“Another Man’s Life with Barley”

Dr Reg C.M. Lance.

Acknowledgments:

I would like to thank the Chairman of the Farrer Memorial Trust, Dr Scott Hansen,

Director General, the Department of Primary Industries - New South Wales. To the other members of the Farrer Memorial Trust I would like to thank you for bestowing on me this honour. I am very honoured and humbled to accept it.

I would like to thank the NSW DPI Deputy Director General Kate Lorimer-Ward for her very kind words for my introduction and for the presentation of the Farrer Memorial Medal for 2018.

Dedications:

I would like to dedicate this oration to the memories of two of the early United States of America pioneers of barley breeding and genetics: Harry V. Harlan (1882-1944) and Gus A. Weibe (1899-1975)

Gus A. Weibe (1899-1975)

Harry V. Harlan (1882 – 1944)

Harry Harlan. From "One Man's Life with Barley – The Memories and Observations of Harry V Harlan" (1882 – 1944). Harry was a pioneer of the barley agronomy, breeding and early barley germplasm collections. He conducted research into barley genetics, agronomy and breeding with the United States Department of Agriculture.

Harry Harlan and another early barley researcher Gus Weibe collected barley extensively in Ethiopia.

Gus Weibe also worked with the USDA as a barley breeder and geneticist. From 1946 until 1969 he was the leader of barley investigations for the USDA Agricultural Research Service. He was responsible for collecting at least 1,100 barley lines and land races from Ethiopia. He was the inaugural President of the International Barley Genetics Symposium. The universal Leaf Rust susceptible variety "Gus" is named after him.

My first exposure to the value of barley germplasm collections was to access some 543 Ethiopia barley lines from the USDA collection (Lance and Nilan 1980). Ethiopia is a major "Centre of Diversity" for barley. The variability in this small collection was truly amazing for many agronomic, grain quality and disease resistance traits. Once harvested I screened the population for "acid-soluble viscosities" and selected low and high lines which formed the basis of my PhD thesis studies; "Genetic studies of the beta-glucan content of barley". I have been truly indebted to Ethiopia ever since and have wanted to somehow return my appreciation to them by making a contribution using my acquired knowledge and skills. Through a project between the Ethiopian Institute of Agriculture Research (EIAR) and the University of Queensland (led by Prof. David Jordan and Dr Emma Mace) funded by the Bill and Melinda Gate's Foundation I have now been able to be involved in modernising their breeding programs and in a small way return the favour.

Introduction:

The transition from public to private barley breeding in Australia has occurred over the past 15 years. Prior to the formation of the Grains Research and Development Corporation barley breeding programs were funded by State Governments and either pre-Barley Research Council or Barley Research Council funding. The commodity Research Councils were amalgamated into the GRDC which was able to then take on a national role for co-ordination and funding. At this stage there were 6 state based programs; Queensland, New South Wales, Tasmania, Victoria, South Australia and Western Australia. The "Waite Institute" program being at the University of Adelaide's Waite Campus.

A transition arrangement saw a rationalisation of the NSW and Vic programs to form Barley Breeding Australia. BBA was the association of three nodes; BBA-north, BBA-south and BBA-west from the DAFQ, University of Adelaide and the DAWA programs. A further transition saw the BBA-west program being privatised to InterGrain Pty Ltd. The DAFQ program has transitioned to a pre-breeding program for foliar disease resistances. The BBA-south program has dispersed with some of the breeders taking positions with either AGT or SECOBRA Recherches. The fate of the rest of the Waite Institute program as a pre-breeding entity is uncertain at this point in time.

Elements for a successful barley breeding program:

Successful breeding programs require the integration of a number of disciplines *vis-à-vis*:

Germplasm:

Any program relies on the introduction of new genetic material whether it is a modern cultivar or lines with desirable traits. The continued introduction of new genetic material by such programs as AGG managed within is fundamental to the future growth and sustainability of the breeding programs. My post graduate studies began with utilising land races from Ethiopia. My current research and development into

discovering and pyramiding multiple disease resistance genes into modern pre-breeding germplasm relies inexorably on new germplasm found in various international collections. Some of the resistant lines we are currently crossing into our Nested Association Mapping (or NAM) populations are lines which were originally collected and handled by either Harry Harlan and/or Gus Weibe many years ago.

Yield evaluation and agronomic assessment:

Previously the Waite and DAFWA programs had extensive investments in people and machinery to plant, manage and harvest extensive yield trials. Now the preference is to outsource to private companies such as Kalyx or Eurofins Agroscience Australia

Quality laboratories:

Fundamental to being able to evaluate grain and/or malting quality is to have access to a laboratory that can have a high throughput of a large number of samples in an efficient manner. Since the mid-80s I have been fortunate to be associated with two superb barley quality laboratories; at the Waite Institute lead by Lesley MacLeod and Sophie Roumeliotis and at the DAFWA laboratory led by Allen Tarr. The professionalism of their laboratories was fundamental to be able to confidently measure grain and malting quality traits and to subsequently select for improvements.

The advent of NIR technologies calibrated to specific quality traits has enabled the non-destructive and accurate estimations of a range of malting and grain quality traits such as protein, malt extract, colour, etc. In the context of improvements in the rate of genetic gain, NIR technologies, I believe, are just as important as molecular genetic technologies.

Disease screening and evaluation:

In the past, late stage testing and assessments of the disease resistance profiles of advanced lines was carried out by dedicated pathology groups associated with the breeding programs. It is now imperative that pathology (and molecular genetic) inputs takes place during early generation phases. It is unconscionable to carry forward lines which are susceptible to disease unless they are a part of a germplasm enhancement or parent building program.

Biometricians for advanced statistics:

Nearest neighbour analysis was my introduction to modern statistical design and analysis. Now, spatial analyses, partial replicates, mixed models, multi environment trials, unbalanced designs and pedigree analyses have become the norm. The impact on accounting for the estimation of the variety performance of traits is spectacular to say the least. Having a co-ordinated and independent approach to the analysis of plant breeding data and assessment of National Variety Trials is paying major dividends and the GRDC should be congratulated for the role and leadership in funding this program and process.

Molecular genetic marker laboratories:

Molecular genetic markers have made a significant impact on the breeding of all crops including barley. The GRDC invested wisely into the Australian Winter Cereal Molecular Marker Program (AWCMMP) and its predecessors for barley and wheat. The peak of research activity in barley culminated in the publication in a special issue of the *Australian Journal of Agricultural Research* (AJAR Vol 54 Nov 2003). The barley breeding programs have been able to use marker Assisted Selection for a range of traits from; phenological and agronomic, malting quality to disease and pest resistance and resistances or tolerance to abiotic stress. Early genetic studies have relied on bi-parental populations. Now more complex population structures such as Nested Association Mapping (NAM) or whole breeding program or germplasm collections to undertake Genome Wide Association Mapping and Selection (GWAS) are becoming the preferred mapping and genetic analyses populations.

The DAFWA cereal breeding programs had successfully utilised an F_2 progeny method with a reselection phase in the F_5 generation. However the time taken from cross to release is considered too long and strategies such as alternate generations, doubled haploids, single seed descent or "speed breeding" are considered more desirable in reducing "cycle time" (the time taken from baking the original cross to using the progeny in a new cross). The combination of Marker Assisted Selection (MAS) with either conventional breeding, Doubled Haploids (DH) or Male Sterile Facilitated Recurrent Selection (MSFRS) improved both the rate of gain but enables the selection of early generation germplasm with higher genetic worth. The problem encountered was that those technologies could not be applied to the whole early generation program.

The "Breeder's Equation" should be considered as this encompasses the elements for improving the "rate of genetic gain"; "R" where:

$R = ih^2\sigma_p$ / years per cycle

"i" is the selection intensity,
h^2 is the heritability,
σ_p is the phenotypic variance,
$ih^2\sigma_p$ together represents the response per cycle.

In early generations, off season nurseries or the use of doubled haploids or "speed breeding" greatly speed up the early years and may cut 2 – 3 years off the overall cycle time. The modern imperative is to efficiently and effectively improve the rate of genetic gain. Consideration needs to be made to use the new statistical designs and analyses (including pedigrees) of trials and traits to improve heritabilities and "breeding values". It is also not good enough to just increase the phenotypic variance but to also orchestrate the increase in the desired direction. Again, more detailed analyses of breeding values will facilitate the choice of parents for new crosses.

Varieties – Malting and Feed:

In the 80s the Australian Barley Industry was facing a dilemma. The domestic malting market required lower levels of malt extract, and lowered levels of starch degrading enzymes or diastase and lower fermentability than was now being demanded in the export markets. Domestic brewing used sugar as the adjunct whereas the export brewers used either malt alone or a starch based adjunct such as wheat, corn or rice. As such the export markets demanded higher malt extract, higher diastase and higher fermentability. At the same time there was an increasing demand for improved protein and beta-glucan modification resulting in lower malt beta-glucan, lower wort viscosities, and optimal levels of "Kolbach Index".

Chebec was release as a CCN resistant domestic malting type but did not reach the required extract levels so was rejected as a malting variety. Sloop gained an increase in diastase, of about 25% over Schooner, from the Canadian variety Norbert. Its progeny; Sloop SA and Sloop Vic were the result of crosses incorporating CCN resistance and were released after I moved from the Waite Institute to DAFWA. Both varieties endured for some years.

Shannon was a Barley Yellow Dwarf Virus resistance backcross derivative of Proctor. The source of the BYDV resistance was the *Yd2* gene from CIho 3208-1. This accession was originally collected. 17-Nov-1923. Shewa Ethiopia by Harry Harlan. This line was the one that was the subject of BYDV research in California (Schaller et al., 1963). Franklin barley was bred by Wayne Vertigan, derived from a cross between Shannon and Triumph and represented the best quality variety in Australia at the time. It was however, too late in maturity to be widely grown on the southern and western mainland. It should be noted that both the malting quality and the *Yd2* gene were carried forward to the newer varieties; Baudin, Bass and Flinders.

Dhow was an early maturing, CCN resistant semi-dwarf barley with high extract from the Japanese variety Haruna Nijo. Although it did not reach major acreage, it did give great hope that high yield, good agronomic performance and excellent export malting quality was achievable.

Table 1: Malting and Feed Varieties Released (see also Figure 1) .

Malting Varieties	PBR Granted		Feed Varieties	PBR Granted
Sloop	(1992)*		Chebec	1992
Gairdner	1999		Molloy	1997
Dhow	2004		Doolup	1999
Baudin	2003		Fitzgerald	1999
Hamelin	2003		Keel	2001
Vlamingh	2008		Maritime	2006
Bass	2013		Roe	2007
Flinders	2017		Hannan	2010
Banks	2018+(?)		Lockyer	2010
			Rosalind	2018+(?)

Gairdner barley was bred by Ross Gilmore and Peter Portman at DAFWA but when I arrived in WA I was responsible for choosing a malting variety from amongst 5 candidate lines. Eventually it came down to either Gairdner or Fitzgerald with Gairdner being chosen because of higher and more stable thousand grain weight; i.e. the varieties were very similar for medium quality. Gairdner barley endured in WA and the eastern states for many years, probable less to do with yield and agronomic performance but more to do with the fact that it filled a niche that no other barley occupied.

Baudin and Hamelin were release on the same day. Hamelin, was similar to Stirling (which was the target for replacement) for yield and agronomic performance. Hamelin inherited its superior malting quality from the Canadian variety Harrington but it never took off as a variety, probably because it did not command a higher price.

Baudin was a later maturing variety with superior export malting quality. When test samples were sent to China there was significant disbelief amongst malting customers that an Australian malting variety could achieve such excellent quality. Once accepted it has been in high demand commanding a premium price. As far as disease resistance, Baudin had two weaknesses; powdery mildew and leaf rust. After consultation with the Australian Cereal Rust Control Program at the University of Sydney it was decided to limit the eastern states release to regions where it was less vulnerable and a very prescriptive management package had to be put in place to manage the leaf rust susceptibility. On the south coast of WA farmers sprayed for powdery mildew but new races appeared which were resistant to the triazole fungicides (https://www.agric.wa.gov.au/barley/management-barley-powdery-mildew-2018).

The release of Bass and Flinders addressed some of the disease susceptibility issues. Flinders contained two resistance genes for powdery mildew resistance from Cooper as well as *Rph20* Leaf Rust adult plant resistance.

The last export quality malting varieties (Baudin, Bass, Flinders and Banks) were selected for improved agronomic performance in that they are early maturing, semi-dwarfs with larger grain and stiff straw.

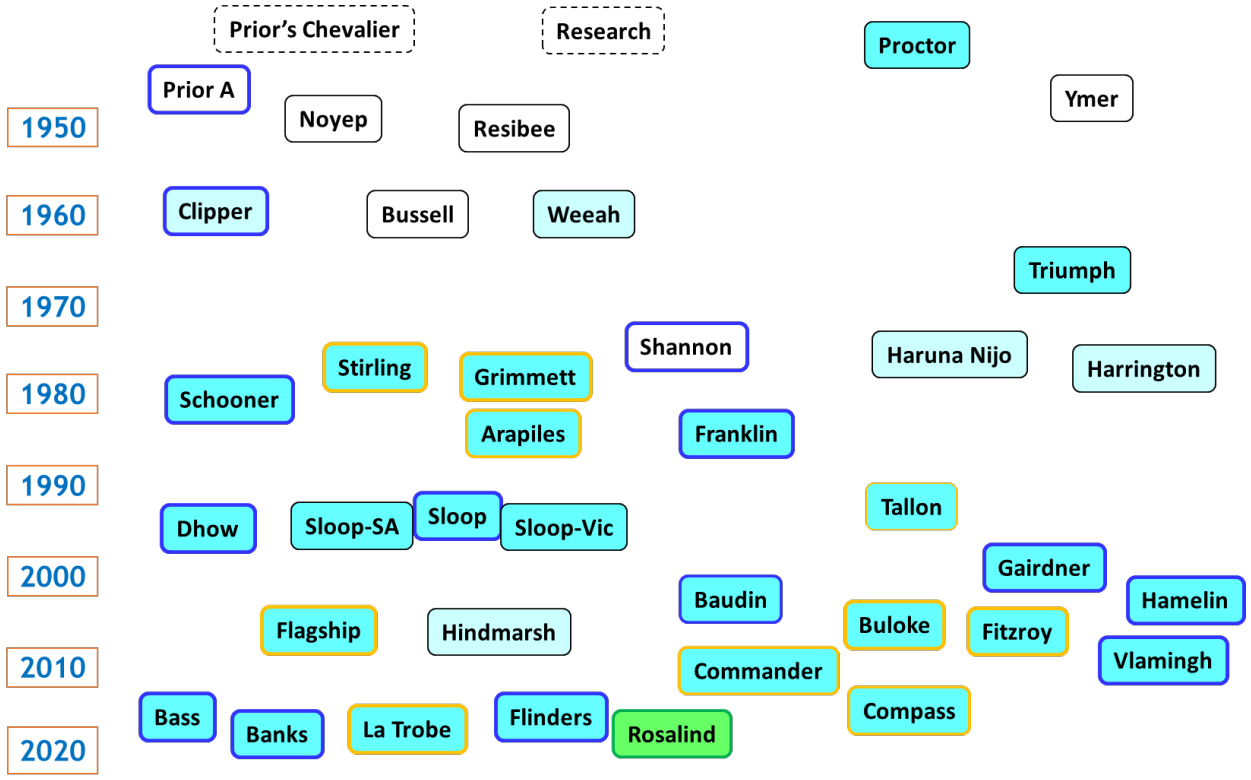

Figure 1: Representation of the release of malting varieties (plus Hindmarsh, a food variety and Rosalind, a feed variety) over the past 70 years in Australia. Those lines with dark blue surrounds are from Farrer Memorial Medal recipients; (Albert Pugsley, Wayne Vertigan, David Sparrow and Reg Lance). There is a significant lineage of varieties through to modern day varieties; Baudin, Banks, Flinders, Dhow, Sloop and Gairdner which have all benefited from their predecessors; Clipper, Prior A, Shannon and Schooner and Franklin.

Graduate Students Co-Supervised:

I have been fortunate to be involved in the co-supervision of a number of PhD students; mostly at the Waite Institute of the University of Adelaide, but also with Murdoch University and now the University of Queensland. Most of these have gone onto have very successful careers. My philosophy for working with post graduates was to empower them to develop their research and critical analyses skills and to think independently but to be able to work as part of a team.

Table 2: Graduate Students – Past and Current co-supervised

Student	Country	Degree	Thesis Title	Status	Year
Mike Sissons	Australia	PhD	Studies of Barley Limit Dextrinase	Passed	1992
Mandy Jenkin	Australia	PhD	The Genetics of Boron Tolerance in Barley	Passed	1993
Jenny Guerin	Australia	PhD	An Investigation of Endopeptidases in Barley	Passed	1993
Ghodratollah Fathi	Iran	PhD	Nitrogen Responses in Barley	Passed	1994
Young Won Choe	South Korea	PhD	Molecular Genetic Markers for CCN Resistance in Barley	Passed	1995

Chengdao Li	China	PhD	Molecular Genetic Markers for Malting Quality in Barley	Passed	1997
Tran van Diem	Vietnam	PhD	Water use efficiency and drought tolerance in barley.	Passed	1997
Jason Eglington	Australia	PhD	Isolation, characterisation and mapping of alternative alleles for malt enzymes from the wild barley progenitor, Hordeum spontaneum	Passed	2003
Retinder Gill	Australia	PhD	Male sterile facilitated recurrent selection in barley: genetic gains and recombination	Passed	2009
Dipika Roy	Pakistan	PhD	Understanding the genetics of spot blotch resistance in barley	Confirmed	2018
Zerihun Tadesse	Ethiopia	PhD	Stem Rust and Stripe Rust resistances in “Vavilov Diversity Panel” Wheats, CIMMYT and Ethiopian Bread Wheats breeding lines with Ethiopian races “in situ”.	Commenced	2018

Current and Future Directions in Barley Improvement:

Crop improvement is entering a most exciting phase with the integration and implementation of a range of breeding technologies and genetic biotechnologies. We are moving from using bi-parental crosses to identify single genes and quantitative trait loci (QTLs) and employing either simple marker assisted selection (MAS) to facilitate the introgression of desirable genes or to more complex pyramiding of multiple simple/single gene traits.

Furthermore, the integration of MAS with either conventional breeding, doubled haploids (DH), “speed breeding” or Single Seed Descent (SSD) with a crossing technology such as Male Sterile Facilitated Recurrent Selection (MSFRS), couples with both genotypic and phenotypic selection, enables a rapid turnover of cycles with “genetic enrichment” phases to develop populations with a significantly enhanced desirable traits. The hierarchical structuring of crosses and selection for different traits can be referred to as a Reciprocal Recurrent Selection. Phenotypic selection for specific Traits such as Scald Resistance can be done at different nursery sites and then selected progeny combined at a “home” site through crossing.

Genome screening and gene/allele based decision making: The advent of whole genome screening at competitive prices brings the cost of genotyping to be about the same order of magnitude of the cost of two replicates of entries in a yield trial (~$50) . A further reduction in the genotyping costs for a reduced set or sub-set of markers down to $10 per line will encourage the increase in the more general use of genome wide markers rather than just using markers for gene discovery and early genetic selection intervention.

Gene discovery and genetic management software:

Future breeding management software must include modules for genetic analysis and gene discovery as well as assisting in the design of crossing strategies to enable the pyramiding of desirable combination of traits.

Pedigree / Trait databases:

To be able to make appropriate decisions on the selection of parents for crossing a breeder must have access to a data base with all the traits of interest adequately documented. This should include data on the traits as well as the genes/alleles controlling the traits. A comprehensive pedigree data base permits the understanding of the inheritance of the trait and tracking through populations.

Future Directions for Barley Disease Resistance Improvement:

A major weakness of current Australian barley varieties is the lack of resistances *vis-à-vis*; MRMS, MR or R for the significant leaf diseases. This includes: Leaf Rust (LR), Net Form of Net Blotch (NFNB), Spot Form of Net-Blotch (SFNB, Powdery Mildew (PM) Additionally we should include Scald (SC) Spot Blotch (SB), Stem Rust (SR), Barley Yellow Dwarf Virus (BYDV), Barley Grass Strip Rust (BGYR) and the Quarantine Trait; Stripe or Yellow Rust (YR). We could also add Loose Smut, Cereal Cyst Nematode (CCN), Pratylenchus spp. and Russian Wheat Aphid (RWA) resistances.

Table 3: Barley disease resistance ratings for foliar diseases in 2018 (Qld et al.)

Variety	Year PBR	LR	NFNB	SFNB	PM
Gairdner	1999	S	MRMS	S	SVS
Baudin	2003	VS	MSS	MSS	VS
Commander	2010	S	S	MSS	S
Fairview	2010	SVS	MSS	S	R
Scope	2011	S	MR	MSS	MR
Bass	2013	VS	MSS	MSS	S
Compass	2015	VS	MRMS	MRMS	S
Granger	2015	MR	SVS	SVS	R
Spartacus CL	2016	MR	MRMS	SVS	SVS
Flinders	2017	MRMS	MRMS	MSS	R
La Trobe	2017	MSS	MS	SVS	SVS
Banks	2018	S	R	MSS	S
Rosalind	2018	MR	MR	MSS	SVS
RGT Planet	2018	MR	S	S	R

Ratings	R	MR	MRMS	MS	MSS	S	SVS	VS

A few varieties were released with *Rph3* resistance to Leaf Rust (LR) but this gene quickly succumbed to new virulent races and varieties such as Bass, and Fitzroy etc. became VS; a very hard lesson to learn. More recently the use of *Rph20* from European and North American sources has given a degree of Adult Plant Resistance and MR ratings.

Net Form of Net-Blotch has proven to be a challenging disease. Genes for resistance have been well documented on chromosomes 6H, 4H and 3H. It would appear that broad QTL resistance on 6H is more complicated and may be resolved into three separate loci (Fowler 2018 PhD thesis).

Spot Form of Net-Blotch resistance has proven to be difficult to find and incorporate resistances. The "classic" gene *Rpt4* does not gives a low level of resistance. Newer genes are to be found in germplasm collections. For example, the USDA core barley collection has been screened against four SFNB races, including a race origination from Australia. Resistance to this race can be found in Mongolian land races. The USDA core collection has been genotyped with the older DArT markers so identifying the new gene

location should be relatively simple. The Australian Grains Genebank (AGG) has been systematically introducing this collection (and others) over many years.

Powdery mildew resistance has similarly been a significant problem. Our experience was that the use of the *mlo* gene resulted in yield reductions of 5-10%. However varieties such as Fairview, Granger and RGT Planet maintain resistant or "R" ratings from using the *mlo* gene.

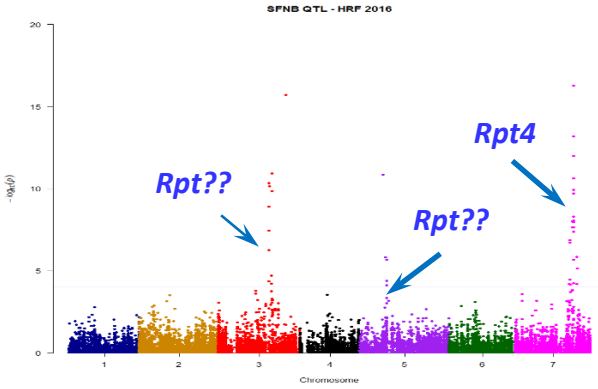

Figure 2a: Spot form of Net Blotch "Manhatten Plots" from NAM_LR (Fowler et al. 2017 unpubl.)

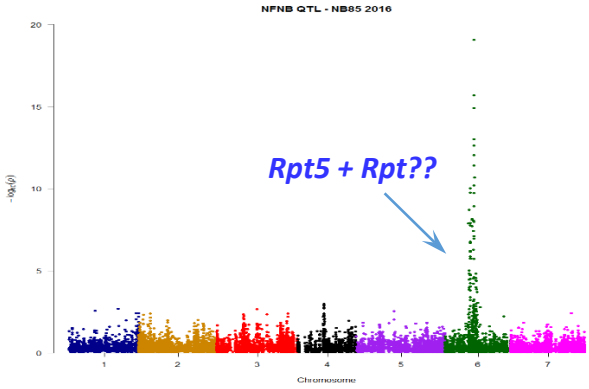

Figure 2b: Net form of Net Blotch "Manhatten Plots" from NAM_LR (Fowler et al. 2017 unpubl.)

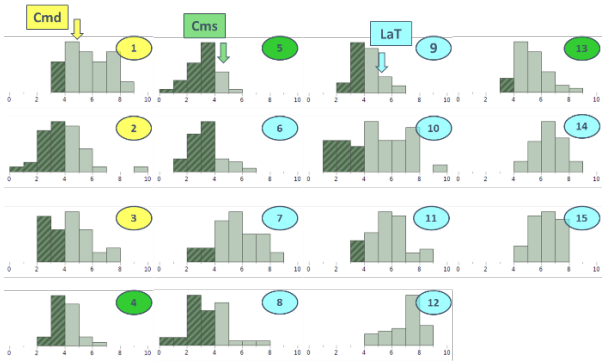

Figure 3a: NAM_DR : Spot Form Net Blotch (SFNB17) populations. 1- 15. Means for reference varieties: Commander (Cmd), Compass (Cms) and La Trobe (LaT) are given for their respective "first" populations. Populations are coded yellow Cmd, dark green Cms and light blue (LaT). Scale is 0-9 where 0 represents more resistance

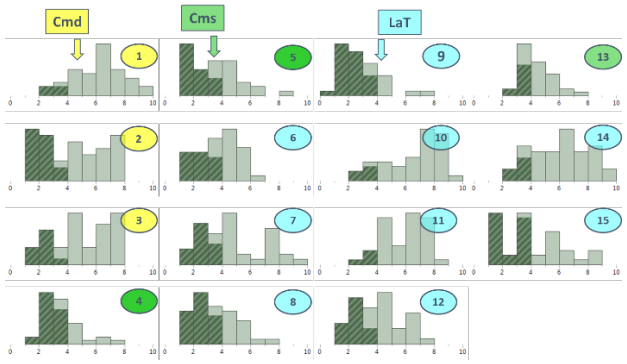

Figure 3b: NAM_DR : Net Form Net Blotch (NFNB17) populations. 1- 15. Means for reference varieties: Commander (Cmd), Compass (Cms) and La Trobe (LaT) are given for their respective "first" populations. Populations are coded yellow Cmd, dark green Cms and light blue (LaT). Scale is 0-9 where 0 represents more resistance

A simplified conceptual scheme is represented in the Figure 4. Disease resistance traits are represented by genes AA, BB, CC and DD. Our starting populations are from Nested Association Mapping (or NAM)

populations where specific lines have been crossed once to several “recurrent” parents. A second and third round of crossing to the “Recurrent” parent will effectively result in BC_2 populations. Populations are both crossed to “recurrent” parents and then selection progeny are intercrossed in pairs of populations e.g. combining AA and BB to give and AABB genotype (or A/a;B/b; homozygote or heterozygote selection). In a second population development CC will be combined with DD and CCDD (or C/c;D/d selected) selected. Finally AABB and CCDD are combined and ultimately AABBCCDD genotypes are selected. An additional round of inter-crossing may be desirable so the final population in Figure 4 is referred to as BC_2 IC_2 populations. Selected lines can be taken through several cycles of “Speed Breeding”

The improvement in the overall disease resistance of Australian barley varieties needs to proceed in two phases. The first is the R&D phase of unique gene/allele discovery including gene mapping. The second phase should be the pyramiding of genes into industry agreed “reference” varieties. The integration of a number of breeding and genetic biotechnologies is the preferred path for success. Male sterile facilitated recurrent selection (MSFRS) would be the preferred technology to effectively and efficiently accumulate the desirable disease resistance genes. The Figure 4 represents a simple scheme to illustrate the general approach. The male sterile (*msg6-rob-sex1*) gene block is back-crossed into the “reference” varieties. The *msg6* is used to facilitate the crossing, selection and inter-crossing of populations which have been selected (both genotypically and phenotypically) for different traits. The NAM populations NAM_LR and NAM_DR have used three reference varieties; Commander, Compass and La Trobe. All populations are therefore 50% reference varieties. Two further crosses to “*msg*-reference” varieties will result in in BC_2 populations. In the simple scheme, two rounds of crossing and inter-crossing would enable the accumulation of genes for four traits. A necessary extension to the simple scheme is to “self” selected F_2 derived lines and then progeny test these for yield, agronomic performance as well as grain quality and NIR predicted malting quality. So not only will selections being made for pyramided disease resistance genes, but the background reference variety germplasm will be selected for overall performance. As an extension to this approach, breeding companies would be encouraged to develop their own propriety “*msg* –reference” varieties or lines to enable the rapid crossing of “selected elite populations” into their own material. Again, the approach would be for the companies to utilise “speed breeding” of their own selected F_2 derived lines for variety development.

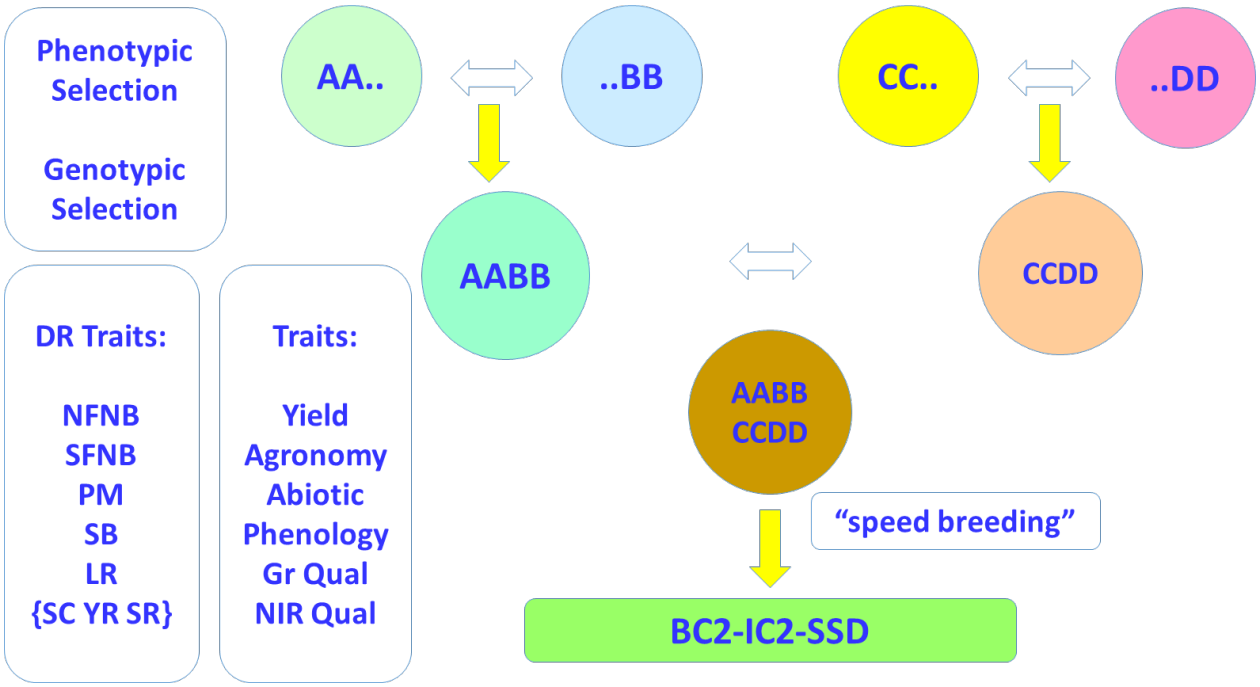

Figure 4: Male Sterile Facilitated Recurrent Selection (MSFRS) for Disease Resistance. A simplified schematic representation of the use of MSFRS, Genotypic and Phenotypic selection to Pyramid multiple genes for disease resistance into various "recurrent" parent backgrounds.

Conclusions:

Barley breeding and genetics has changed significantly over the past forty years or more. Australian malting varieties have improved to the point where they are competitive in the international market place. The longer term future of the barley industry will need to be competitive in relation to other crops. The industry needs to improve the overall barley disease resistance status both; to protect the yield, agronomic and quality improvements achieved but also to reduce the costs of managing multiple disease susceptibilities.

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