

THE WINSTON CHURCHILL MEMORIAL TRUST OF AUSTRALIA

Report by - Andrew Granger – 2003 Churchill Fellow

To investigate the genetic background of sweet cherry

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Introduction

This report details the findings from a 2003 Churchill Fellowship visit to England and Japan to investigate the genetic background of sweet cherry. Specific areas of study included;

- Laboratory based methods for the determination of self-incompatibility genotypes (note cherries cannot pollinate themselves and require cross pollination with other cultivars to set fruit, that is they are self-incompatible). These methods can also be used for phylogeny studies to trace the ancestry of cherry cultivars.
- Germplasm repositories and cultivar collections as a source of biodiversity.
- Cherry breeding programs for both cultivars and rootstocks.

Acknowledgements

To the Winston Churchill Trust for both financial support and its good name which helped open many doors.

To Dr Ken Tobutt and Dr Encarna Ortega, Breeding and Genetics Department, East Malling, Kent, UK for their fellowship and sharing of knowledge, facilities and plant materials. And to all the other scientists that welcomed me to East Malling.

I must also thank Dr Ryutaro Tao, Kyoto University, Japan, for enlightening me with his latest discoveries regarding self-incompatibility research in cherry. Also Dr Masami Yamaguchi, National Institute of Fruit Tree Science, Tsukuba, Japan for organising my itinerary and hosting me in Japan. And to Dr Ishiguro, Yamagata Prefecture, for hosting my visit there.

I thank the Department of Primary Industries and Resources, South Australia through the South Australian Research and Development Institute in supporting me during the Churchill Fellowship.

Last but not least I thank my three referees Mr Bill Bishop, Dr Phillip Ainsley and Ms Jennifer Witherspoon for their support.

Executive Summary

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Project Description

The overall objective was to gain a better understanding of the genetic background of cherries through the use and interpretation of molecular techniques in England and Japan.

Highlights

1. East Malling Research. Long considered the number one horticultural research institute in the world. The Breeding and Genetics Department headed by Ken Tobutt has a major program in cherry breeding and genetics of the self-incompatibility locus (S-gene). Here I spent 4 weeks under the guidance of Dr Encarna Ortega learning laboratory techniques to determine the S-alleles of cherry based on DNA sequences established by the group around the first and second intron of the S-locus and also allele specific primers. Many of the techniques are cutting edge and in the process of being published so it was a very valuable transfer of knowledge by Drs Tobutt and Ortega.
2. Royal Botanical Gardens, Kew. Searched for specimens of *Prunus avium* in the Rosaceous section of the Herbarium and examined accessions of other cherry species for comparison. I was also provided with a map of cherry accessions in the living collection and spent a day locating and examining each tree. Thanks to Dr Nigel Taylor and Dr Aaron Davis for facilitating my visit.
3. Kyoto University, Laboratory of Pomology. Dr Ryutaro Tao provided a tour around molecular biology laboratory facilities, field and glasshouse plantings. Extensive discussions on recent findings regarding self-incompatibility and self-fertility were a revelation for me in the understanding of the function of the self-incompatibility locus. I also provided a seminar regarding my research activities in cherry breeding.
4. Yamagata Prefectural Horticultural Experiment Station. Dr Ishiguro and Dr Yamaguchi conducted visits to breeding plots on the Experiment Station and to grower properties. The breeding program has been successful having released 4 new white fleshed cultivars (white rather than red fleshed cherries are preferred in Japan). Yamagata prefecture accounts for 70% of Japan's cherry production with the majority of trees grown under plastic covers to protect against rainfall that causes fruit to crack.
5. National Institute of Fruit Tree Science, Tsukuba. Dr Masami Yamaguchi guided me on inspections of stonefruit, nashi (Japanese pear) and chestnut breeding programs. Projects included tissue culture, genome analysis, molecular markers and conventional breeding.

Lessons and conclusions

I have made contacts with leaders in the field of breeding and genetics of cherries and the fellowship with these scientists should continue into the future because they expressed a keen desire for on going collaboration. I have also acquired a working knowledge of cutting edge DNA techniques used to determine the S-alleles of cherry. These techniques can be applied to the Australian cherry breeding program immediately. In this way the lessons learnt during the fellowship will be disseminated to the Australian community through new and improved cherry cultivars.

I have also learnt that, in terms of cherry research, Australia's strength is the size of our seedling populations, which on a world scale are large, compared to other programs and this is because land is comparatively cheap and readily available in Australia. The cherry program is weaker in the area of molecular biology but this can be strengthened by collaboration with scientists in England and Japan.

Cherry cultivars and species will be imported from both England and Japan and cultivars and breeding lines from the Australian breeding programme will be sent to both countries.

The results of my fellowship will also be disseminated through scientific papers, reports and conferences. For example I have spoken at the Cherry Growers of Australia annual conference in July 2004. And in collaboration with Dr K. Tobutt and Dr E. Ortega it is intended to present a paper at the 2005 International Cherry Symposium regarding S-alleles in Australian cultivars and the discovery of a new combination of alleles, which constitute a new pollination group.

Research priorities identified during the fellowship include further investigation to determine the mechanism of self-incompatibility and the adoption of molecular markers. Studies to confirm the parentage of Australian cultivars. And analysis of the inheritance of resistance to black cherry aphid, in collaboration with East Malling.

Programme

Date	Travel details	Contact Person
27/4/04	Adelaide - London	
28/4/04-18/5/04	Laboratory work, East Malling Research	Dr Ken Tobutt
19/5/04	Royal Botanic Gardens, Kew, Herbarium	Dr Aaron Davis
20/5/04-21/5/04	Royal Botanic Gardens, Kew, Living Collection	Dr Nigel Taylor
23/5/04-24/5/04	London-Tokyo-Kyoto	
25/5/04	Kyoto University, Kyoto, Pomology Laboratory.	Dr Ryutaro Tao
26/5/04	Travel to Yamagata Prefecture	
27/5/04	Yamagata Prefecture Horticultural Experiment Station. Travel from Yamagata to Tsukuba	Dr Masami Yamaguchi and Dr Ishiguro
28/5/04	National Institute of Fruit Tree Science	Dr Masami Yamaguchi
31/5/04-1/6/04	Tokyo-Adelaide	

MAIN BODY

Introduction

The Australian cherry industry has expanded plantings exponentially over the past ten years in response to unsatisfied demand in both domestic and export markets. The Australian industry currently grows cultivars with three characteristics that significantly restrain the production of large quantities of high quality fruit for overseas markets. Fruit is susceptible to cracking after rainfall, fruit is generally small and many cultivars are self-incompatible requiring other specific cross pollinating cultivars for good fruit set.

Chile is Australia's main competitor for supply of cherries to South East Asia and UK/Europe. Labor accounts for 70-90% of the production cost of cherries and the cost of labor in Australia is five times that in Chile. The problem for Australian growers is that this translates to a higher priced product. Overseas buyers then expect a higher quality product. Large, well presented fruit with consistent supply usually commands a higher price on most markets.

The breeding program aims to incorporate crack resistance, large fruit size and self-fertility for consistent cropping into varieties with a range of harvest dates. A small component of the breeding project includes the development of dwarfing rootstocks because smaller trees require less labor inputs and will help to underpin lower production costs for Australian growers.

The Australian cherry breeding program is the only one of its kind in the Southern Hemisphere. Cherries are exotic to Australia and plant material must be imported from the Northern Hemisphere, consequently the breeding program operates with a small and restricted gene pool. There is little understanding of the inheritance of genetic traits in cherry or of its origin although the use of DNA based techniques in England and Japan to study the self-incompatibility locus (S-gene), in particular, has made large gains in the past 10 years. Compared to annual species woody, perennials such as cherry have proved difficult for DNA extraction and the development of different DNA extraction techniques has allowed these investigations to go forward.

As mentioned previously cherries are self-incompatible that is they are unable to pollinate themselves or cross pollinate with other cultivars that carry the same S-alleles. There are exceptions to the rule in that self-fertile mutants do exist and they have been used extensively in breeding programmes to produce self-fertile cultivars. It is important to identify the S-alleles of new cultivars and in particular determine if they are self-fertile or not to assist growers in planning their orchard plantings. At present most cherry breeders test self-fertility by bagging flowers in the field and recording fruit set this is backed up with microscopic examination of pollen tube growth to ascertain if compatible or incompatible pollen tube growth occurred. In the UK and Japan DNA based methods are used to determine the S-alleles of cherries. The DNA based methods had been tried in Australia with mixed results, probably due to differences in extraction techniques. The identification of alleles also provides a means of tracing parentage if it is unknown or in question. The S-alleles occur across a large number of species within and outside of Prunus, they are highly conserved and provide a tool for phylogenetic work. This will help answer questions such as what came first the plum or the cherry? How

closely related are the various *Prunus* species (cherry, plum, peach, almond etc) and are they reproductively compatible for the production of new hybrids?

The main aims of the Fellowship were to acquire knowledge of successful DNA extraction techniques and PCR based methods for the determination of S-alleles in cherry.

To observe breeding programmes and germplasm collections and assess genetic material for potential use within the Australian breeding programme. Also to compare and contrast techniques between the Australian and overseas breeding programmes.

To become familiar with the taxonomy and origin of species by examining herbarium and living collections at Royal Botanic Gardens, Kew.

To gain a greater understanding of the mechanism of self-incompatibility and the genes controlling it.

And in general to gain an appreciation of the socio-economic environment that science is practiced under in UK and Japan.

East Malling Research, DNA techniques, East Malling, England.

Plant Material:

Fresh leaves were sampled from the cherry collection at East Malling. Cultivars included Sir Tom, Sir Don, Dame Roma, FB1327 and FB113, bred in South Australia and being evaluated at East Malling. Dormant budwood was harvested at Lenswood and mailed to East Malling as a source of buds. Extracts of a range of cherry cultivars grown at Lenswood, including the abovementioned and 24 of their siblings, were made in the Spring of 2003 using the DNeasy® Plant Mini Kit (Qiagen) stored at -80°C and mailed to East Malling in May 2004. Young, emerging leaves were sampled from shoot tips for these extractions.

Fresh fully expanded leaves were sampled at East Malling and a disc was cut from each leaf by closing the lid of a 2 ml Eppendorf tube over the blade of the leaf while avoiding the midrib. Buds were sampled by removing them from their subtending branch and the bud scales peeled away to reveal green leaf tissue. Extraction of unopened buds has never been used in Australia for cherry and offers a new way of sampling cherries out of the growing season. Note that none of the material was weighed; if buds were visually determined to be small two were used instead of one. Similarly leaf discs were not weighed this provided an advantage in that plant tissue could be placed immediately into liquid nitrogen and thereby limit the exposure of samples to oxidative processes that produce contaminants and reduce the quality of DNA extracted.

Extraction procedure:

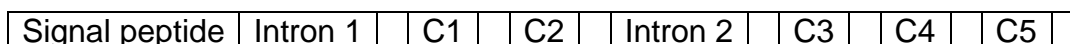
The East Malling laboratory had a stepped approach when deciding what extraction procedure to use for a particular species. The starting point was to use the CTAB DNA mini prep (Doyle and Doyle, 1987) if this didn't work a modified CTAB method was used which involved the addition of 2% polyvinyl

pyrillidone (PVP-40) and 1% (final volume) β -mercaptoethanol. If further improvement was required a modified version of the extraction procedure developed by Kobayashi et al (1998) involving two extraction buffers was implemented. Cherry was successfully extracted using the modified CTAB method. Rather than detail the entire procedure I will provide tips for optimising the method as found during my stay. After the precipitation of the DNA with cold isopropanol the DNA is mixed by inverting the tube and following this the protocol calls for centrifugation at maximum speed for 5 minutes if this is extended to 10 minutes it ensures the pellet sticks to the side of the tube and there is less chance of losing it when pouring off the supernatant. A 5 minute centrifugation is called for again after the ethanol washing step again if this is extended to 10 minutes it is more effective and for even greater cleaning of the DNA pellet it can be kept in ethanol overnight. In the final step RNase A enzyme is added to the pellet to remove RNA, a nucleic acid contaminant, the protocol calls for an incubation time of 30 minutes however a better result is obtained with a 1 hour digestion.

Grinding of plant tissue into a fine powder was achieved by placing two 5/32 inch ball bearings, purchased from a bicycle repair shop, in the bottom of a 2 ml Eppendorf tube with a rounded bottom – this is to ensure the ball bearings don't jam. This was frozen in liquid Nitrogen, plant tissue transferred to the tube refrozen and then either placed on a shaker or shaken by hand. In the past a micro-pestle had been used to grind each sample.

Primers/PCR techniques:

Dr Ken Tobutt described the Prunus S-RNase gene structure and it is represented schematically as follows;



Where C denotes a conserved region.

Two consensus primer sets were developed by Dr Tineka Sonneveld that span the conserved regions and the first and second introns. Variation in introns gives different amplicons for different alleles. A former student at East Malling, Dr Sonneveld went further and designed allele specific primers by looking for specific sequences in the conserved regions of each allele and then designed primers using Clustal analysis and DNA software. Both approaches were used to analyse samples from Australian cherry cultivars. For greater detail on the structure of the S-locus and primers please refer to Sonneveld et al (2003).

Interpretation:

It should be noted that the first intron consensus primers cannot satisfactorily differentiate between S4 and S6, S1 and S5 nor S2/S7/S9/S12 and in turn S10 and S14, they are unable to detect S13. While the consensus primers for the second intron are unable to differentiate between S1, S3 and S13, S10 and S14, S2 and S5. When results for the first and second intron are

combined difficulties occur in differentiating S2 and S7, S10 and S14 and sometimes S1 and S13.

Findings:

Several cultivars were genotyped for the first time, Sir Hans (S2S4'), Sir Douglas (S3S4'), Dame Roma (S4'S13), Black Douglas (S1S13), Burgsdorf (S3S6) and Stockton Morello (*P.cerasus*) (S6S11). The S4' designation indicates self fertility and in the case of the abovementioned cultivars all have been established as self fertile in previous years by bagging and self pollination in the field. Currently the self fertile allele cannot be detected separately but is assigned through association to S4 genotypes known to be self fertile (Note Dr R.Tao, Kyoto University, has discovered the self-fertile mutation in cherry and so in the future the self-fertile allele can be detected). Another source of self fertility also exists in cherry because Burgsdorf is self fertile as established through field tests yet its S-genotype is S3S6, previous work (Granger, 1998) showed linkage between self fertility and the S3 allele using results of isozyme analysis. And self fertility associated with the S3S6 genotype has been reported for a Spanish cherry cultivar Cristobalina (Wunsch, pers.comm.2003).

Another interesting outcome from these results is the genotypes of Sir Hans and Sir Douglas. Both cultivars were produced by caging two Stella cherry trees in an insect proof enclosure and were assumed to be a result of selfing Stella. However the S-genotype of Sir Hans is S2S4', its assumed parents Stella is S3S4' so the S2 allele must have been provided by a different pollen donor. In this case it is thought that Vega (S2S3) must be the male parent of Sir Hans as it was planted adjacent to the Stella mother trees. The integrity of the insect proof cloth must have been compromised at some stage and bees effected pollination between Stella and Vega. While the genotype of Sir Douglas is consistent with it being a Stella self it could equally have resulted from a cross with Vega gaining the S3 allele from Vega and the S4' from Stella.

It is also the first time that the S1S13 genotype has been reported and this means a new pollination group must be proposed for Black Douglas. Initially it is assigned to Group 0, Universal Pollinisers, until another is found and a new group can be formed.

Also 48 progeny from a family produced by crossing Black Douglas (S1S13) x Stella (S3S4') were analysed using the second intron primers. This cross would be expected to produce 1S3S13: 1S4'S13: 1S1S3: 1S1S4' we observed 4S3S13: 9S4'S13: 9S3S?: 9S4'S? plus 3S6S13, 2S6S? and 1S13S?. This shows that further analysis is required using the first intron primers and allele specific primers to determine the indistinguishable alleles but on first assessment observed and expected ratios appear to be close. The S6 allele appeared unexpectedly and is probably due to either contamination in the field ie an uncontrolled cross. Or it maybe due to a transposon, thought to be located in the S-locus, changing the sequence in the intron resulting in different sized fragments being produced during the PCR process.

The power of the procedure is displayed in these few results alone. It can be used for genotyping and assigning cultivars to pollination groups, paternity analysis and in classical genetic studies. And given that genotypes are the

same in other species eg Stockton Morello (*P.cerasus*) it can be used in phylogeny studies within the genus *Prunus*. It should also be noted that cultivars sampled from the collection at Lenswood in South Australia and analysed at East Malling agreed with previously reported S-genotypes of the same cultivars grown at East Malling. This indicates that the cultivars are true to type and shows another use for the technique in quality control. This is important because mislabelling and propagation errors are probably the biggest cause of incorrect nomenclature in tree crop species.

Discussions:

Dr Ken Tobutt emphasised the need to correlate molecular differences with test cross results.

Nomenclature for the S-alleles has been established by the John Innes Institute when they created the compatibility groups for cherries or the functional pollination groups, thus alleles should be based on the functional protein they produce and not on small molecular differences (although it is acknowledged that one could argue that this could be a result of different alleles). Apparently there is very little crossing over in the S region of Rosacea due to its centromeric location and this is why different parts of alleles are not seen together.

All hand hybridisations of cherry are carried out in glasshouses at East Malling due to the high risk of poor weather during flowering. Mother trees are grown in pots and kept on sand beds. Breeding objectives include dwarfing rootstocks and to achieve this, hybrids between *Prunus lannesiana* and *P.mugus* are being evaluated. Cherries are also being screened for resistance to cherry aphid (*Myzus cerasi*), two biotypes have been identified, one attacks sweet cherry and the other sour cherry. Resistance to aphids is tested by releasing 5 cherry aphids near the apex of glasshouse grown seedlings. To assist consistent scoring of infestation the side shoots of seedlings are removed so that each has a single leader. It was noted during this discussion that populations of field grown cherry seedlings at Lenswood in South Australia appear to be showing resistance to cherry aphid. Dr Tobutt recommended that we reassess the resistance and have the aphids positively identified and in particular the biotype. Further the material at Lenswood displays resistance as an apparent recessive trait so it was suggested that we should conduct a cross between two resistant sibs if the trait is recessive we would expect all progeny to be resistant. In turn if a backcross were made to a susceptible seedling we would expect half of the progeny to be resistant and half susceptible. Dr Tobutt also recommended publishing the preliminary finding of resistance.

A recent release from the cherry breeding programme at East Malling is 'Penny' a late season black cherry. They are also close to releasing a columnar apple with better fruit quality for home enthusiasts. East Malling no longer owns the rights to Colt cherry rootstock but when it did it brought in £50, 000 per year in royalties, now they receive very little return. Meiosis is a commercialisation company on site that handles the East Malling strawberry cultivars and returns 2-3 pence per plant sold to the breeding programme.

Cherry/*Prunus* taxonomy has not been reviewed for 50 years and there is a real need to revisit it in a collaborative approach including taxonomy and molecular biology.

Dan Sargent is using a wild strawberry *Fragaria vesca* in his PhD studies it is ideal for genetics, mapping etc because it is small, has a short generation time (20 days from flowering to fruiting), produces 2 or more crops per year and one pollination creates 100-200 seeds. It also has a small diploid genome of 7 chromosomes. As such it is ideal for mapping *Rosacea*.

Brogdale is the national repository for apple, pear, medlar, cherry and apricot administered by Imperial College and the Ministry of Environment. It has a role in maintaining diversity with 300 cherry cultivars in the collection. Budwood is provided free to bona fide R&D organizations on request.

P.avium is truly wild in the UK also continental Europe to Southern Sweden and across to Nepal. English cherries represent the western limit of *P.avium* brought in with the last ice age rather than as previously thought with the invading Romans.

Royal Botanic Gardens, Kew, England herbarium and living collections.

Documents within the herbarium collection contained the comment re *Prunus* "the whole group needs intensive monographic study" this reinforces discussions held at East Malling indicating a review of the genus was necessary. One key indicated the genus *Prunus* belongs to the family Rosacea and the tribe Pomeae (including Malus, Pyrus etc) yet another key assigned Prunus to the tribe Pruneae with Maddenia, Prinsepia and Stephenandra. Synonymns of Prunus include Amygdalus, Armeniaca, Cerasus, Prunophora, Microcerasus, Emplectocladus, Amygdalopsis and Aflatunia. Cultivated *P.avium* has been assigned to the Subgenus Cerasus Section IV Eucerasus.

Specimens had been collected from Cypress, Turkey, Armenia, Persia (Iran), Afghanistan, England, Madeira (Atlantic Island), Spain, Austria, Bulgaria, Norway, Macedonia and Romania.

P.avium var plena has double petal flowers, there are very few stamens and it seems they have differentiated into petals rather than reproductive organs. Also in some cases the styles had differentiated into bracts. No ova were observed in the receptacle. This variety could possibly provide an opportunity to study the development of reproductive organs in cherry.

In the forest situation *P.avium* occurred with oak, beech, chestnut and conifers.

Kyoto University, Japan.

Discussions with Dr Ryutaro Tao.

- A tissue culture, transformation technique is being developed for *Prunus* to confirm function of the pollen part of the S-gene. A regeneration system has been developed for sweet cherry at Yamagata.

- They have developed a tetraploid *P. avium* to see if it is self-fertile. The ploidy has been checked using flow cytometry, and all tissue was 4n.
- Peach has S-alleles shared with almond and yet it is self-fertile. It has 2 S-Rnase genes and it is thought that a modifier gene found in Solanaceae for self-incompatibility may be missing in peach.
- Dr Ryutaro has found the deletion associated with self fertility in cherry and developed a marker for it which will soon be published in the Journal of the American Society of Horticultural Science.
- Dr Tao's findings indicate that the pollen specific part of the incompatibility reaction in cherry is a result of F box genes. There are hundreds of F box genes in Arabidopsis and they are thought to be involved in cell-cell recognition, circadian rhythm and the plant immune system (or a substitute for it). There are no introns in the F box region so they cannot develop markers. Southern blot analysis resolves bands from other F box genes in the genome, which makes S-allele typing difficult.

Yamagata Prefecture Horticultural Experimental Station, Yamagata, Japan

Field visits and discussions with Dr Ishiguro

- Yamagata accounts for 70% of Japan's cherry production. Producing 14 000 tonnes from 2570 ha in 2003. The main cultivar grown is Satonishiki and it produces a white fleshed fruit. Note that white cherries are preferred in Japan. The main polleniser for Satonishiki is Rockport Biggareau. The average size of cherry orchards in Yamagata is 400 m², and they are family owned. This is because corporations cannot own land in Japan for the purpose of agricultural pursuits, although this is set to change in the next few years.
- All trees were grown in greenhouses. Plastic covers are usually installed at stage III the rapid cell expansion phase. Although some houses were heated to induce early harvest, in this case winter chill is monitored and when 1050 hours is reached covers are applied to break dormancy (usually around January) resulting in harvest as early as April 10. Rest breaking chemicals such as hydrogen cyanamide have only been trialled in recent years resulting in even flowering and weaker vegetative growth. Greenhouses usually contain 14-15 trees spaced at around 6 metres apart. They are grown on vigorous rootstocks mainly *P. lannesiana*. Trees are trained as open vases and 20 year old trees were 6 metres high and wide. Pruning is carried out in winter and summer to remove strong shoots. Trees are not irrigated but sprinklers combined with roof mounted fans are used to cool the greenhouses that are maintained at 25°C. Fruit cracking sometimes occurs at stage III inside the house when it rains, even though the trees are protected, high humidity especially after 2-3 days of rain causes severe cracking. No cracking occurs at maturity.
- Growers market fruit to the wholesale market or directly to consumers via mail order.

- The breeding program at Yamagata Prefecture Horticultural Experiment Station began in 1978. Its objective is to breed high quality new varieties with a range of maturities with emphasis on early cultivars. Three cultivars have been introduced from a population of 6,848 seedlings. The cultivars are as follows: - Benishuhou a cross between Satonishiki and Tenkonishiki, harvested late June to early July, 8-10 gram fruit, white flesh. Sugars 20% Brix, titratable acids 0.6%. Benisayaka a cross between Satonishiki and Seneca, harvested early June, 5-7 gram fruit, dark-purple flesh (note harvested at pink flesh stage). Sugars 16% Brix, titratable acids 0.8%. Benitemari cross between Vic and Satonishiki, harvested early July to mid July, 9-11 gram fruit, cream flesh. Sugars 20% Brix, titratable acids 0.7%. Several un-named cultivars were also observed in field plantings they included Yamagata C3 – a yellow cherry that matures 1 week before Satonishiki. Yamagata C6 – same maturity as Benisayaka, susceptible to rain cracking (note 1800 mm of rainfall had been received to date). Yamagata C10 – (Rainier x Stella) self-fertile, matures mid June, white flesh, 21% Brix, heart shaped fruit. Yamagata C8 – 1 week before Satonishiki, very bright blush, 20% Brix. Yamagata C9 – Very high brix over 25%. Firm fruit harvested mid June.
- Dr Ishiguro collected cherries in the wild from Turkey in 2003, south of Bracksy. He has 40 specimens at the Experimental Station all very large and sweet. Only one domestic cultivar from Turkey was of interest it was unnamed and produced fruit 12 grams in weight. The main cherry germplasm collection at the station contains 80 cultivars and includes Turkish and Chinese material.
- Satonishiki was introduced in 1920 it is a cross between Napoleon and Governor Wood its S-genotype is S3S6 and it is self-incompatible. The Spanish cultivar Cristobalina, also a white fleshed, blushed cultivar is S3S6 but self-fertile.

National Institute of Fruit Tree Science (NIFTS), Tsukuba, Japan.

Discussions and field visits, Dr Yamaguchi.

- Breeding activities were focussed on four main tree crops peach, Japanese pear or nashi, chestnut and Japanese apricot (*Prunus mume*)
- The nashi cultivars Kosui and Hosui were released from Tsukuba and now comprise 50% of nashi plantings in Japan.
- Chestnut cultivars have been bred for resistance to Chestnut gall and their varieties now occupy 70% of Japanese plantings.
- Breeding research includes molecular studies such as genome analysis of peach and nashi and SSR's to distinguish cultivars. They have many markers in peach including cling vs free stone, yellow/white flesh, nematode resistance, peach/nectarine (fuzz/fuzzless). The latter constitutes a health issue for peach growers whom react to the fuzz on peaches. In the past they have relied on the importation of nectarine varieties from USA for production but there is consumer resistance to them due to sour taste and cracking. In the future sub acid nectarines

will be in demand. The peach breeding program has an objective to produce high quality fruit, brown rot is a major problem with fruit ripening in the rainy season (June/July), however there is no known resistance to it. *Xanthomonas* a bacterial disease of peaches is also a problem but resistance to it has been found in a wild type peach (*Prunus mira*). Dr Yamaguchi undertook exploration of *P.mira* in Nepal and has introduced several accessions to his breeding program. Japanese peaches are almost always white fleshed although recently yellow flesh has become more acceptable. A weeping habit has been introduced by crossing with an ornamental peach 20 years ago after 6 generations fruit size and quality are only now reaching an acceptable level. The approach to peach rootstock breeding was one of making interspecific hybrids with the objective of producing dwarfing rootstocks. Species used included *P.cerasifera*, *P.tomentosa*, *P.kansaensis*, *P.mira*, *P.davidiana* (imported from China 2000 years ago), *P.persica* and *P.japonica* (not suitable as a rootstock due to graft incompatibility with peach). Three rootstocks of interest were Tsukuba 6 a cross between red leaf nemaguard and Chinese peach and two dwarfing rootstocks Tsukuba 4 and 5, which produced smaller trees through precocity of fruiting. The peach breeding program works on a 5 year cycle turning over 1500 seedlings per cycle. Markers for dwarfingness are difficult to find there are many bands associated with the characteristic and they vary from year to year.

- Japanese apricot or *P.mume* is more popular in Japan than China. They are harvested mature and used to produce pickles or harvested green to produce a juice combined with sugar and water; they are also dried and processed in brine. Around 100, 000 hectares are grown in Japan producing about 100, 000 tonnes. The breeding programme has made hybrids between mume, apricot and Japanese plum the latter is red fleshed and produces a pink juice. They have also crossed mume with Turkish apricots that are sweeter and lower in acid. Some of the mume are self-compatible but the majority are self-incompatible. The Shiroka cultivar is male sterile having no pollen. The European cultivars are in general susceptible to bacterial shot hole although Harcourt is resistant. Pakistan germplasm doesn't fruit at Tsukuba due to too many disease problems. Mume is relatively resistant to *Xanthomonas*.
- Dr Yamaguchi is trying to breed triploid grapes for seedlessness. The same has proved difficult in Prunus.
- Breeding Japanese pears for resistance to black spot (*Venturia pyrii*). Winter Nellis a European pear (*P.communis*) and Chinese pear (*P.bretschneideri*) are being used as a source of resistance to black spot. Self-fertility has been found in nashi although it needs to be combined with self-drop otherwise too many small fruit result compared to the normal self-incompatible cultivars.
- Soft flesh in cherries is strongly correlated with crack resistance and it can be used to select for crack resistance at maturity. Cultivars can be firm fleshed at stage III and crack at that stage even though they may be soft at maturity. Results from water bath immersion of fruit were well correlated with field observations of cracking, and this is thought to

depend on field management, there is however a large genetic component to crack resistance.

- Dr Yamaguchi and his colleagues at the NIFTS were interested in collaborative work with Australian scientists.
- There seemed to be a trend toward more outside funding and shorter term appointments of scientists in Japan.

CONCLUSIONS

1. The use of molecular biology in cherry research in Australia is trailing behind UK and England. Collaboration with fellow scientists in these countries will bring us up to speed.
2. There is a need for further importation of cherry cultivars and other species to bring new genes into the Australian cherry breeding programme. For example pest and disease resistance.

The information contained in this report can be best disseminated through continued interaction with the contacts made during this fellowship. And through public addresses at conferences and meetings. Media articles particularly in trade journals will help communication to the cherry industry. Ultimately the knowledge gained during the fellowship will be integrated into new cherry cultivars and this will benefit both growers and consumers of cherries across Australia.

RECOMMENDATIONS

1. Incorporate the use of PCR for the determination of S-alleles into the Australian cherry breeding programme for new cultivars, further studies on the inheritance of self-incompatibility and self-fertility, confirmation of parentage and genetic relatedness. This will provide efficiencies within the breeding programme and supply growers with greater information regarding the performance and needs of new cultivars.
2. The timing is right for collaboration with colleagues in England and Japan. For long term benefits to Australian science all efforts should be made to participate in on-going collaboration.
3. Cultivars from the English and Japanese breeding programmes as well as other species such as *P.lannesiana* will be imported into Australia to introduce other valuable characteristics into the cherry breeding programme and to broaden the gene pool being utilised for breeding. This will help prevent problems associated with inbreeding and also provide genes not currently available in the breeding populations eg resistance to cherry slug. In addition cultivars from the Australian cherry breeding programme should be made available to breeders in Japan and England.
4. Research priorities in cherry research within Australia should include investigations to determine the mechanism of self-incompatibility. There should be a collaborative program with East Malling Research to study the inheritance of resistance to black cherry aphid. Finally if mapping the genome of Rosaceous species, such as Prunus, becomes a national objective serious thought should be given to using diploid

strawberry species as the mapping organism because it is a much better genetic organism.

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