CSIRO
PLANT
INDUSTRY

Travel Report
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April 18–24

13th Australasian Plant Breeding Conference – Christchurch NZ

Master Class in Population Plant Breeding – Lincoln University NZ

Executive summary

Purpose of the visit

The 13th Australasian Plant Breeding Conference was held in Christchurch NZ, April 18-21. This is the primary conference relating to plant breeding activities in Australia and New Zealand and had six core themes: benefits from plant improvement, added value products, population improvement, plant gene technologies, genetic resources and environmental challenges. I presented a paper outlining the impact of transgenic cotton on the Australian cotton industry and the lessons learnt for plant breeders (copy attached).

Of major importance to my research in this area is to remain up-to-date in all areas relating to plant improvement and specifically in the areas of plant gene technologies and population improvement. Other specific topics of interest discussed were: economic assessment of plant breeding benefits; market focus; and influence of market size and recruitment and retention of plant breeders.

Immediately following the conference was a three day Plant Breeding Master Class run by Prof Duane Falk from the University of Guelph, Canada and Prof Wallace Cowling from the University of Western Australia. The objective of the Master Class was to challenge participants' understanding of the value of application of quantitative genetic principles to plant improvement, and to discuss the role of current, breeder-driven technologies to achieve improved outcomes. The idea was to challenge they way things are currently done, with the aim to have participants step back and re-evaluate their procedures and efficiency.

Conclusions and benefits

Overall, the Plant Breeding Conference was well organised and well run. There was a good mix of presentations detailing a range of crops and techniques, however, as with many conferences in recent years, molecular presentations dominated more than they should. The conference provided an opportunity for interaction with a very diverse group of breeders. It was particularly interesting and beneficial to compare notes with the NZ breeders regarding the organisational and funding structure of breeding in NZ. The quasi-commercial model using state owned entities really seems to work for their situation. I also gained some valuable insights from the case studies on fruit breeding that were presented and I think some aspects could be more broadly applied in Australia. Some of the statistical analyses examining the success of various breeding programs were interesting and as a benchmark the CSIRO breeding program appears to be doing very well. Several of these programs also specified very ambitious targets, similar to what we have proposed for our program in doubling the rate of yield increase.

Participation in the three day Plant Breeding Master Class was an excellent experience. It re-introduced some of the basic plant breeding concepts and simplified what breeders are trying to achieve. It also introduced some advanced concepts and techniques in population plant breeding, with practical examples of how they can be applied. It did allow me to step back and assess the way that we currently do things. My observation is that the techniques and procedures that we are currently using compare very well to the best examples that were presented. However, there are some areas where I will be evaluating some new procedures and these are detailed below in the presentation of my case study.

13th Australasian Plant Breeding Conference

Overview

The main theme for the conference was *Breeding for Success: Diversity in Action*. This theme aimed to highlight the economic, sociological and environmental benefits of plant breeding and its associated sciences to our region. The conference papers highlighted progress in addressing a range of challenges that face our plant-based primary industries and outlined opportunities that can contribute to their future development and success. The broad diversity of crops that are in commercial use in Australasia was reflected in the presentation of results from more than 90 different crops. This allowed considerable opportunity for discussion of key issues and a mutually beneficial exchange of ideas and methodology between crops. This cross-fertilisation also offered opportunities for the development of new collaborations between groups with complementary skills.

Following is a summary of some of the more interesting/relevant papers.

Breeding for success: Diversity in action – Derek Woodfield, AgResearch NZ: This paper outlined the contribution that plant breeding has made to the economic growth of NZ agricultural, horticultural and forestry industries. Exports from these sectors account for 64% of NZ total export earnings. The contribution of agriculture to GDP has increased from 13.5% in 1990 to 17% in 2005, despite significant decreases in government research funding. Plant breeding has been a significant contributor to the success of these industries, eg. Non-toxic endophytes, Zespri Gold kiwifruit and Jazz apples. However, with the continuation of reductions in funding and the regulatory environment there is a risk that NZ primary industries will be less well placed to deliver the economic benefits that can be delivered from competing industries globally.

The economic benefits of forage improvement in the USA – Joe Bouton, University of Georgia: Outlined the diversity of forage species to fill varied management options (1500 grass and 4000 legume species). The economic benefits from forage improvement have been immense, however future resources are going into fewer crops, the ones with greater economic value and where biotech can be applied. This will favour lucerne and penalize forage grasses. This concentration of resources in fewer hands requires development of consortia where organisations can leverage their resources with others who possess complementary resources.

Impacts of institutional and technological change on plant breeding in NZ – Mike Danbier, Christchurch: In the period from 1970 to present, plant breeding in NZ changed from a traditional public sector model to a quasi commercial model. These changes were a result of government legislation and policies that affected plant breeding directly (Plant

Varieties Rights) and indirectly (commodities levy act etc.). The extensive use of NZ for out of season breeding nurseries has assisted the development of plant breeding in the private sector and markedly enhanced the integration of NZ plant breeding into international plant breeding activities.

NZ wheat breeding–from public research to private commercial cultivar development – Bill Griffin, Crop and Food, Christchurch: Since the introduction of PVR in the mid 80's, private programs have competed with a publicly funded, fully integrated breeding research program. Shifts in research priorities for these public funds have now split this program into a commercial cultivar development stream and several linked, public, fundamental research platforms. It was shown how this re-shaping has continued to deliver highly productive varieties for NZ.

Fruit breeding in NZ; some significant examples of success – Errol Hewett, Massey University, Auckland: NZ has gained an international reputation for producing new and innovative fruit cultivars. Of significance: Hayward and Zespri Gold kiwifruit, Pacific Rose and Jazz apples, blueberry and hops varieties. Each of these crops have been developed because of the vision, drive and perseverance of a product champion. However, continued development of superior cultivars will require adequate funding from both industry and government sectors in combination with visionary scientists who have to capacity to predict market trends and fashions in the future.

Analysis of sugarcane productivity data: Increases from new cultivars and improved management in Australia – Mike Cox, BSES Bundaberg: REML methods were used to analyse sugarcane productivity data from all Qld mills from 1980 to 2004. Linear regression of the cultivar effects and year of release was used to estimate the average productivity increase per year due to new cultivars. Substantial genetic gains were found; 1.28t cane/ha/year, 0.03 units CCS/year and 205 kg sugar/ha/year. Benchmarking these increases over 30 year periods showed that the rate of increase in sugar yield has increased from 93 to 224 kg sugar/ha/year from cultivars released 1960-1989 and 1974-2003. *Note: since there were no control varieties continuously grown, it was not possible to estimate the contribution of new cultivars vs improved farming practices*. Based on these analyses, BSES now have the ambitious target of increasing sugar yield by 300kg sugar/ha/year by 2015.

Recent advances in wheat breeding in Australia - Robin Wilson, Dept Ag Western Australia: This paper outlined the advances that has been made that are having a profound effect on the efficiency and success of the Australian wheat breeding programs. These were:

- Unreplicated trial design where breeding lines can be placed at more sites without loss of precision and the same resources.
- Trial analysis, using ASREML, where greater discrimination can be made by accounting for spatial trends.
- Mechanisation of seed preparation.
- Application of new quality calibrations for NIR on all breeding lines before yield testing.

- Rust testing outside the wheat growing areas of all lines prior to yield testing using races possessing virulences not yet widespread.
- Routine uses of molecular markers at several stages in the program, including backcrossing, for traits that are difficult to phenotype.
- Utilisation of sources of abiotic stress tolerances in the breeding program, particularly from India and China.

Note: In terms of relating these to our cotton breeding program, we have considered but dismissed the unreplicated designs based on reliability of data under our irrigated situations; we already use ASREML to account for spatial trends; mechanisation of seed preparation would be very costly to implement in our system ($250K); we already measure many quality traits prior to yield testing; we wish to use molecular markers in our program, however markers for cotton are difficult and funding for the development of those markers is uncertain; we routinely use introductions as sources of stress tolerance in our program.

Golden rice: Introgression, breeding and field evaluation – Swapan Datta, IRRI, Philippines: Outline of the development progress of high carotenoid rice in Asian indica rice cultivars. This was developed based on the PMI selection system and made marker free by segregating out the marker gene from the gene of interest. Anther culture was used to develop homozygous stable lines. Enhanced carotenoid levels (up to T3) were observed in a number of lines compared to T0-T1 seeds, which could be due to transgeneration effect of GH vs field conditions. However, some lines showed reduced carotenoid levels compared to the donor parent. Incorporation of the genes did not change any significant agronomic characteristics.

Note: this is not the Golden Rice that was developed by Syngenta, though I am unsure if the source of the gene is the same.

Breeding for flesh colour in apple – Richard Volz, HortResearch, NZ: An interesting paper examining a novel marketing trait in apples. I was not aware that pink, red, purple, yellow and gold fleshed apple germplasm existed. This research is a multidisciplinary approach combining conventional breeding methods with molecular biology and genetic mapping, with the goal of developing a marker assisted selection (MAS) program. They have identified a number of genes and their inheritance that confer red flesh and cortex in apples and are well on the way to implementing a MAS program. The reason for needing a MAS, is that the traits do not express well until the trees have reached some level of maturity.

Balancing marker and phenotypic selection – David Bonnett, CSIRO Canberra: Provided data based on two strategies; a) selection applied only to inbreds, b) Partial F2 enrichment followed by selection of target genotypes in inbred lines generated from selected F2s. Based on predictions, partial enrichment strategies that increase but don't fix target marker alleles in early generations followed by phenotypic selection in later generations are an effective way to combing genotypic with phenotypic selection.

Bridging the domestication barrier – a model for introgressing useful minor alleles from wild relatives – Wallace Cowling, UWA, Perth: A very interesting and relevant paper considering the cotton introgression program I am currently working on. The breeding potential of wild relatives is well recognised, but cost, time and genetic drift often work against the introgression of valuable wild alleles into elite populations. Backcrossing to the elite is an effective way to restore domestication traits and elite performance, but potentially valuable minor wild alleles will be lost if the focus is on selection of major alleles, too many BC occur and the BC population is small. A model was presented where selection for the domestication genes occur during BC, while retaining sufficient individuals in each generation to fix an unknown but potentially valuable allele in fully domesticated BC2 derived lines. The introgression process takes between 2-3 years with most work done in the GH. Population breeding principles must then be applied to ensure that the new alleles are not lost from the elite population during subsequent breeding.

Broadening the genetic base of sugar beet: introgression from wild relatives – Lee Panella, USDA, Colarado: The genetic base of sugar beet is thought to be narrower than most open pollinated crops. Systematic attempts to screen wild beet germplasm for disease resistance were initiated in the early 1900s. Many undesirable traits from wild beet were reportedly introgressed with the disease resistance and it is only since the late 1900s that the use of wild genetic resources is being used in breeding programs. In 1983 the sugarbeet crop germplasm committee was formed, with a high priority on improving the germplasm pool. Currently, they have over 2500 accessions screened for 10 major disease and insect pests. Resistance genes from wild beet have recently been commercialised.

Note: This has taken a focused, concerted effort over 20 years to commercialise resistance sourced from wild relatives, even though this is a species that readily hybridises with its wild relatives with no apparent genetic incompatibilities.

Intragenic vectors for gene transfer without foreign DNA – A. J. Conner, Crop and Food, Christchurch NZ: Presented details of the intragenic vector system which involves identifying functional equivalents of vector components from within the genome of specific plant species and using these DNA sequences to assemble vectors for transformation of that plant species. Claimed that the use of these vectors for the transfer of genes from within the gene pools of crops may help to alleviate some of the public concerns over the deployment of GM crops in agriculture.

Note: I am unsure if this has been examined in detail in cotton. However, because we tend to introduce foreign genes into cotton anyway, having a plant based vector probably has little advantage (unless a gene is being transferred from a related species).

Diverse Arrays Technology, a novel tool for harnessing crop genetic diversity – Andrzej Killian, DArT P/L, CSIRO Forestry: DArT is a novel method to discover and score genetic markers. It is sequence-independent, high throughput method able to discover hundreds of markers in a single experiment.

Note: This is interesting technology and our cotton molecular group in Canberra is in communication with DArT. However, it works best with species where there is a high level of polymorphism, so for cotton, there would have to be a large amount of work done prior to applying the technology.

Physiological traits and cereal germplasm for sustainable agricultural systems – Richard Richards, CSIRO Plant Industry: This paper reviewed opportunities where plant breeding can contribute to improvements in sustainable farming practices from a cereal perspective. The main contribution for breeding is to a) increase crop water and nutrient use so that less escapes from the root profile; b) preserve the soil resource with conservation farming systems by developing cultivars specifically adapted to changed farming practices and competitive cultivars that reduce herbicide use.

At the root of it all: a QTL analysis of root distribution in perennial ryegrass – M.J. Faville, AgResearch NZ: Breeding for a deeper root profile is desireable in many crops and has a major influence on drought tolerance. They presented data to show they had successfully identified two QTLs which influence vertical root distribution in ryegrass. This is the first step in developing a MAS strategy for better rooting in ryegrass.

Note: This area is of interest to us and the techniques used here may be useful.

Arable-vegetable field trip

The final day of the conference program was a choice of three field trips in the Canterbury area. The arable and vegetable field trip was centred around the Crop & Food Research station and Lincoln University.

The program commenced with an overview of Crop & Food Research. This is a Crown Research Institute owned by the NZ government that carries out both government-funded research and work for commercial clients. This research is undertaken in partnership with a range of local and international industry and government clients. Many of the innovation and ideas are commercialised with their business partners.

The research is organised under five Centres of Innovation:
Sustainable land and water use
High performance plants
Personalised foods
High value marine products
Biomolecules and biomaterials

The following pictures show some of the areas that were looked at in detail.

Forage Brassicas: A nationally targeted program in collaboration with PGG Wrightson. Targeted species are – Turnips, forage rape, kale and swede.

Potatoes: They have been involved with potato improvement for over 65 years. Target markets are French fries, fresh and crisping in both NZ and Australia.

Onions: The onion genetics group focuses on understanding some key onion traits through developing and applying molecular genetic technologies, then using partnerships with private breeding companies to apply this knowledge within new commercial cultivars.

The National Centre for Advanced Bio-Protection Technologies: Established in 2003 as a centre of excellence supported by the NZ government. The research is divided into four themes; biosecurity, biocontrol, agri-biotechnology and Maori bio-protection. These are complemented by the NZ Biotron, a purpose built plant growth facility enabling observation and measurement of plant-microbe and physical interactions above and below ground.

Pictures below: Exterior of the biotron; one on the 'pots' filled with soil (about one m^3) showing camera setup for monitoring root distribution; pot in place in the floor of the one of the growth rooms.

Master Class in Population Plant Breeding

This three day master class was held at Lincoln University, traditionally an agriculture-based university situated on the Canterbury Plains. The purpose of the master class was to explore the evolutionary basis of plant breeding in greater detail, integrate theory from diverse sources, develop improved breeding methodology, apply improved methods to cultivar development in a variety of crops and to encourage strategic plant breeding.

The course had two presenters:

Assoc. Prof. Duane Falk, University of Guelph, Ontario Canada. Duane developed the RIPE system of barley improvement that makes use of male sterility to improved the efficiency of the population breeding program. His philosophy is: “Breeding is a short-term, accelerated, artificial evolution to maximise genetic improvement in specific traits in specific populations”. Duane’s barley varieties are the most successful current varieties in eastern Canada.

Assoc. Prof. Wallace Cowling, University of Western Australia. Wallace learned the skills of plant breeding first as a lupin breeder with the Western Australian Department of Ag, and more recently as canola breeder at the University of Western Australia. The canola breeding is conducted in a private company associated with UWA, Canola Breeders Western Australia.

The following subjects were covered in detail, I have outlined some of the key points:

Evolutionary theory – rediscovering the basic principles of evolution and breeding.

- Retain the best of the existing systems : rapid fixation of alleles
- Add the most desirable mechanisms from alternative systems/new technology : more opportunities for recombination
- Use population methods in self-pollinated plants : need an efficient genetic male sterility system

Managed populations: genetic basis of evolution and breeding.

i) Tools

- Concept of evolution from Darwin
- Mechanism of genetic variation from Mendel
- Mathematical models for quantitative genetics from Hardy and Weinberg
- Models of population dynamics from Wright, Fisher, Falconer, and others

ii) Directed evolution

- short term
- accelerated
- specific objectives
- specific populations
- many methods
- many traits

iii) Breeders only influence two factors in a population:

1. determine which individuals will contribute gametes to next generation (=selection)
2. determine how gametes from selected individuals are combined to produce the next generation (=mating design)

Managing gene flow, drift and introgression: the principles of population genetics.

- What is genetic drift?
- What is the effective population size of my breeding programme?
- Is the effective population size of my breeding programme large enough to counter drift?
- How can I increase effective population size and not jeopardize response to selection?
- What is generation time in my plant breeding programme?
- How can I increase rate of genetic progress by decreasing generation time?
- How can I understand the following apparent contradictions?
    - Genetic drift dominates when selection pressure is low on small populations… because selection counters the negative effect of drift.

    - Genetic drift dominates when selection pressure is high on small populations… because selection reduces effective population size.
- What combination of population size and selection pressure should I strive for?
- Can I achieve economical response to selection and maintain genetic diversity through immigration, knowing that most immigrants are less well adapted to my target environment?
- How long will it take to reach a selection "plateau" due to inbreeding if I take no action to counter genetic drift?

Recurrent selection

- Cyclic, alternating selection and intermating
- Generally practiced in cross-pollinated crops
- Accumulates desirable alleles in a population
- Eliminates undesirable alleles
- Breaks linkages
- Most effective in closed populations
- Combines best features of inbreeding and outcrossing into a single system
    - multiple crosses among selected lines
    - evaluation of progeny
    - selection of best lines
    - recycle as parents for next cycle
- Maintains variability through moderate selection intensity
- Progress is cumulative over cycles
- Length of cycle a major factor in efficiency

Breeding tools: Xenia, probability, 3-way crossing, mating design and population distribution

i) Xenia

- Based on xenos = strange/different
- Sometimes known as 'pollen effect'
- Can be shown by any tissue originating from fertilization (zygotic tissue, embryo, endosperm, aleurone)
- May show dosage effect

ii) Half-sib crosses for breeding

A = unadapted, good agronomic type
B = well adapted, high yielding
C = unadapted, good disease resistance

A/B + B/C (not A/C)
A/B//B/C F1's intercrossed

RIPE system and modifications
An open-ended, hierarchical structure for introgressing new germplasm into an Elite population with recurrent selection operating at the Elite level and varieties being continually generated at the top end

- ***R***ecurrent selection effective in improving yield of Elite material
- ***I***ntrogression effective in improving agronomics and disease resistance of Elite material
- ***P***opulation approach effective in a self-pollinated crop
- ***E***nrichment of Elite population achieved without losing adaptation of original parents

Breeding tools: Double haploids, index selection,
i) Haploidy (dihaploidy) in Plant Breeding

- Fixation of alleles in one step
- Qualitative
- Quantitative
- No natural selection
- Gametic array of source material
- One-step process (usually)
- Pure-breeding lines

ii)Index Selection

- Method of selection on 'total value' (combined worth)
- Simultaneous selection for multiple traits
- Each trait given 'economic weight'
- Application of DArT system to plant breeding

Presentation of case studies: "How has this course influenced me to examine one aspect of my breeding program"
Prior to this final session we had to prepare a case study based on the above question. Ten people were selected to present to the group, I was one of them. In my presentation I outlined the following:

- Our program uses a traditional pedigree-based breeding system
- It is a relatively closed system and we do practice a form of recurrent selection
- I believe there is potential to incorporate more rapid recurrent selection within our existing program
- We do have both dominant and recessive male sterility (MS) in cotton, though they have not been well utilised
- Based on the information presented at this course, I would consider initiating the following as a test of a recurrent selection system in cotton:
    - Building an elite recurrent selection population based on six elite, homogeneous lines
    - Using a dominant MS to facilitate development and maintenance of the elite population

    - Selection in the RS population will have to take into account the numerous negative correlations between yield and quality traits in cotton
- The other strategy that would be interesting to test is bulk population selection in some of our more hostile environments, such as dryland. This would involve growing several populations in an environment for 4-5 generations and then assessing the populations against a standard pedigree system to determine if we have been more successful in fixing favourable alleles (*ie* accumulating good genes and losing bad genes).

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Lessons learnt in developing transgenic cotton (*Gossypium hirsutum*) varieties

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Abstract. Since its inception in the early 1960s, the modern Australian cotton industry has had to contend with high numbers of damaging insects, particularly *Helicoverpa spp.* The first transgenic cotton (Ingard® - by Monsanto) was approved for commercial release in Australia in 1996. In 2003 the next generation of transgenic insect control became commercial. Varieties containing Bollgard®II, also by Monsanto, express the *Cry1Ac* and *Cry2Ab Bt* proteins targeted at *Helicoverpa*. Use of this technology has resulted in a 97% reduction in *Helicoverpa* insecticide usage compared to conventional cotton. Transgenic herbicide tolerant cotton varieties became available in 2000 with the introduction of Monsanto's Roundup Ready® technology and was rapidly adopted by growers. Weed control programs using Roundup Ready® cotton varieties allow reduced tillage and fewer applications of residual herbicides. We have built up a large amount of knowledge and expertise in breeding with this type of technology. Replicated field experiments were conducted to evaluate the effect of number of backcrosses on yield and quality parameters using three genotypes as recurrent parents and a donor cultivar containing a *Cry1Ac* gene. It was found that backcross number did not have a significant effect on any trait other than staple length. Additional data was collected from field experiments using two transgenic breeding families to demonstrate variability for a range of traits. We have concluded that production of transgenic cotton varieties is not a simple backcrossing exercise, rather a process involving segregation for all properties, such as

presence of the transgenic trait, expression of the transgenic trait, disease resistance, fibre quality and yield. Even backcross 5 derived lines require considerable screening and selection to ensure that the ideal combination of traits is identified. Based on these data, we conclude that, at least for cotton a large number of backcrosses are not necessarily required and the breeder should therefore place more emphasis on subsequent selection and testing using appropriate population sizes to adequately recover the desirable traits of the recurrent parent.

Introduction

Since their introduction in the 1990s, transgenic cotton cultivars have become dominant in the USA and Australia and of increasing importance in a number of other countries (South Africa, China, Argentina, Mexico and India). Incorporation of the transgenes into elite genotypes as quickly as possible has been a commercial imperative and the backcross (BC) method has been used almost exclusively (Verhalen *et al.* 2003). Breeding using backcrossing (BC) is a relatively simple, predictable method for improving a cultivar by incorporating one, two but no more than a few traits from another cultivar. The plan of the backcross is relatively simple. Two parent genotypes are selected and crossed. The recurrent parent (RP) is an adapted, productive genotype which lacks some superior characteristic that is found in the donor parent (DP). Beginning in the F_1, the hybrid material is successively backcrossed several times to the RP. After each backcross, selection is made for the desired trait from the DP. For the method to be successful the trait transferred must retain expression through several backcrosses and a sufficient number of backcrosses must be used to recover all the desirable traits of the

RP. After backcrossing is completed, the resulting genotype should essentially equal the RP except that it should express the trait from the DP (Allard 1960). If selection is applied to the desired trait only, then the proportion of the DP is expected to be reduced by 50% at each generation, except on the chromosome(s) carrying the desired trait(s). On these chromosomes the rate of decrease is slower, due to linkage drag (Stam and Zeven 1981). Therefore, it is theoretically possible to recover (on average) 93.75% of the genes of the RP after three backcrosses, 96.875% after four and 98.44% after five (Fehr 1987). The backcross method is most easily carried out if the desired traits are simply inherited, dominant and easily identified in the hybrid plants.

Backcrossing in cotton was apparently first used in the development of 'Griffin' (released in 1867), over 50 years before geneticists showed the method was scientifically sound for plant improvement (Ware 1936). The method has since been used to successfully transfer desirable fibre properties from *G. barbadense* to *G. hirsutum* (Jenkins and Harrell 1950), improved combinations of lint yield and fibre strength (Meredith, 1977) and bacterial blight resistance into *G. hirsutum* from three other species (Knight 1945). In recent years, the backcross method has been used to develop all transgenic cotton cultivars carrying a variety of traits (Verhalen *et al.* 2003).

Transgenic cotton cultivars have been commercially available in Australia since 1996 with the introduction of Monsanto's Ingard® technology. However, CSIRO has been involved in the development of transgenic cotton cultivars since the mid 1980s. The uptake of the transgenic technology is unquestioned, with almost 90% of the industry planted to cultivars containing Bollgard®II and/or Roundup Ready® in 2005-6. Since the time of our initial involvement in the development of transgenics, we have built up a

large amount of experience in breeding with this type of technology. This paper discusses the lessons we have learnt over the last 20 years in developing transgenic cotton varieties.

Materials and methods

Experiment A

Two field experiments were conducted in successive seasons (1996-97 and 1997-98) at Narrabri NSW to evaluate the effect of BC number on various yield and quality parameters of cotton. Using cultivar Coker 312 transformed with a *Cry1Ac* gene as the DP, three, four and five backcrosses were done with three commercial cultivars. Each group was taken through to the F_2 generation and plants homozygous for the transgenic trait were selected and bulked. No further selection was carried out and F_3 families were grown in RCB design irrigated field experiments with four replications. The experiments were machine harvested to measure yield and a sub-sample taken to measure quality parameters using a high volume instrument (HVI). Data was subjected to analysis of variance techniques using Genstat 8.

Experiment B

Data was collected on unreplicated irrigated progeny row experiments using F_3 lines from breeding families that subsequently progressed to produce commercial varieties. Measured traits included Fusarium Resistance Rank (FRR), a measure of the plant survival of a cultivar when compared to a known standard under Fusarium wilt conditions. The greater the FRR, the higher the level of resistance expressed by the cultivar. Yield and quality parameters were measured as for experiment *A*. Frequency

distributions were used to demonstrate variation from the recurrent parent for each of the traits measured.

Results and discussion

Across the families of the three cultivars tested, BC number did not have a significant effect on lint yield, lint percent, fibre strength or fibre micronaire (Table 1). BC number did have a significant effect on fibre length, with BC_4 consistently producing the longest fibre across the three families. However, the increase over BC_3 and BC_5 was of negligible commercial importance. There was no significant interaction between BC number and cultivar background for lint yield, fibre length and fibre strength, however lint percentage and fibre micronaire did have a significant interaction (data not shown). This indicates that the cultivar backgrounds did behave slightly differently in regard to BC number for these two traits, however, as for fibre length, the magnitude of the differences were of negligible commercial importance.

Figure 1 shows frequency distributions for various traits among BC_4 F_3 lines in a Bollgard®II breeding family. Even with this number of backcrosses there is large variation for all traits among lines, particularly disease resistance (FRR range from 40-140). In addition, the mean of all lines does not necessarily reflect the RP value, as evidenced by lint yield and fibre strength. This can be clearly seen in Table 1, where, for the same family, the percentage of lines that are equal to or better than the RP are calculated. The lint percentage, lint yield and fibre strength of the lines were far below expectation based on the RP. This makes the task of recovering all traits of the RP in a transgenic line very difficult. Figure 1 also indicates the value of the line that was

ultimately selected for commercial release. In this example, the highest yielding line was able to be selected with no compromise in disease resistance or fibre length and only a slight reduction in fibre strength.

The frequency distributions in Figure 2 show a similar pattern. These BC_3 F_3 lines are from a Bollgard®II/Roundup Ready® breeding family. In this example, the RP was not tested against the lines, but the variation among lines is similar to the previous example. Again, the highest yielding line was able to be selected for commercial release, but there has been some compromise in fibre strength (compared to the mean of the lines).

All the traits that we are selecting for (apart from the transgenic traits) are multigenic. The variation among lines, even after a substantial number of backcrosses, poses a huge challenge for breeders who have the task of realigning genes for all the important agronomic traits of a cultivar. Our data does not support the theory that extensive selection and testing of backcross-derived cultivars is not required (Poehlman 1987).

Based on these data, we conclude that, at least for cotton a large number of backcrosses are not necessarily required and in for the families tested here, BC_3 was adequate. However, as demonstrated in Figures 1 and 2 and Table 2, population size can have a large influence on the final product produced. Using a population size equivalent to, or approaching the size of a conventional breeding population increases the chance of recovering all desirable traits. The breeder should therefore place more emphasis on subsequent phenotype selection and testing using appropriate population sizes to adequately recover the desirable traits of the RP.

Table 1: Effect of backcross (BC) three, four and five on yield and quality traits of cotton. Mean of three families.

BC	Lint Yield (kg/ha)	Lint %	Fibre length (mm)	Fibre strength (g/tex)	Fibre micronaire
3	1822	41.2	28.8	28.8	3.72
4	1903	41.5	29.3	29.0	3.76
5	1861	41.6	28.8	28.6	3.68
SED	37.0 (ns)	0.26 (ns)	0.15 (P<0.01)	0.23 (ns)	0.05 (ns)

Figure 1: Frequency distributions of Fusarium Resistance Rank (FRR), lint yield, fibre length and fibre strength among BC4 F_3 lines in family 20405. The donor parent for this family contained the *Cry1Ac* and *Cry2Ab* genes (Bollgard® II) and the recurrent parent contained the *Cry1Ac* gene. Solid arrow indicates the value of the recurrent parent, dashed arrow indicates the value of the individual line that was ultimately selected for commercial release.

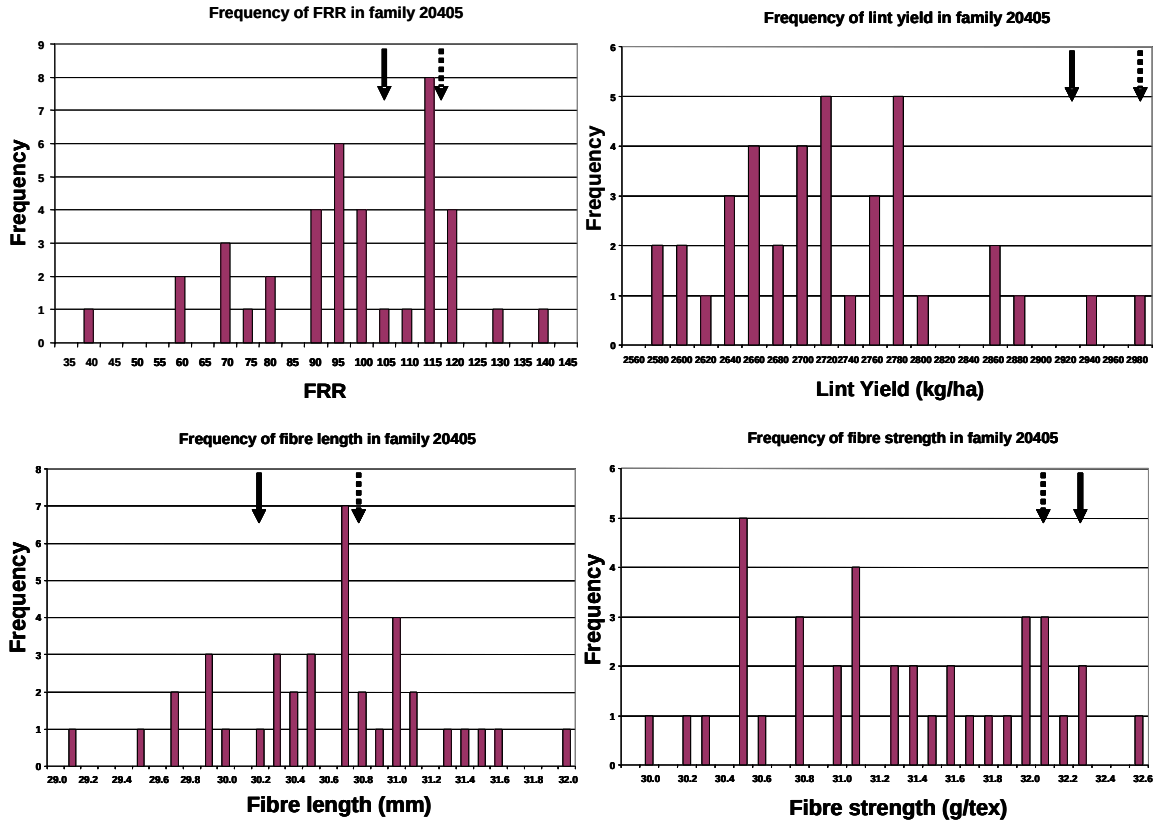
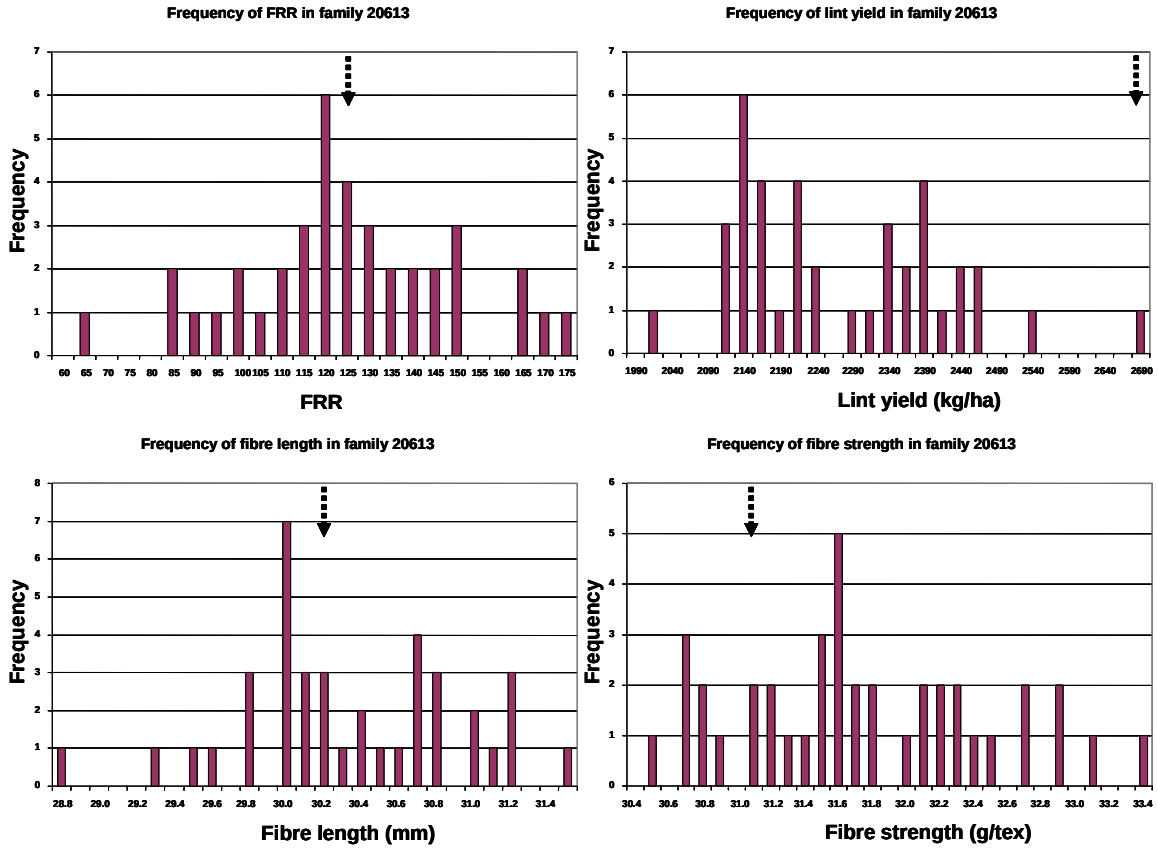


Table 2: Percent of 20405 F_3 lines equal to or better than the recurrent parent for disease resistance (FRR), yield and quality traits.

Trait	%
FRR	42
Lint percent	3
Lint yield	5
Fibre length	68
Fibre strength	8
Micronaire	66

Figure 2: Frequency distributions of Fusarium Resistance Rank (FRR), lint yield, fibre length and fibre strength among BC3 F_3 lines in family 20613. The donor parent contained the *Cry1Ac, Cry2Ab* (Bollgard® II) and CP4 (Roundup Ready®) genes with a conventional recurrent parent. Dashed arrow indicates the value of the individual line that was ultimately selected for commercial release.



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