*Aust. J. Agric. Res.*, 2001, **52**, 1349–1356

# Implementation of markers in Australian wheat breeding

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*Abstract.* Genetic associations of morphological, biochemical, and DNA markers with economically important traits can be used for indirect selection of the traits. Chromosomal linkage between pseudo-black chaff and the stem-rust resistance gene *Sr2*, and between the red glume gene (*Rg1*) and the stripe rust resistance gene *Yr10*, have been used in this way for many years. Similarly, linkages between disease resistance genes, such as *Sr38*, *Lr37*, and *Yr17*, have been used to achieve resistance to multiple diseases while selection is performed for resistance to one disease. Alleles at the *Glu* loci, assessed as protein differences, have been used as predictors of dough strength. More recently, DNA markers have been developed and used, especially to select for resistance to cereal cyst nematode, a trait which is difficult and expensive to assess with conventional bioassays. We found that the major use of DNA markers was for selection for traits of substantial economic importance, which were primarily determined by a single gene, and where the non-marker assay was expensive and unreliable. The other uses of markers were for pyramiding several genes influencing one trait, or for rapid backcrossing.

*Additional keywords:* DNA markers, protein markers, marker-assisted selection.

## Introduction

The concept of using linked genes to follow the inheritance of genes controlling other traits was first envisaged at the beginning of modern genetics. For example, Sax (1923) observed an association between seed colour in beans (*Phaseolus vulgaris* L.), a simply inherited trait, and seed weight, a quantitatively inherited trait. Although not explicitly stated, Sax obviously understood that selection for appropriate combinations of seed colours would indirectly influence seed weight. Later, Rasmussen (1935) observed an association between flower colour in peas (*Pisum sativum* L.), again simply inherited, and flowering time, a quantitatively inherited trait, and conclusively showed that this was due to chromosomal linkage between genes controlling these traits. These reports implied that easily characterised genes can be used as markers in practical plant breeding for other linked genes.

Markers used in plant breeding programs fall into 4 broad categories: morphological, linked disease resistance genes, biochemical, and DNA-based. Morphological markers produce phenotypes which can be readily identified, but which are not usually of direct economic importance. Their value is due to close linkage with economically important traits. In the case of closely linked disease resistance genes, selection for one trait ensures the presence of the other trait. The other two categories are broadly classified as molecular markers. Biochemical markers produce an enzyme or storage protein, which can be identified by biochemical assays. As with morphological markers, their value can be due to close linkage with a trait of economic importance. However, sometimes these


10.1071/AR01067 0004-9409/01/111349

proteins are the determinants of the economic trait, for example, glutenin proteins and dough properties (Payne 1987). Because recombination does not occur between these markers and the trait of interest, they have sometimes been called 'perfect' or 'diagnostic' markers. Strictly, because they are the determinants of traits, it could be argued that they are not markers. However, some are important in wheat breeding, and will be considered as markers in this paper. The fourth major category consists of DNA markers, which identify molecular differences among genes determining traits of interest, or of DNA segments linked to genes determining the traits of interest.

Many types of DNA markers have been developed (see Gupta *et al.* 1999 for a recent review), but only restriction fragment length polymorphisms (RFLPs), allele-specific polymerase chain reaction markers (AS-PCRs) and microsatellites, or simple sequence repeats (SSRs), have been used to date for marker-assisted selection of wheat (*Triticum aestivum* L.) in Australia. These are described in detail elsewhere in this issue (Langridge *et al.* 2001; Rampling *et al.* 2001). RFLPs have been used to study the genetic diversity of Australian wheat varieties, and group elite germplasm (Paull *et al.* 1998).

To be of value for marker-assisted selection in practical cultivar development, a marker must be closely linked to a gene controlling an economically important trait, must be polymorphic amongst parents so that the desirable allele can be distinguished from the undesirable one, and must be cost-effective. These requirements can be relaxed for other aspects of wheat breeding, such as the classification of germplasm, proprietary identification of cultivars or lines, or selection of parents.

Our objective in this paper is to describe, and provide a rationale for, the current use of markers for the development of wheat cultivars in Australia, especially those used for marker-assisted selection.

**Table 1. DNA markers being used for cultivar development of wheat in Australia from July 1999 to June 2000**

| Organisation | Trait | Gene | Approximate number of assays |
|---|---|---|---|
| Agriculture Victoria | CCN resistance | *Cre1* | 2600 |
| | CCN resistance | *Cre3* | 1320 |
| | Noodle quality | *Wx-B1* | 164 |
| | Boron tolerance | *Bo1* | 510 |
| | Boron tolerance | *Bo2* | 510 |
| | Stem rust resistance | *Sr2* | 197 |
| University of Adelaide | Yellow flour colour | —[A] | 500 |
| | Boron tolerance | *Bo1* | 150 |
| | Grain protein | *Nor1* | 20 |
| | Stem rust resistance | *Sr2* | 200 |
| | *Pratylenchus neglectus* resistance | *Rlnn1* | 200 |
| Murdoch University | Noodle quality | *Wx-B1* | 3700 |
| CSIRO Plant Industry | Carbon isotope discrimination | —[A] | 500 |
| | Coleoptile length | *Rht8* | 250 |
| | Tiller inhibition | *tin* | 60 |
| | BYDV | *Bdv2* | 250 |
| University of Sydney | VPM rust resistance | *Sr38/Lr37/Yr17* | 150 |
| | Stem rust resistance | *Sr2* | 200 |
| | Stem rust resistance | *Sr39* | 150 |
| | Noodle quality | *Wx-A1,Wx-B1,Wx-D1* | 300 |
| | Grain protein | —[A] | 60 |

[A]Quantitative trait locus: not yet named.

## Morphological markers

Several linkages between morphological markers and traits of economic importance have been used in Australian wheat breeding. Of particular economic importance has been the use of pseudo-black chaff, or high-temperature-induced seedling chlorosis, to select for resistance to stem rust (causal organism *Puccinia graminis* Pers. f. sp. *tritici*) conferred by the gene *Sr2* (Brown 1993). Resistance conferred by *Sr2* has been particularly durable, and is characterised by slow rust development and low terminal rust responses on field-grown, adult plants (McIntosh *et al.* 1995). It is recessively inherited, making it difficult to detect in segregating populations, especially in the presence of other rust-resistance genes (Brown 1993; McIntosh *et al.* 1995). The use of morphological markers has greatly facilitated the use of *Sr2*. A PCR-based DNA marker for the detection of *Sr2* is now available, and is currently being used by breeding programs (Table 1).

Two other morphological markers have been used for breeding for resistance to rust in Australia. Firstly, there exists a close genetic association (2% recombination) of the red-glume gene (*Rg1*) with *Yr10*, a gene conferring resistance to stripe rust (causal organism *P. striiformis* Westend. f. sp. *tritici*). This linkage was first reported by Metzger and Silbaugh (1970) and glume colour was used by the National Cereal Rust Control Program to select for the presence of *Yr10* in targeted crosses. Glume colour, determined by the *Rg1* gene, was also used in South Australia to select for *Yr10*, and to select for specific glutenin alleles at the *Glu-B3* locus, which is also located on chromosome 1B (McIntosh *et al.* 1998). Secondly, leaf-tip necrosis, conditioned by the *Ltn* gene, is closely linked with *Lr34*, a gene conferring resistance to leaf rust (causal

organism *P. recondita* Rob. Ex Desm. f. sp. *tritici*), and *Yr18*, a gene conferring resistance to stripe rust. These associations were studied in detail by Singh (1992*a*, 1992*b*). Leaf-tip necrosis, along with leaf rust development pattern, is used in the National Cereal Rust Control Program to predict the presence of *Lr34/Yr18* in advanced breeding lines.

Reduced tillering shows promise for slowing water use and increasing grain size in environments characterised by terminal droughts (Richards 1988). Genotypic differences in tiller number can be difficult to assess among segregating families unless evaluated in the field. Chromosomal linkage between the glume pubescence (*Hg*) and reduced tillering (*tin*) gene on 1AS enables visual selection of reduced tillering via differences in glume pubescence. Glume pubescence is highly penetrant, enabling selection in the glasshouse. Linkage for the *Hg* and *tin* alleles is not especially strong (10 cM), allowing non-pubescent, low tillering recombinants to be readily identified during inbreeding.

## Linked disease resistance genes

Several examples of linked disease resistance genes are listed in McIntosh *et al.* (1998). These include *Sr39/Lr35*, *Sr38/Lr37/Yr17*, *Sr24/Lr24*, *Sr9g/Yr7*, *Sr31/Lr26/Yr9/Pm8*, *Sr23/Lr16*, and *Lr19/Sr2*. These sources offer the advantage of indirect selection for resistance to more than one disease while selection is performed for one. The combinations *Sr38/Lr37/Yr17*, *Sr24/Lr24*, and *Sr9g/Yr7* have been routinely used in breeding for disease resistance in Australian breeding programs through collaborations with the National Cereal Rust Control Program. The use of disease resistance genes derived from cereal rye (*Sr31/Lr26/Yr9/Pm8*) has, until recently, been limited to soft- and feed-wheat breeding programs. Secalins associated with the chromosome segment carrying these genes produce undesirable dough properties for bread-making (Barnes 1990). Lukaszewski (2000) reported secalin-lacking sources of *Sr31*, which are now being used in the National Cereal Rust Control Program (H. S. Bariana unpublished data).

## Biochemical markers

### *Enzyme markers*

Isozymes have been used as markers for linked traits of economic importance in several crops (Stuber 1992), but have not been used in applied wheat breeding in Australia. In tomato (*Lycopersicum esculentum* L.), close linkage between a rare allele at the acid phosphate locus (*Aps-1*) and a gene controlling nematode resistance (*Mi*) greatly facilitated the transfer of resistance into adapted cultivars by companies throughout the world (Tanksley 1983).

The appearance of black point, a dark discoloration at the embryo end of a grain of wheat, has been associated with specific peroxidase isozymes (Williamson 1997). The absence of these isozymes could be used as a marker for selection against this undesirable grain characteristic. However, development of an assay for the isozymes through to the implementation stage has been difficult and the emphasis is now on the development of DNA-based markers for use in selection (P. M. Williamson pers. comm., 2001).

### *Storage protein markers*

Glutenins are a major component of the storage protein in wheat (see Gras *et al.* 2001, this issue, for a recent review). Two major types of glutenins have been recognised: high molecular weight and low molecular weight glutenins. Genes coding for the high-molecular-weight subunits of glutenin are located at the *Glu-A1*, *Glu-B1*, and *Glu-D1* loci on the long arms of chromosomes 1A, 1B, and 1D, while those coding for the low molecular weight glutenins, *Glu-A3*, *Glu-B3*, and *Glu-D3*, are on the short arms of the same chromosomes (Jackson *et al.* 1983; Gupta and Shepherd 1990). *Glu-3* loci are very closely linked with genes coding for gliadins (*Gli-A1*, *Gli-B1*, and *Gli-D1*).

Proteins produced by these genes are polymorphic and readily detected by polyacrylamide gel electrophoresis. For example, a total of 31 glutenin alleles across the 6 loci were identified in the breeding programs of the University of Adelaide, Roseworthy, and Victorian Institute for Dryland Agriculture, Horsham (Eagles *et al.* 2001*b*). The minimum number of alleles detected was 3 each for *Glu-A1* and *Glu-D3*, with a maximum of 9 alleles for *Glu-B1* (Eagles *et al.* 2001*b*). Furthermore, many more alleles have been detected world-wide (McIntosh *et al.* 1998), making these genes useful markers for linked genes on Group 1 chromosomes.

Glutenins have a major influence on dough properties, especially dough strength (Payne 1987). A careful selection of combinations of different alleles at homoeologous Group 1 loci often predicts dough strength with reasonable accuracy. They have been used in Australia for this purpose, especially in the wheat breeding programs at the Wagga Wagga Agricultural Institute, New South Wales (P. Martin pers. comm., 2001), and at Roseworthy, South Australia. In the Roseworthy program, in addition to carefully considering glutenin allele composition of parents, more than 600 breeding lines are evaluated each year for their glutenin allele composition. Lines are selected or discarded for grain quality based on this glutenin allele characterisation. Selection over time has had a substantial effect on allele frequencies. For example, *Glu-B1e*, which codes for band 20 and was widespread in cultivars in the 1980s (Lawrence 1986), had reached a low frequency by 1996 and was almost completely eliminated by 1998 (Eagles *et al.* 2001*b*). Currently, the elimination of *Glu-A3e* is a major focus of this South Australian program.

Eagles *et al.* (2001*b*) used data from the Roseworthy and Horsham breeding programs to estimate dough strength and extensibility for the 31 alleles detected in these programs.

For 18 of the alleles, all 2-way interactions were present, enabling predictions of Rmax and extensibility for the 648 genotypes (3 *Glu-A1* × 3 *Glu-B1* × 2 *Glu-D1* × 3 *Glu-A3* × 4 *Glu-B3* × 3 *Glu-D3*) which are possible for these 18 alleles. These predictors are now being used in these breeding programs to select for the quantitative traits dough strength and extensibility, based solely on glutenin allele composition. Furthermore, the predictors have been linked to QU-GENE (Podlich and Cooper 1998) to provide predictions of progeny frequencies from crossing specific parents, as described by Eagles *et al.* (2001*a*).

## DNA markers

DNA markers are now being used for at least 19 genes, or chromosome segments, for cultivar development in Australian wheat breeding programs (Table 1). There is extensive use of DNA markers for selection of resistance to cereal cyst nematode (causal organism *Heterodera avenae* Woll.), determined by the *Cre1* and *Cre3* genes, in the program of Agriculture Victoria based at Horsham. A RFLP marker, which is completely linked to the *Cre1* gene, has been developed (Ogbonnaya *et al.* 1998, 2001) and used for more than 2500 assays over the 12 months from July 1999 to June 2000. Although expensive, implementation was considered cost-effective because of the importance of the disease to wheat production in southern Australia and the high cost and unreliability of the bioassay (Ogbonnaya *et al.* 2001). Attempts are being made to convert the *Cre-1* RFLP marker to an AS-PCR. The second gene, *Cre3*, was originally identified in *Triticum tauschii* (Eastwood *et al.* 1991) and has subsequently been transferred into hexaploid wheat. An AS-PCR, which shows complete linkage with the resistant allele, has been developed (Ogbonnaya *et al.* 1998, 2001), and more than 1300 assays were performed using this marker from July 1999 to June 2000 (Table 1). The development of markers for both resistance genes is allowing *Cre1* and *Cre3* to be combined in the same cultivar, a difficult objective to achieve with bioassays in a classical backcrossing procedure (Ogbonnaya *et al.* 1998).

DNA markers for *Bo1* and *Bo2*, genes for tolerance to high levels of boron, were used in the Agriculture Victoria and University of Adelaide breeding programs (Table 1). The genetic control of boron tolerance appears to be complex, with 2 or more genes necessary for full tolerance (Jefferies *et al.* 2000). Thus, a major benefit of using DNA markers for boron tolerance is for combining independent genes for tolerance. Currently, many breeding lines carrying the marker alleles for *Bo1* and/or *Bo2* do not contain the boron tolerance genes (F. C. Ogbonnaya and N. C. Subrahmanyan unpublished data), possibly due to recombination between the marker and boron genes. This is limiting the use of marker-assisted selection for boron tolerance.

A DNA marker for *Wx-B1b*, the null allele important for quality of white-salted noodles (Yamamori *et al.* 1994), was used for the development of wheat lines with white-salted noodle quality by Agriculture Victoria (Table 1). It was used extensively by Murdoch University to develop cultivars for Western Australia (McLauchlan *et al.* 2001, this issue), where white-salted noodle production is a high priority (Anderson *et al.* 1997). An ELISA assay has now been developed for *Wx-B1* (Gale *et al.* 2001, this issue) and is likely to be much more cost-effective than the DNA marker currently being used.

Marker-assisted selection is being used to develop waxy wheat cultivars in a project funded by the Quality Wheat Cooperative Research Centre (Shariflou and Sharp 1999). They are using SSRs linked to each of the homoeologous waxy genes, *Wx-A1*, *Wx-B1*, and *Wx-D1*, individually and in multiplex assays, to identify triple nulls in a backcross breeding program (M. R. Shariflou pers. comm., 2001). SSR markers are also being used to enrich the background genotype of the recurrent parent, and hence reduce the number of backcross generations required to obtain agronomically elite lines (Shariflou and Sharp 1999).

When milled, some important cultivars produce an undesirable, yellow-coloured flour. A RFLP marker for flour colour (Table 1) has been used by the University of Adelaide in a rapid backcrossing program to develop versions of these cultivars with desirable white flour. This marker was developed from a partial linkage map of the region of chromosome 7A important for determining flour colour in this genetic material (Parker *et al.* 1998).

As discussed previously, *Sr2* is a valuable gene for resistance to stem rust in Australia. A PCR-based marker is now being used to select for the presence of this gene in the Agriculture Victoria, University of Adelaide, and University of Sydney breeding programs (Table 1). The DNA marker has overcome the environmental sensitivity problems associated with the expression of the morphological marker. However, for successful exploitation of this DNA marker, parental polymorphism needs to be checked for each cross (Sharp *et al.*, this issue). In some instances the DNA marker is being used in conjunction with the morphological marker.

In the National Cereal Rust Control Program at the University of Sydney, marker technology has been combined with pathogen testing to produce lines with combinations of rust resistance genes. Markers linked with *Sr38/Lr37/Yr17* and *Sr39* have been used for pyramiding of the stem rust resistance genes (Table 1). Bariana *et al.* (2001) used *Xpsp3000*, a SSR marker, and *Gli-B1* markers to differentiate *Yr10* (brown glumes) from *Yr10vav* (white glumes). The absence of *Yr10vav* linked to the *Xpsp3000* allele in 65 current Australian cultivars indicated that the marker could be used in marker-assisted selection. Parental polymorphism at the *Xpsp3000* locus needs to be checked if this marker is to be used for selection for *Yr10*.

An AS-PCR, described by Khan *et al.* (2000) as a marker for a gene from *Triticum turgidum* ssp. *dicoccoides* for high grain protein content, is being used in the breeding program of the University of Sydney (Table 1). Initially, it was used to detect the gene in donor lines. Now, this marker is being used for selection in a backcrossing program aimed at improving grain protein content.

Molecular markers are being used to complement phenotypic selection for physiological traits in the CSIRO Plant Industry wheat improvement program. Many of the target traits have been identified in overseas germplasm or superseded Australian cultivars. Hence, backcrossing is used to introgress new genes into adapted, high grain quality, Australian backgrounds. An AS-PCR marker is being used to cull progenies for improved transpiration efficiency (Table 1). Selection for this marker reduces the number of lines assessed for costly $^{13}C/^{12}C$ discrimination using a mass spectrometer. Identification of wheats containing the *Rht8* dwarfing gene has been simplified through the *Rht8*-linked wheat microsatellite, gwm261 (Korzun *et al.* 1998). A new, solid-phase PCR test is currently being developed to facilitate rapid screening of this dwarfing gene (K. Gale pers. comm., 2001). Markers have also been identified for the Agropyron-derived, barley yellow dwarf virus (BYDV)-resistance gene (Table 1). This is an important marker given the difficulty in phenotypic selection for BYDV resistance in the field (Zhang *et al.* 2001, this issue).

## Use of markers in other plant species in Australia

The importance of wheat in Australian agriculture suggests that it should be among the first candidates for the application of molecular markers. However, in comparison to other species, the wheat genome is large and is estimated to be 16000 Mb, whereas *Arabidopsis thaliana* is 130–140 Mb, and rice 400 Mb (Arumuganathan and Earle 1991). Thus, the complexity of the genome of hexaploid wheat has made it a relatively difficult species for marker application. Consideration of the relative extent of applications of markers to other plant species in Australia may help to define the specific issues associated with marker application in Australian wheat breeding programs, to identify opportunities for further applications, and hence to delineate those factors limiting application of markers.

Some examples of applications of molecular markers in Australian plant improvement programs are given in Table 2. Use of markers in variety identification and characterisation of germplasm has been relatively widespread. Use in breeding selection has been much more limited. Rice (*Oryza sativa* L.) and sunflower (*Helianthus annus* L.) breeding programs have used markers for selection. As with wheat, the targeted traits have mainly been important characteristics controlled by single genes. Resistance to rust (causal organism *P. helianthi* Schw.) in sunflower (Lawson *et al.* 1998), and fragrance in rice (Garland *et al.* 2000), are 2 examples of this type of character. However, the use of markers in Australia appears to be predominantly in barley (*Hordeum vulgare* L.), where 18 DNA markers were being used in 2001 for biotic stresses, abiotic stresses, and quality traits (Barr *et al.* 2000). Additional DNA markers to those listed by Barr *et al.* (2000) are now also being used for malt extract, diastatic power, α-amylase, and low β-glucan (F. C. Ogbonnaya and D. C. Moody unpublished data). A major use of DNA markers is for enrichment of complex cross $F_1$s, with a substantial use also for accelerated backcrossing (Barr *et al.* 2000). The rapid application of markers in barley could be partly attributed to the initial

**Table 2. Examples of applications of markers in other plant species in Australia**

| Species | Type of marker | Application | Reference |
|---|---|---|---|
| Banana | RAPD/STS | Selection of normal lines | Damasco *et al.* 1996 |
| Barley | RFLP/SSR/AFLP | Breeding | Barr and Langridge 1999 |
| Barley | RFLP/AS-PCR | Marker assisted selection | Barr *et al.* 2000 |
| Barley | STS | Variety distinction | Ko and Henry 1994 |
| Barley | Molecular beacon | Transgene detection | Kota *et al.* 2000 |
| Barley | RAPD | Breeding support | Poulsen *et al.* 1996 |
| Barley | STS | Transgene detection | Vickers *et al.* 1996 |
| Grape | AFLP | Variety distinction | Scott *et al.* 2000 |
| Mango | RAPD | Germplasm evaluation | Bally *et al.* 1996 |
| Navy bean | RAPD | Variety distinction | Graham *et al.* 1994 |
| Rice | SSR | Germplasm evaluation | Garland *et al.* 1999 |
| Rice | SSR | Marker assisted selection | Garland *et al.* 2000 |
| Rice | RAPD | Variety distinction | Ko *et al.* 1994 |
| Riceflower | RAPD | Variety distinction | Ko *et al.* 1996 |
| Sugarcane | SSR | Variety distinction | Cordeiro *et al.* 2000 |
| Sunflower | RAPD | Germplasm evaluation | Lawson *et al.* 1994 |
| Sunflower | STS | Marker assisted selection | Lawson *et al.* 1998 |

generation of barley maps through both the Australian National Barley Molecular Marker Program and the North American Barley Genome Mapping Project (Marquez-Cedillo *et al.* 2000), which focussed on economically important traits in germplasm of importance to barley breeders. Such maps are now becoming available for wheat (Kammholz *et al.* 2001, this issue) and should accelerate the application of markers in wheat.

Wheat displays a very low level of DNA polymorphism relative to the other species, resulting in greater difficulty in identifying genetic markers of use in breeding populations. The limited number of known important single genes, and the low level of DNA polymorphism in commercial wheats, have probably limited the rate of marker application in wheat. Progress in genomics through comparative mapping, candidate gene studies, and genome-wide mapping is likely to identify many more genes for analysis, while tools for the detection of single-base differences (Edwards and Mogg 2001) are likely to provide the extra sensitivity needed to find useful polymorphisms at gene loci controlling traits of economic importance to wheat breeding.

## Discussion

Young (1999), referring to DNA-based markers, stated that 'though marker-assisted selection now plays a prominent role in the field of plant breeding, practical outcomes are rare'. He went on to point out that marker-assisted selection is a young field, and was cautiously optimistic about its future. A similar conclusion could be drawn for the application of marker-assisted selection to wheat breeding in Australia. Also, the example he gave for effective, practical use of markers in the USA was for selection for resistance to soybean cyst nematode (causal organism *H. glycines*). This is analogous to the use of markers for selection of resistance to cereal cyst nematode in Australia. In both cases the disease is of substantial economic importance, the resistance is primarily due to a single gene, and the bioassay is expensive and unreliable. Interestingly, the best example of the practical use of an isozyme marker was to develop tomatoes resistant to nematodes (Tanksley 1983).

With the possible exception of selection for *Wx-B1b*, for which a reliable and relatively inexpensive swelling power assay is available (McCormick *et al.* 1991; Crosbie *et al.* 1992), the other use of DNA markers in Australia was mainly for marker-assisted selection for traits where inheritance was primarily due to a single gene and the assay was either expensive or unreliable. The other uses of markers were for pyramiding or rapid backcrossing. In contrast to barley (Barr *et al.* 2000), they have not been widely used for enrichment of complex cross $F_1$s.

Although the relationship between glutenin alleles and dough strength has been known for decades (Payne 1987), these allelles have been used more for defect elimination than for developing cultivars with optimum dough strength and extensibility. Recent work that has allowed a quantitative prediction of dough rheological properties from glutenin composition is likely to greatly enhance these glutenin genes as markers for improving grain quality for specific markets. Because of the complexity of simultaneously using markers at 6 loci to make predictions, a situation compounded by epistatic interactions, Eagles *et al.* (2001*b*) considered computer software capable of predicting dough strength and extensibility from allele composition an important adjunct to the use of these protein markers. This type of software could be used for the simultaneous use of markers for several traits in a marker-assisted selection program (Eagles *et al.* 2001*a*).

RFLPs are still being used for marker-assisted selection, although in almost every case where these are being used, an attempt is being made to convert them to an AS-PCR. Hybridisation-based RFLP assays are not amenable to the large number of lines to be screened in typical plant-breeding programs. A major effort is going into developing SSRs (Harker *et al.* 2001, this issue), but except for the development of waxy wheats (Shariflou and Sharp 1999), these have yet to be used for cultivar development. SSRs are PCR-based, cheaper to use, and amenable to automation. With the identification of SSRs, we expect an accelerating use of this type of DNA marker in Australian wheat breeding over the next few years.

Although the emphasis of this paper has been on marker-assisted selection, mapping associated with marker development provides information on inheritance of traits, especially numbers and chromosomal locations of genes controlling traits of economic importance. Such information can be extremely useful for identifying parents and determining selection strategies. For example, the identification of close coupling linkage between resistance to *Pratylenchus neglectus* and yellow flour colour in South Australian germplasm explained the high frequency of *P. neglectus*-resistant lines in advanced generations which had undesirably high yellow flour (K. Williams pers. comm., 2001). This knowledge led to a major effort to identify recombinant lines and should greatly assist the development of cultivars that combine resistance to *P. neglectus* with white flour colour.

## Acknowledgments

We thank Professor N. C. Subrahmanyan, Dr Russell Eastwood, and Mr David Moody, Victorian Institute for Dryland Agriculture; Dr Peter Williamson, Leslie Research Centre; Dr Kevin Williams, University of Adelaide; Dr Neil Howes, South Australian Research and Development Institute; Dr N. Ahmed and Dr M. Shariflou, University of Sydney; Dr Peter Martin, New South Wales Agriculture; and Dr Richard Richards and Dr Kevin Gale, CSIRO Plant Industry, for provision of information.

Much of the work reported in this paper was funded by the Grains Research and Development Corporation. Their contribution is gratefully acknowledged.

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Manuscript received 16 March 2001, accepted 11 June 2001