Guyot x Rogue Red
8 - 15 Feb

I-15-48
Guyot x Rogue Red
5 – 14 Feb

K-48-85
Rogue Red x Guyot
8 Feb – 12 Mar

Photo 4: Light pink-blushed series.

F-20-05
BPM x Comice
21 Jan – 8 Feb

K-21-05
Guyot x Rogue Red
8 – 15 Feb

F-12-45
Guyot x Comice
8 Feb – 15 Mar

Photo 5: Late season series of green pears with fine buttery texture.

G-09-36
Packham x Comice
3 – 12 Mar

E-24-28
Comice x Josephine
Mid Mar

H-47-68
Eldorado x Rogue Red
Early April

Photo 6: Inter-specific series.

ANP-07-27
Inter-specific
Tree ripen mid Feb

C-41-67
Comice x Yali
Tree ripen early Mar

Photo 7: Potential disease resistance.

G-27-27
Guyot x Corella
13 Feb – 1 Mar

D-11-57
Harrow Delight x Packham
25 Jan – 22 Feb

Two of the European selections C-31-42 (BPM x Corella) [photo 8] and F-11-82 (Corella x Comice) [photo 9] progressed to stage 3 large-scale evaluations managed by APFIP in 2008.

Photo 8: An early season European pear selection with pinkish-red blush.

Photo 9: A mid season European pear selection with attractive green/red skin.

Genetic diversity study

3.1 Microsatellite Markers

3.1.1 Justification of final suite of SSR's

From the original suite of 15 SSR markers, 13 markers were used in the codominant diploid data analysis, whilst 14 were suitable for use in the dominant, binary data. One marker (CH01H01) failed to produce scorable signals during sequencing, whilst a second marker (PS12A02) failed to make the codominant dataset. All remaining markers conformed to codominant formats (2 alleles per loci only) following removal of alleles of insufficient signal or individuals with dubious multiple amplifications; NB109a (1 deleted), KA16 (1 deleted), GD147 (1 deleted), BGA35 (4 individuals removed), KA14 (2 individuals), NH001c (1 individual). Tsu Li failed to produce amplicons under any of the markers and was therefore excluded from further analyses. This was the only representative of the Chinese species, *P. ussuariensis*.

3.1.2 Marker polymorphism

The ability of a marker to detect individuals or cultivars is a function of its polymorphism. The degree of polymorphism provides an indication of the strength of each marker to detect differences between individuals and hence act as a diagnostic tool. Table 3 (*Appendix 2*) highlights the total number and percentage of alleles amplified under each species grouping, under each marker. NH015a (20) and NB109a (19) are the most polymorphic amongst the suite of 13 markers, followed by NH001c (17), BGT23b (16) and KA16 (16). Results also indicate that loci BGA35 (9) was the least polymorphic, whilst NH029a (10) and KA14 (10) were similarly low.

The percentage variations amongst individuals that can be explained by the polymorphism exhibited at each of the loci, as calculated by Arlequin. Loci 8 (KA14) explained the greatest proportion of the variation among population singularly (36.31%). Loci GD147 explained a similarly portion of among population variation (34.60%), relative to other markers. Locus 10 (CH02D12) explained the greatest proportion of the variation within populations (87.35%); this loci is also derived from apple. For all markers together, the variation among the populations was 22.54% and within populations 77.46%.

3.1.3 Unique Allele Work (GeneAlex)

All 13 (codominant) markers produced species – specific alleles for at least one species. Therefore, all are useful for cultivar identification. The majority of individual species groupings also exhibited alleles not observed in any of the others (*figure 1)*. These have the potential to act as diagnostic, species – specific markers. It is apparent that whilst each of the Asian species and Interspecific hybrids exhibited an average of less than 0.5 private alleles, *P. communis* by far expressed the most number of putative species – specific alleles (3.846).

Figure 1; The mean number of private alleles across 10 different populations of Pyrus

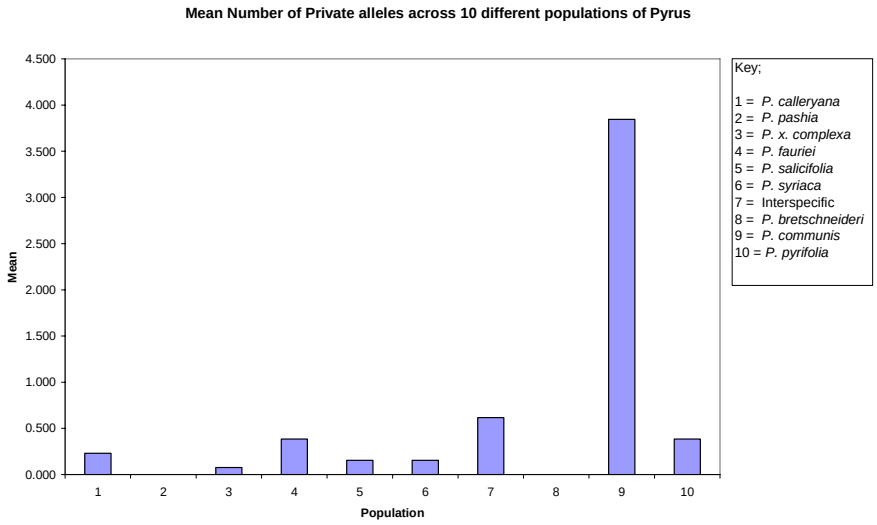

Figure 1; The mean number of private alleles (population – specific) which amplified in each of the 10 populations, as calculated by GeneAlex.

3.2 Population Structure

Statistical analyses were subsequently performed to assess the likelihood and conformation of population structure amongst the 95 accessions using *Structure v2.2* (data not shown), *Arlequin v3.1, GeneAlex v6.1 and NTSYSpc v2.1*.

Fst values, as calculated by *Arlequin* with the 1 – 10 pre - defined population structure reveal that population 9 (*P. communis)* is significantly distinct from all the Asian species (except *P. syriaca*). This is consistent with the dendrogram generated by *NTSYS (Figure 3).* In addition, there is significant distance between *P. communis* and *P. pyrifolia;* consistent with much of the literature. Values of population – specific Fst calculation, by *Arlequin* were all very similar. Results suggest both *P. pashia* and *P. salicifolia* are equally the most divergent groupings from the other species groups (0.28243). Following this is *P. x. complexa* (0.27474), joint *P. calleryana* and *P. fauriei* (0.26321), joint *P. syriaca* and *P.x bretschneideri* (0.25936). Therefore, the immediate structure appears to be strongest amongst the Asian species.

Analysis using *GeneAlex* to produce a matrix of the average Fst values between each pair of pre – defined populations gave similar results to the *Arlequin* analysis. Using the *average* measure of genetic distance again appears to indicate structure is strongest and most apparent between the Asian species (Pop 1 – 6) and much less significant between the better represented populations (Pop 7 – 10).

GeneAlex software was used to run principal component analysis (PCA) on the codominant and binary data, using both Covariance and Distance – Standardised methods (*codominant data using distance – standardised method only shown; Figure 2*). Trends produced from the codominant data are consistent with that of the binary, although the groupings are more apparent in the binary data, exaggerating the differences between cultivars. Review of the PCA analysis of the codominant data indicates a

concentration of *P. communis* cultivars on the far bottom left of the graph, *P. pyrifolia* on the far bottom right and a mixed intermediate, the composition of which is determined by its proximity to the species at either extreme. The majority of the remaining Asian species and the Chinese pair, *P. bretschneideri*, are central in this intermediate grouping, except for *P. syriaca*, which is nested in the *P. communis* cloud.

The software *NTSYS* was used to construct a dendrogram based on the codominant dataset (*figure 3*). More reliable estimates of genetic relationships can be sought from codominant data, compared to binary (data not shown). Two very distinct (major) clusters are apparent.

The upper major clade is further subdivided into two; the upper part of this consisting of predominantly of *P. pyrifolia* with few Interspecific hybrids (clumped together, but polyphyletic) and the lower part containing the single, remaining *P. pyrifolia* (Shen L; potentially a mis - classified *P.x bretschneideri),* the only *P.x bretschneideri,* two Interspecific hybrids (*P. communis* x *P. bretschneideri* and BPM x *P. bretschneideri*) and five of the 6 Asian species. These Asian species sit in three branching pairs; *P. salicifolia* and a hybrid (*P. communis* x *P. bretschneideri*), *P. calleryana* and *P. pashia*, *P. x. complexa* and *P. fauriei.*

The lower major clade consists of all the *P. communis* individuals, three Interspecific hybrids (in a basal clade; some Asian parentage) and one Asian species, *P. syriaca.* Sub – structure within this large clade (ultimately monophyletic) may exist, however, PCA analysis of *P. communis* cultivars revealed no conspicuous trends.

Figure 2; PCA Analysis of Codominant data using Distance – Standardised method

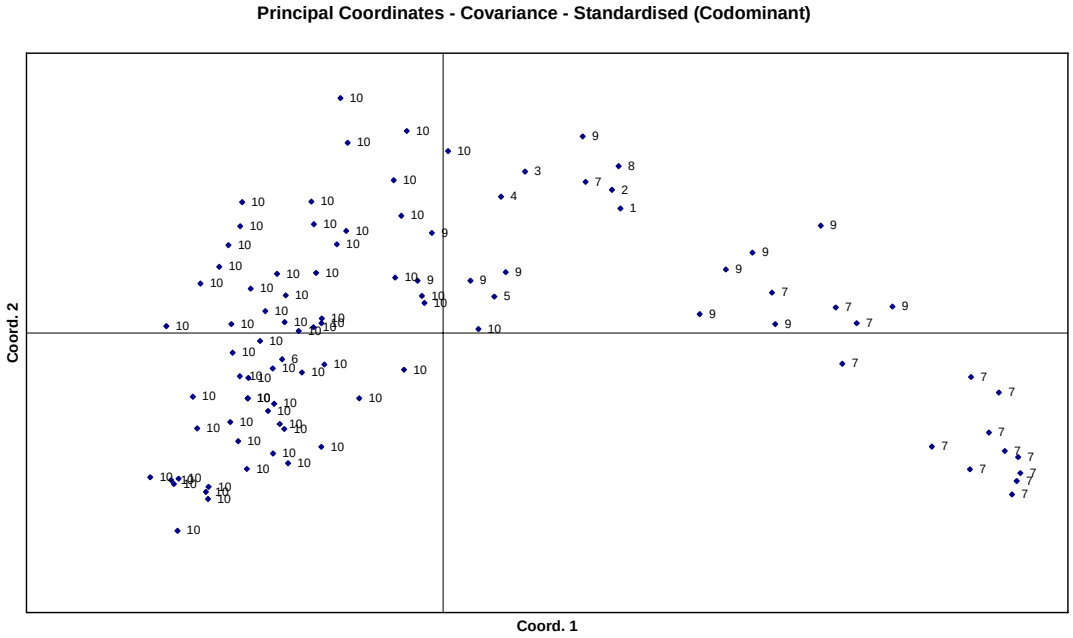

Figure 2; Distance – standardised PCA analysis, as performed by *GeneAlex*, on the codominant dataset. The 95 individual cultivars are labelled according to their species or hybrid grouping; 1 = *P. calleryana*, 2 = *P. pashia*, 3 = *P. x. complexa*, 4 = *P. fauriei*, 5 = *P. salicifolia*, 6 = *P. syriaca*, 7 = *P. pyrifolia*, 8 = *P.x bretschneideri*, 9 = Interspecific and 10 = *P. communis.*

Figure 3; Dendrogram showing the genetic relationships amongst 95 accessions of *Pyrus* Spp.

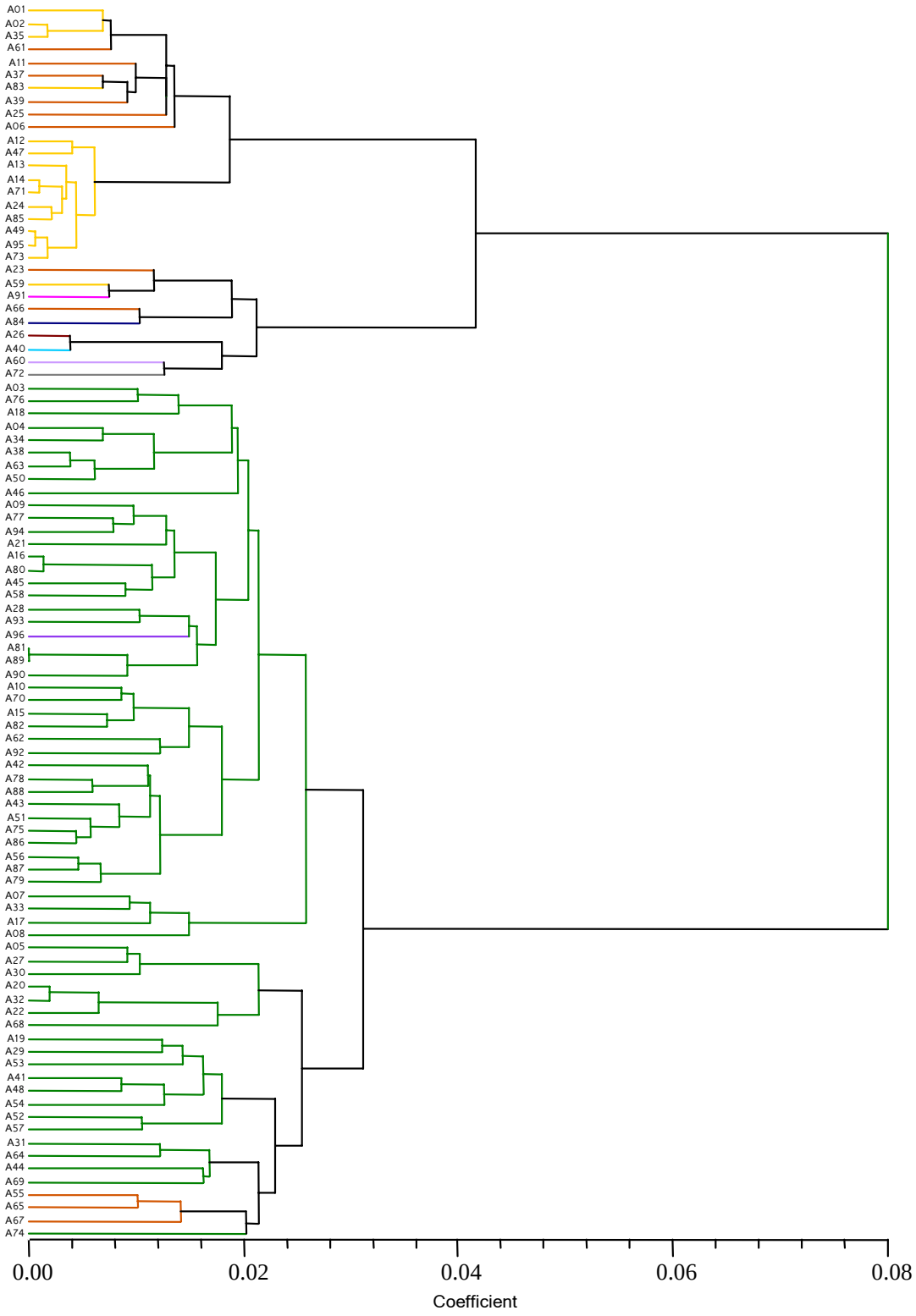

Figure 3; Dendrogram showing the genetic relationships amongst 95 accessions of *Pyrus*, encompassing 9 species and interspecific hybrids, based on codominant data from a suite of 13 SSR markers.

Discussion

Market and consumer preference research

The focus of the pear program is changing from initial identification of new selections to the evaluation of existing selections, and identification of elite selections with market potential. Market and consumer preference research is important to consider in identifying new pear varieties with potential for commercial success. In a USA survey (The Perishables Group, 2001) 26% of consumers planned to buy pears, 37% were impulse buyers and 37% were non pear buyers. Of the planned pear buyers 51% based their buying decision on flavour and 27% on appearance. These pear consumers tend to be older, conservative in their choice of pears, and reluctant to substitute their favourite pear for another. But are the future pear consumers who will purchase new varieties from the pear breeding program just the traditional pear consumer? To re-invigorate the pear industry in Australia new pear varieties that are distinctive from the current mainstream varieties are required to attract consumer interest in eating pears, and more importantly to attract new pear consumers to increase per capita consumption.

Promising market research from Australia reveals that 71% of pear consumers are willing to try other pears (Dignam, 2000). More than 50% of impulse buying is based on appearance, followed by price and advertising in store (The Perishables Group, 2001). The non pear buyers are also potential future pear consumers that can expand the pear market. But what type of pears are they likely to buy? Convenience foods are more common today and European pears have traditionally been viewed as messy to consume and difficult to judge when ripe and ready to eat. Pears tend to be consumed as a snack food (79%), rather than as a dessert, or included in salads (The Perishables Group, 2001). It is likely that different groups of consumers (i.e. traditional, impulse buyers, non-pear buyers and groups within these) may require different types of new pears.

There is value in having an appearance that is familiar and meets consumers pre-existing expectations of what a pear looks like such as a pyriform shape to encourage initial purchase (Gamble et al., 2006). Consumers have been found to prefer sweet and juicy pears within a range of flavours and textures from unripe “green/grassy” flavours to over-ripe fermented, sweet flavours (Jaeger et al.., 2003). Hence new varieties can have a range of flavours and textures but must be sweet and juicy and not too soft to ensure convenience to eat as a snack food. A distinct colour from traditional pears is desirable as commercial success in branding/trademarks often require a visual point of differentiation. Unique cosmetic appearance as well as unique flavour is ranked highly among retailer requirements for new fruits (Reid and Buisson, 2001). Our method of rejecting pears on fatal faults, particularly for flavour, fits well with consumer preference for a range of flavours and textures, but it is essential to get the ripening process right for the selections to provide consistent eating quality and match this with a desirable appearance.

Identification of elite selections

Seven distinct series of promising pears have been identified in the program that ripen across the harvest season. Series 1 are bi-coloured pears with an intermediate straight shape and flat base. This series of pears have a strong red blush on a dark green background colour and will be distinct from other pear varieties currently in the market but still display a typical pear shape. One of these series (F-11-82) has progressed to stage 3 large-scale commercial evaluation. This mid-season selection has a strong red blush on a green background with a smooth reasonably symmetrical shape. It develops a soft, juicy, texture with aromatic flavours similar to the variety Comice. It has the potential to handle and store long term and can offer consumers an alternative to the traditional variety Packham but with more attractive appearance and improved eating quality. A further earlier maturing selection from this series (F-01-88) is also showing great promise.

Series 2 are the Corella series of red-blushed pears principally from the Guyot x Corella family. They have a crisp juicy texture and some with a slight musk flavour. The early ripening selection C-31-42 has progressed to stage 3 large-scale commercial evaluation. It tree ripens in early January and has an attractive appearance of a uniform pyriform shape and pinkish-red blush on a yellow background. It would come onto the market before WBC and potentially open the pear season for consumers with a new appealing pear variety. The small pears in this series have the potential to appeal to children and have a niche in the lunchbox/snack market as they are small and firm and have a crisp juicy texture and very mild pear flavour that would be appealing to the majority of consumers. They would also be attractive as a fruit snack in lunch packs provided on airline flights. Feedback from informal tasting

sessions with the mid-season selection, G-32-17, have supported this potential marketing niche. The type of blush on selections in this series can vary from a strong red even blush to a lenticel type blush as in the variety Corella. The effect of rootstock and tree management on blush expression requires some investigation as the selections will need to preferably display a consistent level and type of blush.

The Rogue Red series 3 of pears consist of small full red pears and medium-large red blushed pears. The majority have a fine buttery texture and aromatic pear flavour with varying levels of grit. This series offers the potential to provide exceptional eating quality but the shape can be slightly variable and some tend to have the flesh extend right up the stem, particularly on the larger sized selections. The small red full red pears present a new unique looking pear in the market place with good eating quality. In the light pink-blushed series of pears, the majority have eating quality similar to the Rogue Red series but lack the strong red blush development and show blemishes more readily. Some are thin skinned so will also be more easily marked. However the unmarked fruit are extremely attractive in appearance and distinct with their light pink blush. Some selections in this series have a more round or "plum pudding" shape.

Series 4 are late season green pears with a fine buttery texture. Their appearance is not unique but they represent the traditional green pear but with an improved eating quality. As they're late season pears they can be harvested after the majority of other pear varieties and stored or put into the market as a late season freshly picked pear. Only a few inter-specific crosses were made, as breeding inter-specific hybrids is only a minor component of the program. However the inter-specific series of pears currently has two promising selections. In particular C-41-67 which tree ripens in March. It is a cross between the Chinese pear Ya Li and the European pear Comice. It has a crisp juicy texture and a sweet aromatic pear flavour unlike a typical nashi. It has very limited storage potential so would need to be directly marketed. It has a light yellow to cream skin colour with slight stem russet and can be prone to skin rub. Comments from informal tasting sessions of fruit eaten directly off the tree have been very positive.

The potential disease resistance series of pears encompass fire-blight and scab resistance. D-11-57 has a fire-blight resistant parent (Harrow Delight) so if selections such as this show potential for commercial release they should be tested for fire-blight resistance overseas, to realise their full commercial value. Studies on scab resistance within the project found that more than 60% of individuals in the Guyot x Corella family are scab susceptible due to a dominant gene, *I*, in Corella which suppresses the expression of resistant genes from Guyot (Liu et al. 2008). G-27-27 is one of the exceptions in the Guyot x Corella family and in testing displayed a high level of scab resistance in both the leaves and fruit. Overall Ya Li, Hood, BPM and Guyot had the more scab resistant genotypes and as such, families of Guyot x Hood and BPM x Ya Li, had up to 96% of progeny resistant to scab. Only 2 to 3 selections have been identified in these particular families and none of these selections are within the top 20% of selections listed in appendix 1. Under the disease resistant model proposed by Liu et al (2008) the segregation ratio of the other major families of which we have selections is 5 R : 11 S in the family of Guyot x Corella, and 1 R : 1 S in the family of Guyot x Rogue Red, where R encompasses progeny from slight to high scab resistance. However it is likely that several generations of backcrossing or top-crossing may be required to obtain varieties with both desirable fruit quality and high disease resistance as there is more than one major dominant gene and possibly other minor genes involved in scab resistance (Liu et al. 2008).

Commercialisation strategy

APAL is forming a commercial company, similar to Prevar™ in New Zealand, to provide variety management and commercialisation services for new apple and pear varieties in Australia. Through this new entity, APAL have developed a draft variety management proposal for a pipeline of new pear selections from the current national pear breeding program. The APAL variety manager and commercialisation model is based upon large-scale evaluation nationally, co-ordinated local and overseas testing and marketing with measures to ensure Australian growers attain first access to variety benefits, marketing models developed and implemented on a variety potential basis, a strong focus of branding an intellectual property (PBR and trade-marking), and controlled licensing and management of the supply chain. DPI, through AVS, will oversee the commercialisation process and interface with the new APAL entity on behalf of all stakeholders, should APAL be appointed via an exclusive evaluation and commercial licence option agreement.

Genetic diversity study

Overall, 95 pear accessions, including 36 European and 16 Asian cultivars, 31 numbered selections from the Australian Pear Breeding Program, 6 interspecific hybrids and 6 naturally occurring species were genotyped across a suite of 13 codominant SSR markers. The markers in this study appear adequately polymorphic to differentiate between all cultivars, with an average of 14.2 alleles per marker. This emphasises the efficiency of using SSR's in cultivar identification and diversity analysis.

Whilst numerous markers derived from other species within *Pomoideae* (e.g. *Malus*), for example, or *Rosaceae* (e.g. *Prunus*) may be transferable and informative, not all markers used were suitable for data analysis. Uunique differences in the genome structure of *Pyrus* means discrepancies are inevitable and transferability is not complete (Yamamoto *et al.* 2002a; Hemmat *et al.* 2003). The *Pyrus* genome, for example, constitutes approximately 2/3 of the nDNA content of the *Malus* genome (Yamamoto *et al.* 2002a).

The genetic relationships amongst the 95 accessions depicted by both PCA and NTSYS analyses are consistent with pairwise Fst values generated by both *Arlequin* and *GeneAlex* and simulations performed by *Structure*. The subdivision of the sample population into two clades is clearly apparent; European pear (*P. communis*) cultivars form a monophyletic clade and are significantly distinct from the Japanese (*P. pyrifolia*) and all other Asian species (except *P. syriaca)* (supported by Oliveira *et al.* 1999; Yamamoto *et al.* 2002b; Ghosh *et al.* 2006).

The average Fst values, calculated by both Arlequin and GeneAlex, indicate there is substantial structure amongst these Asian species. This is reflected in the dendrogram, as 6 of the Asian species form distinct branches in a sub – cluster from the remaining *P. pyrifolia* cultivars. Only *P. syriaca* appears to be nested amongst the *P. communis* clade. In fact, this underlying structure is so strong that it gives greater values of divergence than the division of the European cluster. However, it must be appreciated that each of the Asian species, except *P. pyrifolia*, is represented by a single individual and may bias results and explain why some of the better represented populations (e.g. *P. communis*, *P. pyrifolia*) are not necessarily the most divergent.

This study highlights there is wealth of diversity between species and amongst cultivars, whilst relationships generally conform to the geographic proximity of cultivars or their parents. The greater *within* population variation, relative to *among* population variation, conforms to what is typical for any outcrossing species, such as *Pyrus.* Whilst it also highlights the population structure, the boundaries are blurred and diversity is overlapping. This resolution of the genetic relationships has application in improving pear breeding (Ghosh *et al.* 2006).

Further resolution amongst *Pyrus* species and within the European clade requires analysis of more germplasm and greater, more balanced representation of species. European and American cultivars tend to be better characterised, which may bias attention and the sources from which markers are derived (Hemmat *et al.* 2003). Exploration for further SSR markers will also improve the resolution between cultivars and help pyramid major QTL with linked loci, for application in MAS. Ultimately breeding programs aim to maintain good genetic diversity within their breeding germplasm to enable greater genetic gain in traits of economic importance.

Future research

In the longer term DPI will be exiting from investment in cultivar development as the lead organisation of the Australian Pear Breeding Program to invest in the upstream activities of biotechnology and germplasm enhancement. The Australian pear industry has the potential to develop its own business models to continue breeding/cultivar development activities into the future. Hence over the next few years the pear breeding program will focus on the development of existing selections to a commercial product to provide Australian consumers with a new and appealing range of pear varieties. The commercial pipeline should provide feedback on the most suited selections for release. Ultimately the program only requires a few successful new varieties to make an impact on the pear industry. But these new varieties will need a quantum step change in appearance and flavour to be able to expand the pear market with new consumers (non-pear buyers) that are looking for a new exciting, novel fruit to try.

Technology Transfer

A presentation was given to the FGV young growers group in the Goulburn Valley in August 2006. In April 2007 the Northern Victoria APFIP assessment group viewed and tasted some of the superior pear selections from the breeding program. Presentations were given to the APAL Pear Advisory Group in June 2006 and July 2007. An update on pear breeding program was presented to SA growers by Angie Grills at the Intensive Pear field day in SA in August 2007. A similar report to Victorian growers was cancelled due to insufficient numbers attending the field day. Shiming Liu presented a paper at the 2007 Annual Conference of the International Fruit Tree Association in Hobart Tasmania on 4th February 2007 entitled: Breeding new pear varieties to strengthen the local industry in Australia. Fruit assessment sessions were held with fruit growers in a pear workshop at the annual AFCO Conference in May 2008.

These activities focus on communicating progress rather than the transfer of new technologies *per se*, as no selections have yet reached the commercialisation stage. Two elite selections have progressed to third stage large-scale commercial trials through APFIP and will be propagated in 2008. The program is expecting to release new varieties from 2013 onwards.

Recommendations

Two selections progressed to advanced large-scale commercial trials through APFIP on fruit grower properties in 2008. One selection ripens early January and can be tree ripened with a short storage potential. The other selections ripens mid-season and has the potential to handle and store long term similar to Packham.

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Ross Wall (part-time 2007 onwards)	Mick Jordan (2004-2007)
	Kelvin Cornwall (part-time 2008 onwards)

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Stuart Gray (APAL)	Andrew McNab (N. Vic pear grower)
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APAL	Apple and pear Australia Limited
HAL	Horticultural Australia Limited
APFIP	Australian Pome Fruit Improvement Program
FGV	Fruit Growers Victoria
DPI	Department of Primary Industries, Cobram
AVS	Agriculture Victoria Services, DPI
DAFWA	Department of Agriculture and Fisheries Western Australia
RS	Rural Solutions, South Australia

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Appendix 1: Harvest data measured on top 50 selections after 10 weeks cool air storage at 1°C, pre-ripening 2008. * Fruit taken from original tree in primary selection block.

Selection	Female	Male	Harvest range	Weight (g)	Height (mm)	Width (mm)	Firmness (Kg)
C-31-42	BPM	Corella	11-25 Jan	150-200	75-85	60-70	4.5-8
F-01-88	Comice	BPM	18 Jan-13 Feb	200-300	80	65-75	6.5
F-20-05	BPM	Comice	21 Jan-8 Feb	150-300	80-90	60-50	5-6
F-02-70	Comice	BPM	21 Jan-12 Mar	100-250	60-80	55-75	3-5
F-49-98	Guyot	Corella	25 Jan-14 Feb	150-250	90-100	65-75	3-7
D-11-57	Harrow Delight	Packham	25 Jan-22 Feb	100-200	80-90	55-65	5-6
F-05-36	WBC	Howell	25 Jan	150-250	75-85	65-75	7.5-9.5
F-11-82	Corella	Comice	30 Jan-21 Mar	150-250	75-85	65-75	5.5-7.5
C-41-67	Comice	Ya Li	30 Jan-26 Mar	150-250	80-90	60-70	3-4
K-21-78*	Guyot	Rogue Red	4-15 Feb	100-150	70-75	55-65	5-6
I-08-69*	Guyot	Rogue Red	4-27 Feb	75-125	65-70	55-60	3-5
I-21-19*	Guyot	Rogue Red	5-14 Feb	50-100	50-60	50-60	3
G-23-24*	Guyot	Corella	6-15 Feb	50-100	60-70	45-55	3-4
I-19-47	Guyot	Rogue Red	6-15 Feb	100-500	60-110	60-100	5-7
G-20-54*	Guyot	Corella	6-22 Feb	150-250	65-85	65-75	4-5
G-24-31	Guyot	Corella	6-27 Feb	150-200	75-85	60-70	3-5
G-21-61*	Guyot	Corella	6 Feb-1 Mar	100-150	60-70	55-65	5-6
G-30-57*	Guyot	Corella	6 Feb-1 Mar	50-100	65	50	6
G-32-17*	Guyot	Corella	6 Feb-1 Mar	50-100	60-70	50-60	5-6
I-09-29*	Guyot	Rogue Red	7-15 Feb	50-150	55-65	50-65	5.5-7.5
I-15-48*	Guyot	Rogue Red	7-15 Feb	100-200	70-80	60-70	4-6
K-19-77*	Guyot	Rogue Red	7-15 Feb	200-300	95-105	65-75	5-6
K-22-31	Guyot	Rogue Red	7-27 Feb	NA	NA	NA	NA
I-14-09	Guyot	Rogue Red	7 Feb-21 Mar	150-250	75-85	65-75	3-5
G-20-09	Guyot	Corella	8-15 Feb	NA	NA	NA	NA
K-21-05*	Guyot	Rogue Red	8-15 Feb	100-150	65-75	60-70	4-5
K-23-55*	Guyot	Rogue Red	8-15 Feb	175-225	80-85	70-75	4-5
I-38-18*	Rogue Red	Josephine	8 Feb-1 Mar	75-125	60-70	50-60	3-5
K-19-20*	Rogue Red	Guyot	8 Feb-4 Mar	100-150	60-70	60-70	5-6
I-48-85*	Rogue Red	Guyot	8 Feb-12 Mar	100-150	65-75	60-70	2-5
F-12-45	Guyot	Comice	8 Feb-16 Mar	150-250	70-80	65-75	4-5
I-38-76*	Rogue Red	Josephine	9 Feb-4 Mar	50-100	50-55	50-55	3-4
G-27-27	Guyot	Corella	13 Feb-1 Mar	150-200	85-95	55-60	6-7
H-51-59*	Guyot	Corella	15-20 Feb	150-200	65-75	60-70	3-4
F-49-56	Guyot	Corella	19 Feb-8 Mar	150-400	90-110	65-90	3-5
F-50-46	Guyot	Corella	20-Feb-5 Mar	150-250	75-85	65-75	5-6
F-18-71	Comice	Howell	20-Feb-21 Mar	150-250	65-80	65-75	5-6
K-19-21*	Rogue Red	Guyot	22-Feb-1 Mar	50-150	55-65	55-65	5.5-9
ANP07-27	20th Century	op	24 Feb	150-250	75-85	65-75	4-5
F-46-45	Guyot	Corella	3-12 Mar	200-300	80-90	70-80	3.5-6.5
F-49-86	Guyot	Corella	4-12 Mar	150-250	75-85	70-80	5-6
G-09-36	Packham	Comice	4-13 Mar	150-300	55-95	55-85	5.5
C-22-18	BPM	Corella	4-Mar	150-250	75-85	70-80	3-4
F-44-60	Guyot	Corella	5-21 Mar	250-350	85-95	75-85	4.5-6.5
F-46-90	Guyot	Corella	5-Mar	150-250	80-90	65-75	3-7
C-26-10	BPM	Corella	6-Mar	100-200	60-75	60-70	4.5-5.5
A-27-26	Comice	Howell	7-Mar	200-300	80-90	70-80	6-7
E-24-28	Comice	Josephine	15-Mar	NA	NA	NA	NA
C-16-21	Packham	Corella	19-Mar	100-200	80-90	60-70	4-6
H-47-68	Eldorado	Rogue Red	5-Apr	NA	NA	NA	NA

Appendix 2.

Table 1: *Cultivar and Specific name of all 96* accessions of *Pyrus analysed in this study, in addition to their individual positions in the ABI and Liz plates.*

Pear accession	*Code*	*Species*	*ABI Lane*	*Plate Well*
P._calleryana	A26	*Asiatic species*	12	C2
P._pashia	A40	*Asiatic species*	26	D4
P._x_complexa	A60	*Asiatic species*	88	E12
P._fauriei	A72	*Asiatic species*	86	F12
P._salicifolia	A84	*Asiatic species*	84	G12
P._syriaca	A96	*Asiatic species*	82	H12
FLA_58-45OP	A06	*Interspecific*	48	A6
D-54-15	A11	*Interspecific*	95	A11
D-42-63	A23	*Interspecific*	93	B11
Hwa_Hong	A25	*Interspecific*	11	C1
Dan-bae	A37	*Interspecific*	9	D1
H-14-69	A39	*Interspecific*	25	D3
Flordahome	A55	*Interspecific*	55	E7
Bong_Ri	A61	*Interspecific*	5	F1
Hood	A65	*Interspecific*	37	F5
K-6-2	A66	*Interspecific*	38	F6
FLA_58-45	A67	*Interspecific*	53	F7
Ya_Li	A91	*P. bretschneideri*	49	H7
G-27-27	A15	*P. communis*	29	B3
Butirra_Rosata_Morettini	A16	*P. communis*	30	B4
E-22-58	A50	*P. communis*	8	E2
H-40-55	A51	*P. communis*	23	E3
Yellow_Huffcap	A52	*P. communis*	24	E4
Green_Madeline	A53	*P. communis*	39	E5
C-31-42	A54	*P. communis*	40	E6
E-37-5	A62	*P. communis*	6	F2
I-38-76	A63	*P. communis*	21	F3
Gin	A64	*P. communis*	22	F4
F-49-86	A70	*P. communis*	70	F10
F-10-58	A74	*P. communis*	4	G2
I-48-54	A75	*P. communis*	19	G3
Passe_Crassane	A76	*P. communis*	20	G4
F-1-88	A94	*P. communis*	66	H10
F-27-48	A03	*P. communis*	31	A3
Josephine_de_Malines	A04	*P. communis*	32	A4
Forelle	A05	*P. communis*	47	A5
Red_Sensation	A07	*P. communis*	63	A7
Corella	A08	*P. communis*	64	A8
Lemon_Bergamot	A09	*P. communis*	79	A9
D-11-57	A10	*P. communis*	80	A10
Aurora	A17	*P. communis*	45	B5
B-14-40	A18	*P. communis*	46	B6
Red_Face	A19	*P. communis*	61	B7
Vicar_of_Winkfield	A20	*P. communis*	62	B8
Howell	A21	*P. communis*	77	B9
F-22-64	A22	*P. communis*	78	B10

Pear accession	***Code***	***Species***	***ABI Lane***	***Plate Well***
H-11-35	A27	*P. communis*	27	C3
Beurre_Bosc	A28	*P. communis*	28	C4
Precoce_de_Fiorani	A29	*P. communis*	43	C5
F-11-82	A30	*P. communis*	44	C6
Pound	A31	*P. communis*	59	C7
Winter_Cole	A32	*P. communis*	60	C8
Coboy_Packhams	A33	*P. communis*	75	C9
F-1-103	A34	*P. communis*	76	C10
E-22-34	A38	*P. communis*	10	D2
June_de_Coloures	A41	*P. communis*	41	D5
E-42-98	A42	*P. communis*	42	D6
Eldorado	A43	*P. communis*	57	D7
LInconnue	A44	*P. communis*	58	D8
Clapps_Favourite	A45	*P. communis*	73	D9
F-55-78	A46	*P. communis*	74	D10
Morcroft	A48	*P. communis*	90	D12
Rogue_Red	A56	*P. communis*	56	E8
Beurre_dAnjou	A57	*P. communis*	71	E9
E-4-33	A58	*P. communis*	72	E10
Dawn	A68	*P. communis*	54	F8
Conference	A69	*P. communis*	69	F9
Harrow_Delight	A77	*P. communis*	35	G5
K-19-46	A78	*P. communis*	36	G6
Doyenne_du_Comice	A79	*P. communis*	51	G7
Butirra_Precoce_Morettini	A80	*P. communis*	52	G8
Packham_-_Russett	A81	*P. communis*	67	G9
F-49-98	A82	*P. communis*	68	G10
F-12-45	A86	*P. communis*	2	H2
I-51-13	A87	*P. communis*	17	H3
Williams_Bon_Chretien	A88	*P. communis*	18	H4
Packhams_Triumph	A89	*P. communis*	33	H5
K-23-22	A90	*P. communis*	34	H6
Dr_Julies_Guyot	A92	*P. communis*	50	H8
Beurre_Hardy	A93	*P. communis*	65	H9
Shinsetsu	A01	*P. pyrifolia*	15	A1
Okusankichi	A02	*P. pyrifolia*	16	A2
Nijisseiki	A12	*P. pyrifolia*	96	A12
Hakko	A13	*P. pyrifolia*	13	B1
Kikusui	A14	*P. pyrifolia*	14	B2
Shinseiki	A24	*P. pyrifolia*	94	B12
Hosui	A35	*P. pyrifolia*	91	C11
Chojuro	A47	*P. pyrifolia*	89	D11
Shin_Soo	A49	*P. pyrifolia*	7	E1
Shen_Li	A59	*P. pyrifolia*	87	E11
Kosui	A71	*P. pyrifolia*	85	F11
Heang_Soo	A73	*P. pyrifolia*	3	G1
Niitaka	A83	*P. pyrifolia*	83	G11
Yakumo	A85	*P. pyrifolia*	1	H1
Shinsui	A95	*P. pyrifolia*	81	H11

Table 2; *Review of the 15 SSR Microsatellite markers used in this study and details of their PCR conditions.*

Pool	SSR marker	Primer Sequence 5' - 3'	Repeat Motif	Size Range (bp)	PCR Program	Fluorochrome Dye	Origin/ Literature
1	NH015a	**F** TTGTGCCCTTTTTCCTACC	$(AG)_{19}$	95-127	Touch 2	FAM	(Volk et al. 2006; Yamamoto et al. 2002b)
		R CTTTGATGTTACCCCTTGCTG					
	BGT23b	**F** CACATTCAAAGATTAAGAT	$(TC)_{18.5}$	203	Touch 2	HEX	(Yamamoto et al. 2002a)
		R ACTCAGCCTTTTTTTCCCAC					
	NB109a	**F** ATGCTCTATAAAACCCACCTACC	$(AG)_{18}$	140-375	Touch 2	TAMRA	(Ghosh et al. 2006; Yamamoto et al. 2002b)
		R AGAGGGACCATTGTGTTGTTATAT					
2	KA16	**F** GCCAGCGAACTCAAATCT	$(CT)_2T(TC)_{17}$	137	Touch 2	FAM	(Yamamoto et al. 2002a; Yamamoto et al. 2002b)
		R AACGAGAACGACGAGCG					
	NH029a	**F** GAAGAAAACCAGAGCAGGGCA	$(AG)_8$	87-382	Touch 2	HEX	(Ghosh et al. 2006; Yamamoto et al. 2002b)
		R CCTCCCGTCTCCCACCATATTAG					
	NH025a	**F** CTGGACACAAACATTCAAGAGGG	$(AG)_{21}$, $(AG)_4$	78-387	Touch 2	TAMRA	(Ghosh et al. 2006; Yamamoto et al. 2002b)
		R CACACCAGAAACTCCAAAACAGG					
3	BGA35	**F** AGAGGGAGAAAGGCGATT	$(AG)_8$	124	Touch 2	FAM	(Yamamoto et al. 2002a)
		R GCTTCATCACCGTCTGCT					
	KA14	**F** TCATTGTAGCATTTTTATTTTT	$(CA)_5G(AC)_2G(CA)_5$	180	Touch 2	HEX	(Yamamoto et al. 2002a; Yamamoto et al. 2002b)
		R AGTGCAAGGGAGATTATTAG					
	NH001c	**F** AATACTAATCCTTTTTGCTAA	$(GA)_{21}$	103-403	Touch 2	TAMRA	(Yamamoto et al. 2002b; Ghosh et al. 2006)
		R TCCATTCAATCTGTCTCGGTC					
4	CH01H01	**F** GAAAGACTTGCAGTGGGAGA		97-123	Touch 1	FAM	(Volk et al. 2006; Liebhard et al. 2002; Gianfranceschi et al. 1998; Yamamoto et al. 2002b)
		R GGAGTGGGTTTGAGAAGGTT					
	CH02D12	**F** ACCCAGATTTGCTTGCCATC	$(AG)_{21}$	213-255	Touch 1	HEX	(Volk et al. 2006; Liebhard et al. 2002; Gianfranceschi et al. 1998)
		R GCTGGTGGTAAACGTGGTG					
	NZ05g8	**F** CGGCCATCGATTATCTTACTCTT	$(GA)_{18}$	102-345	Touch 2	TAMRA	(Ghosh et al. 2006; Guilford et al. 1997; Yamamoto et al. 2002b)
		R GGATCAATGCACTGAAATAAACG					
5	GD147	**F** TCCCGCCATTTCTCTGC		120-148	Touch 2	FAM	(Hemmat et al. 2003; Volk et al. 2006)
		R AAACCGCTGCTGCTGAAC					
	PS12A02	**F** GCCACCAATGGTTCTTCC		162-380	Touch 2	HEX	(Ghosh et al. 2006; Gianfranceschi et al. 1998; Yamamoto et al. 2002b)
		R AGCACCAGATGCACCTGA					
	CH01D08	**F** CTCCGCCGCTATAACACTTC		226-300	Touch 1	TAMRA	(Volk et al. 2006; Liebhard et al. 2002; Gianfranceschi et al. 1998)1335;1377;1380
		R TACTCTGGAGGGTATGTCAAAG					

Table 3; *Number of putative alleles for 13 SSR markers identified for Pyrus accessions*

SSR Name	Number of Alleles										Total	%
	P. calleryana	*P. pashia*	*P. x. complexa*	*P. fauriei*	*P. salicifolia*	*P. syriaca*	*Interspecific*	*P. bretschneideri*	*P. pyrifolia*	*P. communis*		
NH015a	2	1	1	1	2	2	10	2	4	16	20	
BGT23b	2	1	1	2	1	2	9	2	5	9	16	
NB109a	2	1	1	2	1	1	5	0	2	14	19	
KA16	2	2	1	2	1	2	8	2	5	13	16	
NH029a	2	1	1	2	1	2	5	2	3	10	10	
NH025a	1	1	1	2	1	1	8	2	5	8	14	
BGA35	2	1	2	1	1	1	7	2	6	6	9	
KA14	2	1	2	2	2	2	3	2	2	6	10	
NH001c	1	2	1	1	2	1	7	1	5	13	17	
CH02D12	1	2	1	2	1	2	5	1	8	10	15	
NZ05g8	1	1	2	1	1	2	7	1	5	9	12	
GD147	2	1	2	1	1	2	8	1	6	9	12	
CH01D08	1	1	2	2	1	2	5	2	3	11	15	
Total	21	16	18	21	16	22	87	20	59	134	**185**	
%	11.35	8.6	5.4	11.35	5.4	11.89	47.03	10.81	31.89	72.43		

Table 3; The putative alleles identified and scored for 13 SSR markers applied to species of the genus *Pyrus.* Numbers of individuals of each species as follows; *P. calleryana* (1), *P. pashia* (1), *P. x. complexa* (1), *P. fauriei* (1), *P. salicifolia* (1), *P. syriaca* (1), Interspecific (11), *P. bretschneideri* (1), *P. pyrifolia* (15), *P. communis* (62). The total row at the base of the table presents the sum of the column above it. NB; the overall total number of alleles (185) does not equal the sum of the alleles present in each species (414) due to the overlap of alleles expressed between species.

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