# VARIATION AND GENETIC CONTROL OF PROLAMINS IN TETRAPLOID WHEATS AND THEIR ASSOCIATION WITH QUALITY IN DURUM WHEAT

by

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**Dedicated to my wife, son,**

**my parents and relatives,**

**whom I love**

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# SUMMARY

Durum wheat (*Triticum turgidum* var. *durum*) is an important crop used for production of pasta, couscous, and in some areas of the world, various types of bread.

Low-molecular weight (LMW) glutenin subunits are a major protein group deposited in the wheat endosperm and are recognized as having a large influence on gluten quality. In the present work, the variation and genetic control of the LMW glutenin subunits in durum wheat and other tetraploid wheats have been studied. The association between some of these LMW, and also high-molecular weight (HMW) glutenin subunits with physical dough properties has been investigated. In particular, the effect on dough properties of transferring chromosome 1D from bread wheat into durum wheat has been investigated.

The variation and genetic control of the LMW glutenin subunits in tetraploid wheats had not previously been systematically investigated, mainly because of the unavailability of an appropriate protein fractionation technique. However, the development of a 1-D two-step SDS-PAGE [Singh and Shepherd (1988a), Gupta and Shepherd (1990a)], and more recently a more simple procedure (Singh *et al*, 1991) has stimulated further work on these topics.

The genetic variation in the LMW glutenin subunit composition of 240 durum wheat lines from a world collection showed much variation, particularly in the lines originating from North Africa and Mediterranean regions. However, the majority of the lines tested had one or other of three frequent patterns, including the LMW-2 type (Pogna *et al*, 1990) commonly occurring in commercial lines. All of the B subunit patterns could be classified into 26 groups assumed to correspond to 17 *Glu-B3* and nine *Glu-A3* linkage blocks based on their association with particular γ-gliadin bands and the mutual exclusivity of the patterns. Some of these allelic designations were confirmed later by inheritance studies. The gliadin patterns were also variable and five types of band groupings could be recognised based on the γ-gliadin standards coded by the *Gli-B1* locus. The γ-45 type was present in 84% of the lines including most of the commercial varieties.

A total of 281 wild tetraploid and some other cultivated tetraploid wheat lines (*T. turgidum* var. *dicoccoides*, *T. dicoccum* and *T. polonicum*) were also analysed for their variation in LMW glutenin subunit and gliadin band patterns. The extent of variation observed was much greater than that recorded within the durum wheats. The complexity of these band patterns indicated that the wild tetraploid wheats are an important source of variation with a potential to improve cultivated wheats.

The inheritance of several different LMW glutenin subunit patterns and the major γ- and ω-gliadin bands encoded by the *Glu-3* and *Gli-1* loci was studied using the test-cross approach. A double 1RS wheat-rye translocation line lacking all LMW glutenin subunit bands (B subunits) was used as the third parent in test-cross. It was produced in durum Langdon, by crossing it with the hexaploid wheat line Chinese Spring-Gabo 1AL.1BL.1RS and selecting

among the progeny for plants lacking 1AS and 1BS prolamin markers. It was found that all of the B subunits in the tetraploid wheats analysed were controlled by genes on chromosome arms 1AS and 1BS, and the tight linkage between the *Gli-1* and *Glu-3* loci was confirmed. Altogether four test-cross progenies were studied including two parents each crossed to Edmore and Langdon, the latter having known bands controlled by 1A and 1B, acting as controls. In the Langdon crosses, all of the B subunits and major gliadin bands were inherited as units (blocks), as previously reported. However, both crosses to Edmore yielded rare recombinant test-cross progeny, involving recombination between genes on 1BS-controlled B subunits with a 3.1% recombination rate, suggesting the existence of another locus coding for B subunits (tentatively *Glu-B4*). Evidence was also obtained for the presence of an additional proximal locus (tentatively *Glu-B2*) in Edmore, controlling a B subunit of glutenin and loosely linked (21.2 ± 3.2 cM) with the major *Glu-B3* locus. In contrast, the major gliadin components were retained as linkage blocks through out all progeny. Two rare recombinants were detected between the *Glu-A3* and *Gli-A1* loci.

Turning to the wheat quality investigations.

Firstly, a large set of durum wheat lines (79 including eight advanced breeding lines) from a world collection and 11 bread wheat cultivars were grown in replicates at two field locations to compare the yield and gluten quality differences between these two species of wheat. The durum wheats tended to possess higher grain protein concentrations (14.0% vs. 11.9%) and gave lower grain yield than bread wheat. The SDS-sedimentation (SDSS) test was used to evaluate the gluten strength. All the durum lines studied gave much lower values than the bread wheat controls (45.8 ml vs. 76.2 ml). Specific gliadin and glutenin markers were found to be well associated with flour quality attributes except the known HMW glutenin subunits at *Glu-A1* locus. Cultivars having the gliadin band γ-45 or (LMW-2) type generally gave stronger gluten than lines with band γ-42 (LMW-1 type), as reported by earlier workers. Cultivars having the HMW glutenin subunits coded by *Glu-B1* genes such as 13+16, 7+8 were highly correlated with dough strength as they have shown in bread wheat. The combined better alleles at *Glu-B1* and *Glu-3* or *Gli-B1* showed cumulative effects for dough strength. The Australian advanced lines had higher yield and better dough strength than other durums from other countries except those from CIMMYT, which also had 1~1.5% higher protein concentration and equal or better grain yield than the bread wheat, suggesting that these lines had better potential for commercial use. However, the best durums tested so far were still poorer in dough strength when compared to that of bread wheat as measured by the SDS-sedimentation test, indicating that future breeding work should be directed into breeding stronger dough durums for both pasta-/bread-making purposes while keeping the yield potential.

In the second investigation, a set of D-genome disomic substitution lines in Langdon background was used to study the main effects of the individual D-genome chromosomes on flour technological properties. Most of these quality characters were highly inter-correlated. Chromosome substitutions 1D, 5D, 2D and 7D gave higher SDSS values, and only

chromosome substitution 1D had positive effects on the proportion of glutenin ($P_1$%) values measured by SE-HPLC, than the euploid parent Langdon. Principal Component Analysis showed that the predominant effect on durum wheat rheological properties was from the chromosome 1D substitution, which confirmed the potential importance of this chromosome for improving the bread-making quality of tetraploid wheats. The chromosome substitution 5D had a major effect on grain hardness (50% decrease) and increased the whiteness of the flour.

A study was carried out to elucidate the effect of chromosome 1D in tetraploid wheat. Two substitution lines of durum Langdon [Langdon 1D(1A) and Langdon (Edmore-1B)] were chosen as parents and $F_2$-derived progeny lines were selected having either chromosome 1D or 1A and with one of two different alleles at the *Glu-B3* locus. Quality tests on the selected lines demonstrated that although the grain protein concentrations were similar, significant genotypic differences for flour quality characteristics existed. The quality parameters such as $P_1$%, SDSS values, and $P_1/P_2$, dough mix time and peak resistance were all increased in genotypes carrying the Edmore LMW-2 bands compared to the Langdon LMW-1 bands, and the effects were even more pronounced (2-fold increases) with the substitution of chromosome 1D for 1A. The quality characters of genotypes carrying both chromosome 1D and the LMW-2 genes of Edmore were cumulative for all characters except for the mixograph parameters peak resistance and resistance break down.

In another experiment, these $F_2$-derived progeny lines were included in trials with ordinary durum and bread wheats with the aim of comparing the flour quality of these diverse genotypes. Grain protein concentration was negatively correlated with grain yield among the hexaploid but not among the tetraploid wheats. The $P_1$% in the flour measured on the HPLC was consistently correlated with all of the mixograph quality parameters. Durum wheat lines carrying the LMW-2 prolamin band gave similar $P_1$% and $P_{1-1}$% values to those of medium strength bread wheats but exhibited large differences in dough mixing properties of these different types of wheat. The 1D(1A) substituted durum lines reached the medium dough strength of hexaploid wheats, but had a longer mixing time than all of the bread wheats. Although the $F_2$-derived types-III and -IV possessed dough strength poorer than the best bread wheats, these $F_2$-derived genotypes exhibited very different mixing properties from both durum and bread wheats. This study suggested that the large functionality differences between doughs made from durum and hexaploid wheats might be due to seed endosperm components other than seed protein composition.

The implications of the results presented in this thesis on our understanding of the genetics and evolution of the genes controlling LMW glutenin subunits and gliadins in durum wheat and other tetraploid wheats and their involvement in improving the bread-making qualities are discussed.

# DECLARATION

This work contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text.

I give consent to this copy of my thesis, when deposited in the University Library, being available for loan and photocopying.

SIGNED: DATE: 17/3/94

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# ABBREVIATIONS USED IN THE THESIS

| | |
|---|---|
| 1-D | one-dimension(al) |
| 2-D | two-dimension(al) |
| AWCC | Australian Winter Cereal Collection |
| BC | backcross |
| BBD | bandwidth break down (of mixograph) |
| BWAP | bandwidth at peak (of mixograph) |
| CS | Chinese Spring |
| cf. | compare |
| cv. | cultivar |
| DTr (4x) | double-1RS translocation line (tetraploid) |
| Edm | Edmore |
| HMW | high molecular weight |
| HMWGS | HMW glutenin subunits |
| HPLC | high-performance liquid chromatography |
| HRS | Hard Red Spring |
| Ldn | Langdon |
| LMW | low molecular weight |
| LMWGS | LMW glutenin subunits |
| MW | molecular weight(s) |
| MT | mix time (of mixograph) |
| $P_1$ | glutenin fraction (by SE-HPLC) |
| $P_{1-1}$ | highly aggregated glutenin fraction (by SE-HPLC) |
| $P_{1-2}$ | intermediate aggregated glutenin fraction (by SE-HPLC) |
| $P_2$ | gliadin fraction (by SE-HPLC) |
| $P_3$ | non-prolamin fraction (by SE-HPLC) |
| PAGE | polyacrylamide gel electrophoresis |
| PBW | peak bandwidth (of mixograph) |
| PC | protein concentration |
| pers. comm. | personal communication |
| PMCS | pollen mother cells |
| PR | peak resistance (of mixograph) |

| | |
|---|---|
| RAC | Roseworthy Agricultural College |
| RBD | resistance break down (of mixograph) |
| SDW | Saddleworth |
| SD | standard deviation |
| SDS | sodium dodecyl sulphate |
| SDSS | SDS-sedimentation |
| SE | standard error |
| SE-HPLC | size-exclusion HPLC |
| TPBW | time to peak bandwidth (of mixograph) |
| TC | test-cross |
| Waite Institute | Waite Agricultural Research Institute |

# CHAPTER 1. GENERAL INTRODUCTION

Durum or macaroni wheat (*Triticum turgidum* L. var. *durum*) is an important crop used for production of pasta, couscous, and in some areas of the world, various types of bread (Boggini and Pogna, 1989). This type of wheat occupies approximately 20 million hectares worldwide, spread over many countries, accounting for 8% of the total world wheat production (Bozzini, 1988). More than half of the total cultivation lies in the Mediterranean area: South Europe, North Africa and Southwest Asia, where tetraploid wheats were domesticated around 10,000 to 15,000 BC (Bozzini, 1988). Around 80% of durums are cultivated in West Asia and North Africa (Srivastava *et al*, 1988). The annual world durum wheat production for the 1979-81 period was estimated to be around 27.5 million metric tons (Srivastava *et al*, 1988). Historically, the yield level of durum wheat is around 80% of bread wheat and this has been attributed partly to less favourable crop growing environments and management practices (Srivastava *et al* 1988).

In the regions of West Asia and North Africa approximately 15% of the durum wheat is consumed in the form of pasta products, 50% is processed into single- and two-layered flat breads and the remainder is used for raised breads. In other areas of the world, such as Southern Europe and the Americas, nearly all durum wheat is used for pasta production and the surplus grain is exported (Bozzini, 1988; Srivastava *et al*, 1988). In the past, Australian farmers have been reluctant to grow durum wheat, due to its reputation of having lower yields than bread wheats. However, newly developed lines have been produced with an equal or even superior yield potential to the highest yielding bread wheat, and since the price of durum is often higher than bread wheat, it is a promising and viable alternative crop. In the current economic situation, there are larger markets for durum wheat, both for domestic consumption and for export to the developing countries where a greater demand of food supply is needed due to ever increasing populations.

The storage proteins in the endosperm of wheat seeds have been shown to be the most important determinants of the functional qualities of the flour products (Elton and Ewart, 1967; Kasarda *et al*, 1976). These proteins constitute more than 80% of the total proteins in the grain (Osborne, 1907) and can be divided into two main groups, the gliadins and glutenins. Extensive studies have been carried out on the variation and genetic control of durum and bread wheat (*Triticum aestivum*) gliadins and glutenins and their association with wheat end-use quality (see reviews by Garcia-Olmedo *et al*, 1982; Kreis *et al*, 1985; Shepherd, 1988; MacRitchie *et al*, 1990). About ten years ago, a strong relationship was first found between the banding patterns of gliadin polypeptides separated by polyacrylamide gel electrophoresis (PAGE) and the gluten quality of the endosperm proteins of durum wheat (Damidaux *et al*, 1978, 1980b). The presence of the γ-gliadin band 45 and the absence of band 42 was found to be associated with strong gluten. This important discovery has provided a strong impetus to the search for similar relationships between other protein components and gluten properties.

The low-molecular-weight (LMW) glutenin subunits, although accounting for about 80% of the total glutenin fraction, have not been studied extensively until recently due to the lack of a suitable protein fractionation technique. When new methods of assaying for the LMW glutenin subunits were developed, such as a 1-D two-step SDS-PAGE [Singh and Shepherd (1988a), Gupta and Shepherd (1990a)], and recently, a more simplified procedure (Singh *et al*, 1991) at the Waite Institute (Chapter 3), this has provided the technique to answer the question of how much variation exists among tetraploids in these LMW glutenin components and their association with the functional properties of dough. This is of particular interest, since not only have the LMW glutenin polypeptides in durum wheat been shown to be highly correlated with gluten strength (du Cros, 1987a) but there is a further suggestion that they actually determine dough viscoelastic properties of durum wheat (Pogna *et al*, 1988; Feillet *et al*, 1989). Hence Chapter 4 focuses on the characterization of the variability of the previously unidentified LMW glutenin types in a survey of a large number of tetraploid wheats including *T. durum* , *T. dicoccoides*, *T. dicoccum* and *T. polonicum*.

Following the identification of the nature of variability of LMW glutenin subunits (LMWGS) and gliadins in the tetraploid wheats, further information was required on the genetic control of these bands, since there have been no detailed studies on the inheritance of LMW glutenin subunits, except for the LMW-1 and LMW-2 types. A test-cross approach was undertaken to study the inheritance of some of the variant band patterns detected in the survey. This included the breeding of a special genetic stock lacking all the LMW glutenin B subunits to be used as the third parent in the test-crosses (Chapter 5).

Although there is a wide range of gluten quality among durum wheats, the durums are not as strong as Hard Red Spring (HRS) wheat grown under comparable conditions (Finney *et al*, 1987). However, good quality durums are unique in a cooking quality possessing a readily produced pasta that maintains good texture, resists surface disintegration and retains a firm structure, characteristics not evident in bread wheat flours (Cubadda, 1989). The basic quality differences between the pasta-cooking and bread-making quality is still not clear. The present study aimed at firstly investigating the major technological quality differences between durum and bread wheat wholemeal/flours from a normal varietal survey (Chapter 6) and secondly studying the rheological changes in the durum background as influenced by genes on D-genome chromosomes of bread wheat (Chapter 6) as well as the storage protein genes from chromosome 1D (Chapter 7).

# CHAPTER 2. LITERATURE REVIEW

## 2.1 INTRODUCTION

The modern era of wheat seed protein chemistry began in the 1960's after the introduction of improved separation techniques for these proteins following-up the classical early studies of Osborne (1907). During the last two decades a great deal of research work has been carried out at the molecular and cytological level on the seed storage proteins of wheats to determine the biochemical and genetical basis of the differences in flour technological characteristics shown by different wheat types and varieties (reviews by Pomeranz, 1971; Shepherd, 1988; MacRitchie *et al*, 1990). Much progress has been made, in understanding the inheritance, quality association and evaluation of wheat seed storage proteins, and more recently, their molecular biology. The present review of literature covers articles published before 1991 and relevant to designing the current research program. Relevant literature published after that date has been included in the results and discussion sections. The extensive literature on the genetical control of proteins in hexaploid wheat will not be reviewed in detail but reference will be made to this work when it is relevant to an understanding of specific issues in tetraploid wheats.

## 2.2 CLASSIFICATION OF SEED STORAGE PROTEINS

Seed proteins can be classified on the basis of their solubility, chemical composition, or biological function.

Osborne (1907) classified plant proteins into four groups, based mainly on their protein solubility. The albumins were soluble in water, globulins in dilute salt solutions, prolamins in aqueous alcohols, and the glutelins in dilute alkaline or acidic solvents. The albumins and globulins corresponded principally to the metabolically active proteins while the other two fractions were storage proteins termed gliadins and glutenins, respectively (review by Wall, 1979) or collectively the gluten proteins (review by Miflin *et al*, 1983). These proteins were synthesized on the endoplasmic reticulum and deposited in protein bodies during endosperm development in the grain (review by Shewry and Miflin, 1985). They were storage proteins and a source of nitrogen and sulphur for the seedling during and after germination. Recently, with improved knowledge of their amino acid sequence, all the gluten proteins have been classified as prolamins (review by Kreis *et al*, 1985).

The classification of wheat seed proteins based on extractability was recently criticized as being too simple in concept, since more recent research has shown that each of these solubility classes included a complex mixture of proteins and a large amount of overlap existed between the Osborne fractions (review by Shewry and Miflin, 1985). For example, ethanol extracts obtained from seed endosperm without reduction and termed 'classical gliadins', have been

shown to contain aggregated fractions called 'aggregated gliadins' (Shewry *et al*, 1983a). Similarly, glutenin fractions have been shown to contain gliadin-like subunits (Bietz and Wall, 1973).

The degree of overlap between the 'classical' fractions was increased when the extraction procedure was modified. When reducing agents were used for extraction, or if the ethanol was acidified, or replaced by a higher alcohol such as n-propanol, or the temperature was raised, the more 'classical' glutenin fraction would be extracted in the 'gliadin' solvent (Jackson *et al*, 1983). Although Osborne's original extraction procedures have been modified in many minor ways, the problem of cross-contamination of protein fractions still remained and new criteria were required to define the gluten proteins more precisely (reviews by Shewry and Miflin, 1985; Khan and Chakroborty, 1987). Although the classical nomenclature is still widely used and accepted by cereal chemists, its inadequacies must be acknowledged.

Gluten proteins have been classified by their ability or inability to form aggregates in the wheat endosperm (Huebner, 1970; Payne and Corfield, 1979). Proteins which remained monomeric corresponded to the classical gliadins, and they comprised a complex mixture of single-chain polypeptides with MW ranging from 30,000 to 80,000 daltons. These were rich in glutamine (30~ 55 mol %) and proline (15~ 30 mol %), had relatively few ionizable side chains and did not form intermolecular disulphide-bonds (Wall, 1979). When fractionated by gel electrophoresis at low pH, they separated into four groups of components, termed $\alpha$, $\beta$, $\gamma$ and $\omega$ with decreasing mobility (Woychik *et al*, 1961). Glutenins were large, heterogeneous aggregated molecules with MW ranging from one to several million, consisting of two groups of subunits, the high-molecular-weight (HMW) subunits and the predominant low-molecular-weight (LMW) subunits (review by Wall, 1979). The HMW subunits comprised a group of at least 20 different subunits (Lawrence and Shepherd, 1980) with apparent MW ranging from 65 to 130 kilodaltons (KD) while the LMW subunits also included many different subunits ranging in size from 12 to 60 KD (Autran *et al*, 1987).

Recently, Shewry *et al* (Reviewed in 1986) proposed a different classification and nomenclature system for gluten proteins based on the biochemical and genetical relationships of the individual subunits rather than their aggregating properties. In this system, both gliadins and glutenins were referred to as prolamins and they were divided into three groups: the S-rich prolamins ($\alpha$-, $\beta$-, and $\gamma$-gliadins as well as LMW glutenin subunits), the S-poor prolamins ($\omega$-gliadins), and HMW prolamins. They are characterised as follows:

### 2.2.1 The Sulphur-rich (S-rich) prolamins

This group accounted for 80-90% of the total prolamins for grain grown under normal nutrient conditions but the proportion decreased disproportionately with low sulphur supply (Moss *et al*, 1981; Wrigley *et al*, 1984). Proteins in this group were distinguished mainly by their relatively large content of S-amino acids, and they could be sub-divided further based on electrophoretic mobility and their N terminal amino acid sequences (review by Kreis *et al*,

1985). One was the α-type, one was the γ-type and another was the aggregated gliadin type, which were now called LMW glutenin subunits (LMWGS) and will be discussed in section 2.4.3.1 (Also review by Kreis *et al*, 1985). The three most mobile groups of gliadins (α-, β-, γ-gliadins) when separated on Acid-PAGE were all S-rich prolamins.

### 2.2.2 The Sulphur-poor (S-poor) prolamins

This group of proteins, characterised by the almost complete absence of sulphur-amino acids, contained a very high proportion of glutamine/glutamic acid and proline and relatively higher content of phenylalanine than other prolamins. These three amino acids made up about 80% of the total amount in the approximate proportions of 4 : 3 : 1. The S-poor prolamins occurred almost exclusively in ethanol extracts and constituted about 10-20% of the total prolamins in the seeds of wheat plants grown under normal nutrition (Wrigley *et al*, 1984). They were the ω-gliadins in Acid-PAGE electrophoresis and had three distinct but related types of N-terminal amino acid sequences (review by Kreis *et al*, 1985).

### 2.2.3 The HMW Prolamins

These proteins were characterised by their high content of glycine (15-20 mol %) (review by Kreis *et al*, 1985) and lower amounts of proline (11-17 mol %) and phenylalanine than other prolamins, and accounted for 10% of the total seed protein in bread wheats (Payne, 1987a), and could be easily distinguished by their low mobility upon SDS-PAGE (Payne and Corfield, 1979). Glutamate accounted for between 30-39% of the total amino acid residues, but they contained relatively low amounts of basic and acidic amino acids (Shewry *et al*, 1984). These proteins occurred almost exclusively as disulphide-linked aggregates and thus needed to be extracted under reducing conditions (review by Kreis *et al*, 1985).

## 2.3 FRACTIONATION OF WHEAT STORAGE PROTEINS

### 2.3.1 Fractionation of wheat storage proteins by gel electrophoresis

Electrophoretic fractionation techniques based mainly on gel electrophoresis (eg. Bushuk and Zillman, 1978), gel isoelectric focusing (Wrigley, 1968a), sodium dodecyl sulfate (SDS) gel electrophoresis (Payne *et al*, 1979) and two-dimensional fractionation by gel electrophoresis (Wrigley and Shepherd, 1973; Payne *et al*, 1985), has formed the basis for evaluation of gluten composition in recent studies (review by Shewry and Miflin, 1985). The advantages of electrophoretic separation techniques were that they required only a very small amount of material (as little as a half kernel or less) and a large number of samples could be analysed in a brief period. The electrophoretic band patterns were genotypically determined and not usually modified by the growing environment of the grain analysed (Lee and Ronalds, 1967; Wrigley and Shepherd, 1973).

### 2.3.1.1 *Gliadin separation*

Moving boundary electrophoresis was first introduced to separate gliadins at acidic pH without reduction. The gliadins were classified into four groups, α, β, γ and ω components depending on their mobility (Jones *et al*, 1959; Woychik *et al*, 1961). Starch-gel electrophoresis at low pH (3 to 3.2) was developed by Woychik *et al* (1961) and was commonly used during the 1970's for gliadin separations and analyses (review by Shepherd, 1988). The resolution of protein bands was generally poor by the previous techniques and great improvement of resolution was achieved by using polyacrylamide gel electrophoresis (PAGE) (Bushuk and Zillman, 1978). This has been used extensively since then for screening cereal germplasm collections (Damania *et al*, 1983), cultivar identification (eg. Bushuk and Zillman, 1978; Tkachuk and Mellish, 1980; Jones *et al*, 1982; Wrigley *et al*, 1982a, 1982b; du Cros *et al*, 1982; Khan *et al*, 1983, 1985; Sapirstein and Bushuk, 1985) and as a means of detecting fraudulent addition of bread wheat flour to pasta products (Burgon *et al*, 1985).

After the improved separation of gliadin components by PAGE, the second achievement in fractionation of gluten proteins was the introduction of gel isoelectric focusing (IEF), which fractionated proteins in a pH gradient according to differences in their isoelectric points (Wrigley, 1968a, 1968b). An important application has been the two-dimensional (2-D) combination of IEF with gel electrophoresis, which has greatly improved the resolution of protein bands. For example, a single cultivar revealed about 45 gliadin components in 2-D separation (Wrigley and Shepherd, 1973) while only 30 bands could be identified in the best 1-D procedure (Joppa *et al*, 1983a). Different systems had since been developed for various purposes of fractionation. Wrigley (1970) separated gliadins using gel electrofocusing in the first dimension, followed by starch gel electrophoresis at pH 3.1 in the second dimension. Du Cros and Wrigley (1979) modified this procedure and used acrylamide gels for separations in both dimensions with the improved separation. Payne *et al* (1984d) adopted a system using Acid-PAGE in the first dimension followed by SDS-PAGE in the second dimension while Lafiandra and Kasarda (1985) modified the procedure used by Mecham *et al* (1978) that carried out electrophoresis in the same gel slab at two different pHs. All of these 2-D fractionation systems have achieved much better resolution of protein components. However, with 2-D procedures, only a limited number of samples could be loaded per gel and the complexity of the procedure prevented its wide application to screen large number of samples in genetics and breeding programs. Furthermore, the glutenin fraction was poorly separated in Acid-gels and IEF gels because in their native state, they were too large to enter the gel matrix as discrete units and were separated as a streak from the gel origin (Woychik *et al*, 1961; Bietz and Wall, 1972).

### 2.3.1.2 *Glutenin separation*

The early one-dimensional separation systems (section 2.3.1.1) were not well suited for the fractionation of the glutenin subunits and the introduction of the discontinuous system of one-dimensional (1-D) SDS-PAGE by Laemmli (1970) improved the resolution dramatically.

When wheat glutenins were reduced they gave a large number of polypeptide components, ranging in apparent MW up to 140 KD while their charge differences were masked by the presence of SDS, so that they could be separated according to their electromobility in 1-D SDS-PAGE. This system was introduced to wheat independently by Payne and Corfield (1979) and Lawrence and Shepherd (1980). Four groups of bands referred to as the A, B, C, D subunits of glutenin could be identified (Jackson *et al,* 1983). Group A was the HMW glutenin subunits (HMWGS) while the other groups collectively were the LMWGS. Gliadins were separated as a series of components in the SDS gel patterns in the size range from 30 ~ 80 KD and overlapped some of the LMWGS when both were present in the sample. The banding patterns determined using SDS-PAGE provided an additional means identifying wheat cultivars (Lawrence, 1986) and for studying evolutionary processes (Lawrence and Shepherd, 1980, 1981a; Payne *et al*, 1981a; Vallega *et al*, 1988b). It has since also been used extensively for the genetical analysis of HMWGS (reviews by Miflin *et al*, 1983; Shepherd, 1988).

When IEF was combined with SDS gel electrophoresis to give two dimensional electrophoretic fractionation, the resolution of proteins was much improved, because fewer components overlapped compared to one dimensional separations. The best resolution of bands achieved by such procedures was the 1300 different endosperm protein components detected by Tkachuk and Mellish (1987). In particular, this procedure permitted for the first time separation of the α-, β-, γ-gliadins from the LMWGS and nongluten proteins (Jackson *et al*, 1983), although it was time-consuming. The later development of a 1-D two-step SDS-PAGE procedure (Singh and Shepherd, 1985) allowed large scale analyses of LMW glutenin subunit patterns in cultivar surveys and inheritance studies, which could not achieve by the 2-D approaches (review by Shepherd, 1988).

The combination of these improved methods of protein fractionation with analyses of the seed storage proteins present in aneuploid stocks of Chinese Spring (Sears, 1954), has formed the foundation for studies on the genetics of wheat proteins (Boyd and Lee, 1967; Shepherd, 1968, 1988). Electrophoretic patterns were used in genetic and biochemical investigations of the complex loci coding for these wheat proteins (Mecham *et al*, 1978) and for distinguishing between species and genera (Ladizinsky and Hymowitz, 1979) and for a range of applications in breeding studies as well (Wrigley, 1980). Over the last 15 years, individual gliadin bands and LMW and HMW subunits of glutenin have been associated with differences in the pasta-/bread-making qualities of wheat flours. These findings have led to screening tests for quality in the early generations of durum (Damidaux *et al*, 1978; du Cros *et al*, 1982; Pogna *et al*, 1985a, 1985b; Taha and Sagi, 1987; Damania and Samaroo, 1988) and bread wheat (Payne *et al*, 1979, 1984b, 1987a, 1987b, 1988a) improvement programs.

### 2.3.2 Fractionation of wheat storage proteins by high-performance liquid chromatography

Chromatographic techniques have been used for many years as an important fractionation tool in gluten protein studies (review by Bietz, 1986). However, the early column chromatographic techniques were poor in resolution, when compared to the electrophoretic techniques for protein separation. The advent of high-performance liquid chromatography (HPLC) methods has advanced these techniques greatly (review by Wrigley and Bietz, 1988), which were based on silica-based packing, could withstand high pressure and flow rate, and had low adsorptive characteristics. Major advances in recent years in chromatography media and methodology, has permitted the application of other HPLC techniques to gluten protein fractionation. These developments have led to separation based on hydrophobicity (reversed-phase HPLC) (RP-HPLC), MW (size-exclusion HPLC) (SE-HPLC), and charge properties (ion-exchange HPLC) (reviews by Bietz, 1985, 1986). Automatic data handling and sorting of elution profiles was also possible with HPLC (review by Bietz, 1986).

The high resolving power obtained with RP-HPLC allowed a dilute salt extract of wheat grain to be separated into nearly 50 components and an ethanol extract of the residue into 24 peaks of protein absorbance (Bietz, 1983) and could be applied to wheat varietal identification (reviews by Bietz, 1984, 1986). The procedure was sensitive (requiring only micrograms of protein) and rapid (taking 60 mins for each chromatogram, or quicker if slightly less resolution was acceptable). Gliadin RP-HPLC patterns have been used for genetic studies and for predicting durum wheat cooking quality (Burnouf and Bietz, 1987).

SE-HPLC has extended our understanding of the size range of gluten proteins both in the native state and as reduced polypeptides complexed with SDS (reviews by Bietz, 1984, 1986). In particular, it provided another method for estimating the MW of individual components of gluten (Bietz, 1985).

## 2.4 VARIATION AND GENETICS OF PROLAMINS OF TETRAPLOID WHEATS

This following review will focus on the genetic variation and molecular biology of wheat gluten protein in the tetraploid wheats.

### 2.4.1 Gliadins

The 1-D gliadin electrophoregrams of durum wheats were generally different from hexaploid wheat due to the absence of the ω-gliadins controlled by chromosome 1D, which migrated less than 18 units from the origin in acid-PAGE (Jones *et al*, 1982; Damania *et al*, 1983).

### 2.4.1.1 *Inheritance and linkage mapping*

The chromosomal location of these genes in hexaploid wheat was first described independently by Boyd and Lee (1967) and Shepherd (1968) after aneuploid analysis and this was followed later by more detailed studies (Wrigley and Shepherd, 1973; Mecham *et al*, 1978; Brown *et al*, 1979; Sozinov and Poperelya, 1980, 1982; Payne *et al*, 1982a, 1984d). Six loci coding for hexaploid wheat gliadins have been located on the short arms of chromosomes 1 and 6 of the A, B and D genomes (Wrigley and Shepherd, 1973; Kasarda *et al*, 1976; Brown *et al*, 1979; Brown and Flavell, 1981; Payne *et al*, 1982a). These results have been confirmed in durum wheat by Lafiandra *et al* (1983). These studies revealed that no major rearrangements have taken place in the group 1 chromosomes during the evolution of polyploid wheats from their diploid progenitors (review by Payne, 1987c).

The genetical control of gliadins in tetraploid wheats has not been investigated in detail, mainly because of the difficulty in producing aneuploids (monosomics or nullisomics) in tetraploids because they had only two pairs of homoeologous chromosomes. However, Joppa *et al* (1978, 1983a) have developed Langdon D-genome disomic-substitution lines, in which all seven D genome chromosomes have been individually substituted for their respective A and B genome chromosomes of Langdon. By analysing these substitution lines, they were able to identify the chromosomal control of 19 out of 30 gliadin bands by 1-D Acid-PAGE in Langdon (Joppa *et al*, 1983a) and these results were confirmed by 2-D analysis (du Cros *et al*, 1983).

Mapping of these gliadin protein loci in durum and bread wheat has largely relied on segregation studies of progeny from specific crosses (Sozinov and Poperelya, 1980, 1982; Lafiandra *et al*, 1983; Payne *et al*, 1984a, 1984d; Singh and Shepherd, 1988a, 1988b), indicating independent inheritance of proteins with genes located on different chromosomes or different arms of the same chromosome. The gliadin components were inherited mainly as different blocks with intrablock recombination being very rare (Sozinov and Poperelya, 1980, 1982). Damidaux *et al* (1980a) failed to detect any recombination between certain ω- and γ-gliadins coded by genes on chromosome 1B in the segregating progeny from two tetraploid wheats. Similar conclusions were reached by Bebykin and Kumakov (1981) from inheritance studies in durum wheat. Margiotta *et al* (1987) suggested that a possible recombination event has occurred at the *Gli-B1* locus in an Italian durum wheat 'Berillo', since the ω-components usually linked to the γ-45 band were present along with the γ-42 bands.

#### (A) *Gli-1* loci

The *Gli-1* loci were located on the short arms of group 1 chromosomes and have been shown to carry genes controlling three groups of endosperm storage proteins (β-, γ- and ω-gliadins) (Payne *et al*, 1982a), and to be closely linked with genes controlling the LMWGS (Jackson *et al*, 1983) in bread wheats. These three types of gliadin showed homology in their primary structure as defined by N-terminal amino-acid sequence and by the nucleotide sequence of cDNA and genomic clones (Bartels and Thompson, 1983; Okita *et al*, 1985), indicating that

the genes have evolved from a common ancestral gene. Nevertheless, the ω- and γ-gliadins belonged to distinctive groups as the former lacked sulphur amino-acids and the latter had much higher proportions of glutamine and proline and lower proportions of charged amino acids (Kasarda *et al*, 1976).

The *Gli-1* complex loci each contained several separate genes. However, the number of genes detected by quantitative southern hybridizations was quite controversial as Harberd *et al* (1985) reported to have the same number while others claimed that they might have two or more different genes at each locus (Thompson *et al*, 1983; Forde *et al*, 1985; Harberd *et al*, 1985). Payne (1987c) reviewed that these loci might possess similar numbers of genes coding for ω-gliadins and LMWGS, presumably to be about 9-15 genes at each *Gli-1* locus.

## (B) *Gli-2* loci

The *Gli-2* loci were located on the short arm of group 6 chromosomes and they probably contained only one gene family due to the closely related primary structures among them (review by Payne, 1987c). The number of genes at *Gli-2* was also quite conflicting as Harberd *et al* (1985) estimated these to be 9-12, Okita *et al* (1985) 33 and Payne (1987c) assumed one or two genes with one or more pseudogenes at each *Gli-2* locus.

The close homology between genes at the *Gli-1* and *Gli-2* loci i. e. between γ-, α-, and β-gliadins (Okita *et al*, 1985) indicated that they probably arose from the duplication and divergence of a common ancestral gene (review by Payne, 1987c). The present location of these loci on chromosomes 1 and 6 might have resulted from an ancient interchromosomal translocation in an ancestral line prior to the evolution of the A, B, and D genome diploid species (Shepherd and Jennings, 1970).

## (C) *Gli-3* locus

Galili and Feldman (1984) observed a ω-component (B30) located at the *Gld-B6* locus (where *Gld* ≡ *Gli*), 28.1 cM from the *Gli-B1* locus on chromosome arm 1BS, whereas Metakovsky *et al* (1986) calculated the recombination frequency for a gene coding for one ω-gliadin band to be 29% from the *Gli-B1* locus. Jackson *et al* (1985) also described an additional locus coding D-subunits of glutenin, hence named *Glu-B2*, about 22 cM from the *Gli-B1* locus. Later Payne *et al* (1988b) found that the *Gld-B6* and *Glu-B2* loci were allelic and renamed this locus as *Gli-B3*, because the protein appeared to conform to ω-gliadins (Payne *et al*, 1988b). Sobko (1984) also observed that a minor gliadin component was coded by a separate locus *Gld-2-1A* on the short arm of chromosome 1A showing 31% recombination with *Gli-A1*, comparable to the *Gli-B3* locus on chromosome 1B. In addition, three other possible loci on chromosome 1A controlling ω-gliadins were detected also, showing 13%, 4% and 1% recombination with the *Gli-A1* locus (Metakovsky *et al*, 1986). Nevertheless, this result needs to be confirmed since these bands were rather faint in their experiments.

### 2.4.1.2 *Allelic variation of gliadin components in tetraploid wheats*

Much allelic variation for gliadins has been detected in bread wheats (Sozinov and Poperelya, 1980, 1982). Considerable banding pattern variation was also found in landraces and the collections of primitive durum cultivars from different countries (Margiotta *et al*, 1987; Dominici *et al*, 1988; Damania and Somaroo, 1988). The gliadin banding patterns in some durum landraces consisted of up to 38 biotypes (Damania *et al*, 1983). In contrast, limited genetic diversity has been detected among the commonly grown durum wheat cultivars (Damidaux *et al*, 1978; Jones *et al*, 1982; du Cros *et al*, 1982).

The variation in γ-gliadins has been surveyed extensively because of the apparent association of γ-gliadins with quality parameters (Damidaux *et al*, 1978, 1980a; du Cros *et al*, 1982). Gliadins γ-45 and γ-42 were the two main types present in durum wheats, coded for by groups of allelic genes at a single complex locus on chromosome 1BS (Joppa *et al*, 1983a; Lafiandra *et al*, 1983; du Cros and Hare, 1985). Some other γ-gliadin variants have also been reported but their association with quality parameters was not studied in detail (Pogna *et al*, 1985b; Margiotta *et al*, 1987).

The frequent presence of the γ-45 band and the absence of a γ-42 band in *T. dicoccoides* from Israel indicated that the γ-45 allele might have originated from a primitive source, and that the γ-42 was of more recent origin, possibly arising as a mutation during the domestication of tetraploid wheat (Kushnir *et al*, 1984). Comparing the variation at the *Gld-B1* (≡ *Gli-B1*) locus in durum cultivars with hexaploid wheats, indicated that the 1B-encoded blocks of all the durum wheats clearly differed from those found in bread wheat, indicating that durum wheat could not be an ancestor or a recent derivative of bread wheat (Metakovsky *et al*, 1989). For instance, the chromosome 1B-controlled bands of bread wheat did not include either the γ-45 or γ-42 alleles of durums.

There have been limited studies of the variation at the *Gli-A1* and *Gli-2* loci in durum wheat. Metakovsky *et al* (1989) were the only one who reported three different 1A-encoded blocks present in most of the durum cultivars similar to those identified in common wheats (i. e. *Gld-1A2*, *Gld-1A18*, *Gld-1A19*). The *Gli-A2* locus on chromosome 6A controlled mostly α-gliadins whilst *Gli-B2* on homoeologous chromosome 6B controlled both α-gliadins and γ-gliadins (du Cros, *et al*, 1983; Lafiandra *et al*, 1983), which agreed with the earlier results on the study of chromosomal control of gliadins in hexaploid wheat (Wrigley and Shepherd, 1973). Kudryavtsev *et al* (1987) found five distinct α-gliadin variants (blocks) in durum wheats whereas Autran and Galterio (1989a) identified four major allelic types at the *Gli-A2* locus and two at the *Gli-B2* locus. The α-gliadins in many durum cultivars appeared to be similar or identical to some chromosome 6A-controlled gliadin blocks in common wheat (Kudryavtsev *et al*, 1987).

## 2.4.2 HMW glutenin subunits

### *2.4.2.1 Characterization of HMW glutenin subunits*

The HMW glutenin subunit (group A subunits) profiles of durum wheat cultivars were generally similar to those of hexaploid wheat cultivars except for the absence of the *Glu-D1*-coding bands (Bietz *et al*, 1975; Galili and Feldman, 1983c). Furthermore, two groups of very sharp faint bands were often observed in the HMW subunit region. One exhibited slower and the other faster mobility than the classical HMWGS of hexaploid wheats, in the SDS-PAGE patterns of tetraploid and wild diploid wheats (Margiotta *et al*, 1987; du Cros, 1987a; Galili *et al*, 1988). These bands were assumed to be post-translational modifications of the major subunits of HMW glutenin alleles of tetraploid wheats and some of them overlapped the faster moving 1D bands of hexaploid wheat (Galili and Feldman, 1983b; Payne *et al*, 1987c). Furthermore, du Cros (1987a) observed several faint bands (7 out of the 16) within the classical HMW subunit region, which did not occur in hexaploids. Some of these bands appeared to correspond to new alleles designated by Vallega (1986, 1988b) and Branlard *et al* (1989) later.

### *2.4.2.2 Genetical control (Glu-1)*

The genetics of the HMWGS of bread wheat was well documented. The genes coding for these HMW subunits have been designated *Glu-A1*, *Glu-B1* and *Glu-D1* and were located on the long arms of homoeologous group 1 chromosomes (Lawrence and Shepherd, 1980; Payne *et al*, 1980, 1982b) and occurred as complex loci (Payne and Lawrence, 1983; Galili and Feldman, 1983a). Each locus has been shown to comprise a tightly linked cluster of genes and displayed multiple allelism (Lawrence and Shepherd, 1980; Payne *et al*, 1981a; Payne and Lawrence, 1983). Similarly, the HMWGS of tetraploid wheats were also coded by the same two complex loci (i. e. *Glu-A1* and *Glu-B1*) (Bietz *et al*, 1975; Lawrence and Shepherd, 1981a, 1981b). The same HMW glutenin subunit allele symbols of hexaploid wheat have been adopted for the genomes of tetraploid wheat, since band(s) with the same mobility in both species of wheat were assumed to be controlled by the same alleles.

Recently it has been confirmed by molecular methods that each *Glu-1* locus contained only two genes (Harberd *et al*, 1986). These two genes were closely linked and coded for 'x' and 'y' type subunits on the basis of electrophoretic mobility and isoelectric point differences (Payne *et al*, 1981a). The x type subunits had only about half the number of cysteine residues as y type subunits (Forde *et al*, 1985; Payne, 1987c). The subunits 1Ax, 1Bx and 1Dx were more similar to each other than to 1By and 1Dy subunits (Galili and Feldman, 1985; Payne *et al*, 1983). In bread wheat, both the 'x' and 'y' subunits of the *Glu-B1* and *Glu-D1* loci were expressed, whereas the 1Ax subunits were present in some cultivars of bread wheat but the 1Ay subunits were never expressed (Harberd *et al*, 1986). The same patterns of expression for the *Glu-B1* and *Glu-A1* subunits occurred at the tetraploid level (Levy *et al*, 1988). However, both x and y subunits of *Glu-A1* were present in most accessions of diploid *T. monococcum*.

It is assumed that the 1Ay genes must have become inactive in the diploid wild ancestor of the A genome, or at the tetraploid level prior to the formation of durum and bread wheats (Waines and Payne, 1987).

Additional, minor HMWGS, such as the 1Bz subunits (Holt, 1981) and the *Glt-B3* subunits (Galili and Feldman, 1983b), were considered to be post-translational modifications of major 1By (= *Glu-B1y*) subunits. Similarly, the minor *Glt-A2* subunits (Galili and Feldman, 1983b) were likely to be derived from the major 1Ax (= *Glu-A1x*) subunits, 1 and 2*, and not from the products of separate genes (review by Payne, 1987c).

### 2.4.2.3 *Allelic variation of HMW glutenin subunits in tetraploid wheats*

#### (A) *T. turgidum* var. *durum*

Galili and Feldman (1983c) observed extensive variation in the HMW glutenin patterns of durum wheat cultivars. Pogna *et al* (1985a) detected nine different *Glu-A1* and *Glu-B1* alleles in a survey of 52 cultivars and found three new alleles which had not been reported among bread wheats (Payne and Lawrence, 1983). Thirteen novel alleles were identified by other workers, four controlled by *Glu-A1* and nine by *Glu-B1* (Vallega, 1986, 1988a; Margiotta *et al*, 1987; Branlard *et al*, 1989). Nearly all the durum cultivars studied lacked major *Glu-A1* subunits, and some *Glu-B1* alleles (eg. *Glu-B1c* and *Glu-B1i*) presented in hexaploid wheats (Vallega, 1986, 1988a).

#### (B) *T. turgidum* var. *dicoccum*

Galili and Feldman (1983c) noticed extensive variation of the HMW glutenin subunit patterns. Vallega and Waines (1987) identified a total of 20 different *Glu-A1* and *Glu-B1* alleles amongst 167 accessions from diverse origins. Three *Glu-A1* and six *Glu-B1* alleles, which had not previously been found in bread wheats (Payne and Lawrence, 1983), were detected in cultivars from Italy, Germany, India and USSR.

The frequency of each of the different *Glu-1* alleles in *T. dicoccum* differed markedly from that in common wheat. The 'null' *Glu-A1c* allele was observed with a frequency of only 21% in *T. dicoccum* (mainly of Ethiopian origin), compared to a frequency of about 44% in bread wheats (Payne and Lawrence, 1983) and with an even higher frequency among durums (82%, Vallega, 1988b). However, the mean number of major *Glu-A1* and *Glu-B1* subunits found in individual *dicoccum* accessions (2.45 subunits per accession) was similar to that observed in the modern hexaploid cultivars (2.35 subunits) studied by Payne and Lawrence (1983), as well as in durums (Vallega and Mello Sampayo, 1987). More than half (53%) of the *dicoccum* studied had *Glu-B1* alleles which so far have not been identified in bread wheat. None of the *dicoccum* was found to carry the allele *Glu-B1c*, which occurred with a frequency of about 30% in bread wheats, nor were the *Glu-B1f* and *Glu-B1i* alleles identified in the durums (Vallega and Waines, 1987). Also the frequency of the *Glu-B1a* allele was very low in *dicoccum* and *durum* wheats compared to that in bread wheats (Branlard *et al*, 1989). The

different allele distributions at the *Glu-A1* and *Glu-B1* loci in durum and bread wheats probably reflected the independent evolution of these two groups of wheat.

### (C) *T. turgidum* var. *dicoccoides*

Rich variation of HMWGS in the wild tetraploid, the immediate progenitor of most cultivated wheats was reported. Nevo and Payne (1987) analysed 231 plants collected from 11 growing locations in Israel and identified 11 *Glu-A1* alleles and 15 *Glu-B1* alleles. A more extensive investigation of Israelian accessions of *T. dicoccoides* was made by Levy *et al* (1988) who studied a total of 456 accessions from 21 locations. An even higher degree of polymorphism of the HMWGS was observed. They found 50 different SDS-PAGE migration patterns for HMWGS and assigned 15 of the band patterns to genome A and 24 to genome B. Although most of these band patterns consisted of five subunits, the range was from three to six. The band patterns coded by the A genome ranged from zero to three subunits with two being most common and those coded by the B genome had one to three subunits with three being most common.

## 2.4.3 LMW glutenin subunits

### *2.4.3.1 Characterization and fractionation by electrophoresis*

LMWGS remained the least well characterized of the storage protein groups in wheat endosperm though they accounted for about 40% of the total proteins. This has been due to the difficulty of separating these proteins from some of other classes of proteins because of overlapping mobilities when separated by SDS-PAGE, IEF or Acid-PAGE methods (Payne *et al*, 1984c, 1984d; Singh and Shepherd, 1984, 1985).

Native LMW glutenin (small to medium aggregated protein form) has been classified in different ways by different workers. Nielsen *et al* (1968) termed them 'LMW glutenins'; Beckwith *et al* (1966) and Bietz and Wall, (1973, 1980) - 'HMW gliadins'; Shewry *et al* (1983a) and Kreis *et al* (review in 1985) - 'aggregated gliadins'; Graveland *et al* (1982) - 'Glutenin III'. This was because this particular native LMW glutenin was less aggregated than the usual native HMW glutenin and about one third of the total LMW glutenin of durum wheat has been found to be soluble in aqueous ethanol whereas less than 15% of the native HMW glutenin was soluble (Autran *et al*, 1987). Upon reduction, native LMW glutenin yielded subunits with apparent MW of 12,000 ~60,000 (LMWGS) and 80~140 KD (HMWGS) by SDS-PAGE (Khan and Bushuk, 1979; Bietz and Wall, 1980). Similarly, the native HMW glutenin also released the same HMWGS and LMWGS, but in different proportions. Subsequent comparisons of their electrophoretic properties (Payne and Corfield, 1979) and amino acid sequencing (Bietz and Wall, 1980) proved that the LMW subunits obtained from these two classes of aggregated proteins were identical.

Since both native HMW and LMW glutenin contained aggregates of HMW and LMW subunits (Bietz and Wall, 1973, 1980), Gupta (1989) suggested that the term subunits of LMW

and HMW glutenin should be referred to as the LMW and HMW subunits of glutenins or referred to as the groups of A (HMW subunits), B and C (LMW subunits) subunits of glutenin (Payne and Corfield, 1979). LMWGS (B and C subunits) differed from HMWGS (A subunits) in size and chromosomal location of genes (Jackson *et al*, 1983). Both differed from gliadins in primary structure and aggregating properties. Therefore, the term LMWGS and HMWGS will be used throughout this review.

The B subunits were the most common subunits of glutenin and had a MW of 42~51 KD (Payne and Corfield, 1979). They tended to have slower mobilities than the α-, β- and γ-gliadins but showed some overlap in 1-D electrophoresis. The C group was minor proteins and had similar mobilities to α-, β- and γ-gliadins, but they had different isoelectric points from gliadins (Jackson *et al*, 1983).

Since the D subunits of glutenin identified by Jackson *et al* (1983) in the ω-gliadin region have been reclassified as gliadins (locus renamed *Gli-3* from *Glu-2*) (Payne *et al*, 1988b) (see section 2.4.1.1C), it was generally recognized that there were two main groups of LMWGS with MW of approximately 44 and 36 KD observed in gel permeation chromatography (Shewry *et al*, 1983a).

### 2.4.3.2 *Genetical control (Glu-3)*

The genes coding for the B, C subunits were located on the short arms of homoeologous group 1 chromosomes (Brown *et al*, 1979; Jackson *et al*, 1983) and were tightly linked to the *Gli-1* loci (Payne *et al*, 1984d; Singh and Shepherd, 1988a). The symbol *Glu-3* was assigned to the locus controlling these subunits (Singh and Shepherd, 1988a).

The N-terminal amino acid sequences of LMWGS were found to be dissimilar to those of ω- and γ-gliadins, although overall they had similar amino acid composition (Shewry *et al*, 1983b). Comparatively, the LMWGS and the γ-gliadins were more similar to each other than either was to the ω-gliadins. Evidence from several sources showed that the number of genes controlling LMWGS was similar to that of the *Gli-1* genes (Thompson *et al*, 1983; Harberd *et al*, 1985, 1986; Payne *et al*, 1987c).

Since the *Glu-3* loci were tightly linked to *Gli-1* on the group 1 chromosomes, it is of interest to know whether there might be another group of LMW glutenin subunit genes on homoeologous group 6 chromosomes associated with the *Gli-2* loci. Recently Gupta (1989) showed that one of the C subunits of glutenin was coded by a gene(s) on chromosome 6DS.

### 2.4.3.3 *Allelic variation at the Glu-3 loci*

The variation and genetical control of the LMWGS in bread wheat were investigated only recently by using 1-D and 2-D procedures (Jackson *et al*, 1985; Gupta, 1989) but detailed genetic studies on the LMWGS of durum wheat have not been undertaken when the present project was initiated.

### (A) Bread wheats

Jackson *et al* (1983) identified 14 major LMW glutenin subunit components, located in different gel positions to the α-, β-, γ-, and ω-gliadins of bread wheat, using a 2-D method and were able to assign the genes controlling these components to particular chromosomes. The only genes controlling LMWGS that could not be located on particular chromosomes were those coding for minor components which migrated to similar positions as the group 6 α- and β- gliadins on IEF x SDS-PAGE gels. Singh and Shepherd (1985) showed that at least four of the LMWGS were controlled by genes on 1DS and 1AS and at least one subunit was controlled by gene(s) on 1BS in bread wheat. Recently, Gupta (1989) in an extensive study of the variation and genetic control of LMWGS in bread wheat identified 40 different LMWGS (28 B subunits and 12 C subunits) encoded by the *Glu-3* genes (six alleles for *Glu-A3*, nine for *Glu-B3*, five for *Glu-D3*, respectively).

### (B) Durum wheats

Earlier studies of variation showed that the LMW-1 and LMW-2 were the two main patterns of LMW bands in a world collection of durum wheats (Autran and Berrier, 1984; Payne *et al*, 1984c). The genes controlling these two patterns were tightly linked with those coding for γ-42-ω-33-35-38 (allele 42) gliadins, and γ-45-ω-35 (allele 45) gliadins at the *Gli-B1* locus of durum wheats, respectively (Payne *et al*, 1984c). Margiotta *et al* (1987) observed some LMW glutenin subunit variants, as they did not possess the two main types mentioned above. Lafiandra *et al* (1988) observed a line which lacked some LMW glutenin subunit bands and the γ-45 component at the *Gli-B1* locus. Gupta and Shepherd (1988) analysed 11 durum cultivars in the absence of gliadin and found two possible variant patterns coded by *Glu-A3* and three variants coded by *Glu-B3*.

No recombination was detected between the genes controlling different LMWGS indicating that the LMWGS segregated in linkage blocks like the gliadin components (Sozinov and Poperelya, 1980).

## 2.4.4 Origin and evolution of prolamin genes

All the cereal prolamins had two distinct structural domains, in which the N- and C-terminal regions had higher proportions of charged and sulphur-containing amino acid compositions whereas the central domains contained repeated sequences of amino acids, suggesting that the genes encoding them evolved initially by the tandem duplication of short sequences of DNA (review by Shewry and Miflin, 1985). HMW subunits contained at least two separate peptide sequences, which were repeated as adjacent or separate repeats. The origin and functional significance of the terminal domains were not known (review by Shewry and Miflin, 1985).

### 2.4.4.1 *Polymorphism*

The polymorphism of prolamin band patterns appeared to have been generated by DNA changes resulting in amino acid substitutions, insertions and deletions. Comparison of the sequences of purified proteins and DNA clones indicated that the polymorphisms were not due to post-translational modification (review by Shewry and Miflin, 1985). The data on the *Glu-1* loci indicated that the ancestral HMW glutenin subunit genes had already duplicated into x and y genes before the formation of the cultivated tetraploids, and probably they had become physically isolated. No further gene duplication has occurred, but these two genes diversified into the many existing allelic forms by unequal crossing-over and point mutations with the present-day x and y genes (review by Payne, 1987c). Levy *et al* (1988) suggested that prolamin genes might have a tendency for inactivation under a weaker selection pressure and thus exhibited a higher amount of allelic variation. Each band observed on a gel needed not be a single polypeptide, since random mutations could be expected to give rise to many polypeptides that did not differ in their electrophoretic properties (review by Shewry and Miflin, 1985).

Variation at the *Glu-B1* locus (14 alleles) was found to be markedly greater than at the *Glu-A1* locus (6 alleles) in T. *dicoccum* (Vallega, 1988a, 1988b) and also in *T. durum* (13 *Glu-B1* vs. 6 *Glu-A1* alleles) (Vallega and Waines, 1987) as well as in hexaploid wheat cultivars (11 vs. 3) (Payne and Lawrence, 1983). These results were consistent with the hypothesis of a polyphyletic origin for the B genome of polyploid wheats, although differences in the extent of variation within each of the A, B and D genomic constituents of hexaploid wheats could not rule out the possibility of a monophyletic origin (review by Vallega, 1988b).

All the band patterns described in cultivated wheats (Galili and Feldman, 1983b) have also been described in *T. dicoccoides,* but there were many band patterns in *T. dicoccoides* (especially in the A genome) which have not been found in cultivated wheats (Levy *et al,* 1988). This indicated that many of the ancient band patterns have been lost during the independent evolutionary process and artificial selection of modern breeding practice (Feldman and Sears, 1981). The differences in frequency of alleles of these cultivated tetraploid and hexaploid wheats suggested that they have undergone independent evolution since they were first cultivated 2,000 to 10,000 years ago (review by Vallega, 1988b).

### 2.4.4.2 *Inactivity*

Certain prolamin genes (especially HMW glutenin genes) were known to be inactive in cultivated tetraploid and hexaploid wheats. Seeds lacking HMWGS controlled by chromosome 1D in bread wheat were first described by Bietz *et al* (1975) in Nap Hal. Lines lacking subunits 1Dx or 1Dy, or lacking both subunits, or the major 1B controlled subunit(s), or particular blocks of gliadin components have been observed in bread wheats (Wrigley and Shepherd, 1974; Damania *et al*, 1983; Payne *et al*, 1984a, 1988c). Similar phenomena have

also been reported in cultivated durum wheats (Vallega, 1986; Vallega and Waines, 1987; Lafiandra *et al*, 1987, 1988).

Silencing of the HMW glutenin genes appeared to be non-random in bread wheats, affecting the *Glu-A1* genes more than the *Glu-B1* genes (Feldman *et al*, 1986). The striking observation was that none of the cultivated bread wheats had a band coded by the y element of the *Glu-A1* gene whereas some of the y genes at the *Glu-B1* locus and all at the *Glu-D1* locus remained active. This non-random inactivation of the HMW glutenin genes was also observed in some wild and cultivated lines of the tetraploid wheat (Galili and Feldman, 1983c; Feldman *et al*, 1986).

The cause of this inactivation of prolamin genes was not clear. Some cultivars which did not produce any *Glu-A1*-coded subunit actually possessed the structural gene(s). For example, a stop codon has been found in the middle of the coding sequence of a 1Ay gene (Forde *et al*, 1985). One theory to account for the inactivation was random point mutations among the storage protein genes which had a high content of glutamine codons (CAA and CAG), which could easily mutate to stop codons (TAA and TAG) (review by Levy *et al*, 1988). However, this theory could not explain why the other wheat HMW glutenin genes retained their activity and why there should be a non-random pattern of inactivity, as inactivation was not critical for the survival of these tetraploid wheats (Vallega and Waines, 1987). Although Levy *et al* (1988) suggested that silencing widely occurred during domestication and early cultivation, Vallega and Waines (1987) observed that 'null' *Glu-A1* and *Glu-B1* variants were also present among primitive as well as modern forms of diploid and tetraploid wheats. This suggested that the frequency of occurrence of 'null' alleles of HMWGS in cultivated species could have resulted from non-random samplings by early agriculturalists and breeders (Vallega, 1988b).

## 2.5 INFLUENCE OF SEED STORAGE PROTEINS OF TETRAPLOID WHEATS ON TECHNOLOGICAL PROPERTIES

Since wheat quality is a very broad term and refers to the functional properties for a given product, the terms used in the present context need to be defined. Flour technological or processing quality refers to the physical and biochemical characteristics of flour that affect the processing of the cereal grain into end-use products required by consumers. Dough handling properties or dough rheological quality are regarded as being synonyms and of prime interest in this thesis while semolina or flour milling and nutritional quality are separate topics and not discussed further in this review.

In Australia, dough strength is a specific term describing the balance between viscous and elastic properties of a dough (review by MacRitchie *et al*, 1990). High dough strength is associated with strong elastic properties, a high resistance to extension and a slow rate of breakdown, as measured by an extensograph. In European countries (eg. France and Italy), dough strength is a measure of energy (the areas under the curve) in the Alveograph test (Branlard and Dardevet, 1985).

### 2.5.1 Protein quantity

The amount of gluten in flour is an obvious factor influencing the mixing characteristics of a flour, the extensibility and resistance to extension of derived doughs, loaf volume of bread wheats and pasta-making quality of durum wheats. Generally, increases in grain protein concentration (PC) were associated with increases in various parameters of flour quality including sedimentation volume (Axford *et al*, 1979) and bread loaf volume (Finney and Barmore, 1948; Bushuk *et al*, 1969). Protein concentration was largely influenced by growing conditions (soil, climate and nutrient levels) and frequently there was a negative correlation with grain yield (Johnson, *et al*, 1985). Protein quantity can be determined accurately on small samples by Kjeldahl or near-infrared spectroscopy analyses.

The protein quantity in durum wheat semolina was also recognized as an important quality factor and ranged from 9 to 18% in commercial durum wheat, which was generally higher than that of common wheat (review by Dick and Matsuo, 1988). A good pasta-quality wheat generally possessed high gluten strength, proper firmness and stability of the cooked products, which was conferred by the protein properties of durum wheat. It is desirable that durum wheats possessed a protein concentration (PC) of about 13%. PC values below 11% generally resulted in a very poor product (review by Dick and Matsuo, 1988). This parameter has been significantly associated with mixing tolerance index (TI), maximum consistency in the farinograph (Irvine *et al*, 1961; Matsuo *et al*, 1972) and gluten elastic recovery (Dexter and Matsuo, 1977b). Major genes were known to occur in the wild tetraploid wheats *Triticum dicoccoides* which resulted in protein concentration up to 17% (Avivi, 1978) and these genes were introduced to durum wheat by conventional breeding to give protein concentration of 18-23% (Joppa *et al*, 1990). Genes for high protein concentration have been located on chromosome 6B in *T. dicoccoides* (Joppa *et al*, 1990) and the group 5 chromosomes of bread wheat (review by Garcia-Olmedo *et al*, 1982). There was some evidence that regulatory genes on group 2 and 5 chromosomes of wheat influenced the expression of structural genes for gluten proteins (Shepherd, 1968; Law *et al*, 1978; Brown and Flavell, 1981; Garcia-Olmedo *et al*, 1982).

However, durum wheat with high protein concentration did not necessarily result in optimum cooking quality, or at least, semolina protein alone was not sufficient to indicate the cooking stability (firmness) of pasta; instead the latter parameter was found to be significantly correlated with gluten strength (Grzybowski and Donnelly, 1979) and highly influenced by the growing conditions (Damidaux *et al*, 1980b). On the other hand, protein quality was a strongly heritable characteristic only partly affected by environment: a wheat variety that had better bread-/pasta-making attributes was known to retain these over a wide range of protein concentration, whereas a poor quality variety would not perform satisfactorily even at high protein concentration (Finney and Barmore, 1948).

### 2.5.2 Protein composition

Initial studies of the effect of protein composition on functional properties of bread and durum wheat flour/semolina relied on fractionation of the gluten protein by solubility. Osborne and other workers attempted to relate the gliadin-glutenin ratio to baking quality of bread wheat early this century, but with limited success (Tracey, 1967). Chen and Bushuk (1970a, 1970b) used the classical Osborne fractionation procedure to compare the distribution of the various protein fractions in durum, rye and HRS bread wheat. They found that durum wheat endosperm was very low in water- and salt-soluble proteins (albumins and globulins) and very high in alcohol-soluble proteins (gliadins). Dexter and Matsuo (1977b) used a similar procedure to fractionate two Canadian durum wheats at several protein levels and reported that albumins and globulins decreased markedly with increasing protein concentration. Walsh and Gilles (1971) used solvent extraction and gel permeation chromatography to fractionate durum protein into albumins, globulins, gliadins, glutenins, and base-solubles, and they correlated protein composition with specific quality factors. They found that firmness of spaghetti was associated with a high glutenin but a low gliadin content. Another study, using gel permeation chromatography, indicated that a high glutenin-gliadin ratio was correlated with superior cooking quality and rheological properties and that the glutenin content alone correlated with gluten properties (Wasik and Bushuk, 1975a). Similar trends were noted by Dexter and Matsuo (1977a) in their study of the effect of the time of protein deposition during grain development on the spaghetti-making quality of durum wheats. Therefore, the gliadins and glutenins have clearly shown to have a functional role in dough while albumins and globulins were mainly metabolically active proteins (reviews by Wall, 1979; Miflin *et al*, 1983).

Dexter and Matsuo (1977b) suggested that the glutenin-gliadin ratio might not guarantee superior cooking quality, because differences in the chemical composition of the glutenin fraction or other chemical constituents might override the ratio as the determining factor of gluten quality. In spite of this, Autran *et al* (1987) recently recommended using the ratio: LMWGS/monomeric gliadins or total aggregative proteins/monomeric gliadins rather than the classical glutenin/gliadin ratio as an efficient predictor of gluten quality in the selection of durum wheat cultivars, since the HMWGS in durum wheats did not show the large quantitative or qualitative effects on dough quality as found in bread wheats (Payne *et al*, 1987b).

Reconstitution experiments were used to show that differences in the cooking quality of spaghetti were attributable to glutenin fractions in durum wheat. Reconstitution of the various protein fractions showed that a decrease in farinograph mixing time and pasta processing absorption was accompanied by a decrease in glutenins and an increase in gliadins (Dexter and Matsuo , 1978a). A similar trend to that was also observed for reconstituted bread doughs (Lee and MacRitchie, 1971). Interchange of durum and bread wheat components imparted better cooking quality to spaghetti made from bread wheat (Dexter and Matsuo, 1978a).

The amount of the glutenin fraction remaining after various Osborne extraction procedures with dilute acetic acid (Mecham *et al*, 1972; Bushuk, 1985b) or with urea solutions

(Pomeranz, 1965; Lee and MacRitchie, 1971) has been shown to be important in baking quality (Orth and Bushuk, 1972). This fraction, termed 'residue protein' (Orth *et al*, 1976) or acetic acid-insoluble residue protein, was found to be the best index of protein quality (Orth *et al*, 1976; Huebner and Wall, 1976; Moonen *et al*, 1982) and mixing strength (Orth and Bushuk, 1972). This fraction also proved to be more important than any other components in imparting gluten strength and cooking quality of durum flour (Walsh and Gilles, 1971; Matsuo, *et al*, 1972; Dexter and Matsuo, 1980) whereas variations in gliadin amount were not associated with physical differences in doughs (Huebner and Wall, 1976). In contrast, Walsh and Gilles (1971) showed that a high gliadin content was related to a lack of firmness of cooked spaghetti and Alary and Kobrehel (1987) found that the glutenin fraction soluble in acetic acid was also associated with the cooking quality of spaghetti.

The quantity of residue protein was strongly influenced by the growing conditions of the wheat crop (location, year, agricultural practices), however, the efficiency of extraction might vary from time to time. Thus the correlation studies between the amount of residue protein and quality performance have shown some inconsistencies, as positive (Huebner, 1970), negative or even insignificant correlations (Orth and Bushuk, 1972) have been obtained in different studies. When the extraction was incomplete, a negative correlation might result (Orth and Bushuk, 1972), whereas complete extraction gave a strong positive correlation (review by Miflin *et al*, 1983), which limited the usefulness of residue protein as a predictor of genetical differences in durum wheat quality (Matsuo *et al*, 1982).

Many attempts have been made to link the content of sulphydryl plus disulphide groups with the physical properties of gluten proteins on the assumption that they conferred viscoelasticity during dough formation. Sullivan *et al* (1940) first suggested an association between -SH and S-S content with bread-making quality of wheat flour, and this was followed by several other research groups (review by Alary and Kobrehel, 1987). A high ratio of reactive -SH to total SH groups (review by Kobrehel *et al*, 1988b) or the accessible sulphydryl and urea dispersible protein (review by Feillet, 1983) was claimed to be a prerequisite for superior spaghetti cooking quality of durum wheat although Dexter and Matsuo (1977b) had been unable to establish this relationship. Recently, Alary and Kobrehel (1987) showed that the total amount of -SH plus S-S content of the glutenins or the total -SH plus S-S content of durum sulphur-rich glutenin, was highly significantly correlated with the cooking quality of durum samples used, suggesting a functional relationship (Kobrehel *et al*, 1988a, 1988b).

### 2.5.3 Measurement of physical properties of gluten proteins

Slightly different dough types are required for many different types of food, such as leavened and Arabic breads, cakes, cookies, pasta and noodles, which depend on different rheological requirements for extensibility, resistance to stretching, mixing tolerance, or ability to enclose gas bubbles in thin films of dough. These physical properties of dough are generally measured by rheometers such as the mixograph, the farinograph and the extensograph, which

record the characteristics of a flour-water mixture as it is worked into a dough, and also the tolerance of the dough to mixing.

Usually, assessment of quality was based on more than one parameter. For example, in the conventional bread-making process, a dough must have good physical handling properties and capacity for expansion in the final baking process (review by MacRitchie, 1984). For pasta-making, the final assessment of quality depends on a cooking test (review by Finney *et al*, 1987). The following is a brief review of some of the most routine rheological and technological tests for gluten strength assessment in durum and bread wheats.

### *2.5.3.1 Rheological tests*

The farinograph curves are normally described by three parameters: dough development time (DDT), the time in minutes required for the curve to reach a peak; maximum consistency, the viscosity measured in Brabender units at the peak of the curve; and tolerance index (TI), the difference in consistency between the values at the peak and four minutes past the peak. Weak-gluten cultivars gave a short DDT and a high TI, whereas strong-gluten cultivars usually had a longer DDT and very low TI. Dexter and Matsuo (1980) showed that farinograph bandwidth was a better indicator of spaghetti cooking quality than any of the above parameters because it was less influenced by protein content. However, the information obtained from farinograms was not as useful for predicting pasta quality as it was for predicting baking quality in bread wheat. The farinograph is now generally used to measure the water absorption capacity of a flour and as a preliminary to the extensograph in bread wheat (Rathjen, pers. comm.). The extensograph involves direct stretching of a dough until it breaks and measures resistance to extension (R), extensibility (L) and the energy expended (area under the curve). This test was regarded as one of the most reliable tests of all in bread wheat (Rathjen, pers. comm.). However, these two techniques were not used to any extent in early generation testing because the amount of sample required is large (50 g or 300 g of flour). Also they were not commonly used in predicting the quality of pasta-products of durum wheat (review by Dick, 1985).

The Chopin Alveograph uses air pressure to blow a bubble from a disc of dough (250 g) and the similar dough parameters were measured as in the extensograph and this test is frequently used in European laboratories (eg. Branlard and Dardevet, 1985).

The mixograph, especially the micro-mixograph test has been the main procedure employed for early generation prediction of Canadian durum gluten strength but it did not distinguish between types within a narrow range in the later generations (Bendelow, 1967). The mixograph is also used routinely in USDA and some Australian laboratories to assess gluten strength (Finney and Shogren, 1972; du Cros *et al*, 1982), usually with a 10 to 30 g of semolina sample. The mixograph measures the rate of dough development, resistance of the dough to mixing and the tolerance of the dough to extended mixing. All the mixograph parameters were shown to be strongly influenced by durum genotypes (Taha and Sagi, 1987). Longer mixing times were indicative of superior quality (Wasik and Bushuk, 1975a). The

mixing curves of good-quality doughs usually had longer times to peak development, a high peak and a large area-under-curve (du Cros, 1987a), but the mixograph indices, were not correlated with loaf volume when durum wheats were used for bread-making (Boggini and Pogna, 1989). The disadvantages of this instrument compared to the farinograph and extensograph were the lack of temperature control and the non-standardised procedure which made it difficult to compare results between laboratories.

One test which measured the force required to stretch a dough (Matsuo and Irvine, 1970) and another was used to break a strand of a gluten (Matsuo, 1978), have been used for assessing the gluten strength of Canadian durum cultivars. Another test devised for assessing the bread-making quality of common wheat has also been adopted for assessing durum gluten quality in Canada (Matsuo *et al*, 1982). The latter test extracted semolina proteins with 3M urea and the absorption at 280 nm was measured (Pomeranz, 1965).

These rheological tests described above provided a direct evaluation of the physical properties of dough made from bread wheat flour or durum flour/semolina. In general, these tests were sensitive to the environment so controlled conditions were required for reproducibility of results. In practice, these physical tests were not entirely satisfactory for selection of cultivars with strong gluten and good cooking characteristics. Gluten strength has been found to vary significantly between years and to a lesser extent between sites. Furthermore, these tests could not be used for testing the early generations derived from breeding programs.

Because the above quality tests have failed to fully explain semolina cooking quality differences from different cultivars, two new methods were proposed in Europe to measure the pasta-cooking quality of durum wheats, known as the viscoelastographic and the aleurographic tests (Cubadda, 1988). The viscoelastograph test is used to measure the absolute elastic recovery values and firmness of a cooked pasta disc during and after the application of pressure. Resistance to flattening and loss of elasticity was related to high product quality (Cubadda, 1988). Absolute elastic recovery values for good cooking quality cultivars were above 1.6 mm and for low or medium cooking quality types were below 1.0 (Damidaux *et al*, 1980b). This value decreases as the wheat PC increases. The aleurograph test measures the cohesiveness of a cooked pasta disc exposed to water pressure. As a general trend, the higher the cohesiveness, the better the quality of the pasta after normal cooking and overcooking treatment (Taha and Sagi, 1987; Cubadda, 1988). However, these two tests were again poorly correlated with the predicted values from numerous studies (review by Cubadda, 1988). Hence only the experimental pasta-making test and assessment of the finished product so far gave reliable results for semolina quality (review by Cubadda, 1988).

### *2.5.3.2 SDS-sedimentation test (SDSS)*

The SDSS test was first introduced by McDermott and Redman (1977) and used as an indirect measurement of the elasticity of gluten in bread wheats (Axford *et al*, 1978, 1979) and

also as an empirical test for assessing gluten strength in durum wheat (Dexter *et al*, 1980; Dick and Quick, 1983). Good bread-/pasta-making quality was associated with the formation of a large sediment, after flour was suspended with water, then mixed with a solution of sodium dodecyl sulphate and lactic acid (Axford *et al*, 1979).

In durum wheat, SDSS values and grain protein percentage together accounted for over 40% of the variability in cooking quality at optimum time and after overcooking. A modified micro-sedimentation test was also shown to be superior to normal rheological quality tests for predicting cooked spaghetti firmness in early generations of a breeding program (Dick and Quick, 1983). SDSS volumes have been shown to be positively correlated at a high level of significance with loaf volume (Boggini and Pogna, 1989), dough development time (DDT), mixing consistency and area under curve determined in the mixograph (Dexter *et al*, 1980), or the farinograph (Taha and Sagi, 1987). For example, selection for strong gluten on the basis of sedimentation volume was 84% as successful as selection on the basis of micro-mixogram score (Quick and Donnelly, 1980). These data suggested that screening tests based on the SDSS test and wheat protein concentration would be adequate for comparing durum wheats for gluten strength and spaghetti cooking quality (Dexter *et al*, 1980).

### *2.5.3.3 Cooking quality tests*

Cooking quality is one of the most important criteria in assessing the quality of durum wheat semolina for the manufacture of pasta products. It depends on three main parameters: dry matter losses and water absorption (or swelling) during cooking, viscoelastic behaviour and firmness after cooking, and pasta disintegration or the surface condition of cooked pasta (Feillet *et al*, 1989). It was well documented that firmness and surface condition were independent parameters (Autran *et al*, 1986) and that viscoelasticity of cooked pasta was correlated to protein concentration and protein composition (Autran, 1981; Autran *et al*, 1987; du Cros, 1987a; Autran and Galterio, 1989b). Strong gluten cultivars with high elastic recovery exhibited good cooking quality whereas weak gluten cultivars with low elastic recovery had poorer quality (Feillet *et al*, 1977). Optimum cooking quality flour required high PC (14% or over) and an intermediate dough strength, beyond which the quality performance might deteriorate (Dexter *et al*, 1981).

Although pasta quality could be measured objectively by instrumental methods, final judgement of cooking quality was best achieved by sensory evaluation (taste panels). Numerous methods have been designed for measuring cooked spaghetti firmness or tenderness, but yet no standardised instrument-based methods were available (Walsh, 1971; Feillet *et al*, 1977; Voisey *et al*, 1978; Matuso *et al*, 1982). Surface stickiness of spaghetti must be evaluated separately as carried out using the Canadian Grain Quality Laboratory compression tester, and these readings were highly correlated with the parameter used in Italy (review by Matsuo, 1988).

### 2.5.3.4 *Baking tests*

Various modified baking techniques have been developed, based on either long or short fermentation time (eg. Chorleywood Bread Process) procedures (review by Ponte, 1971). Ultimately, a baking test will be considered the final and most reliable test for evaluating bread-making quality. Protein quantity and quality affected bread-making quality of common wheat. Bread loaf volume was related to the gluten strength of the flour from which it was baked. Strong gluten cultivars were required, which were capable of producing an extensive viscoelastic matrix during dough formation and resulted in larger loaf volumes than with the weaker gluten cultivars. Although hardness of grain and the PC of flour were not absolute requirements for higher loaf volumes, PC and loaf volume were correlated.

The loaf volumes obtained with durum and bread wheats were clearly different (Quick and Crawford, 1983). In general, gluten in durum wheat was very tenacious and not very elastic, although it might have a high fermentation tolerance to retain dough stability when the dough reached the optimal fermentation (review by Quaglia, 1988). In order to make a leavened bread from durum wheat flour, it should have a protein concentration above 13%, gluten quality value above 17 by Berliner method, P/L ratio over 1.5 and energy (W) about 200 by alveograph measurements (review by Quaglia, 1988).

### 2.5.3.5 *Indirect 'micro' measurement of quantity of specific proteins*

Since the quantity of gluten protein has been shown to be of prime importance for the technological quality, modern techniques have been developed to quantify the specific protein components present in the endosperm. Densitometric scanning of the gel patterns after SDS-PAGE was used by Autran *et al* (1986) and they related LMWGS content with durum wheat quality.

A monoclonal antibody-based enzyme-immunoassay test was developed to predict dough strength and mixing properties of hexaploid wheat (review by Skerritt, 1988).

The gliadin RP-HPLC patterns of durum wheat could be used to predict durum wheat cooking quality (Huebner and Bietz, 1985; Burnouf and Bietz, 1987; Bietz and Huebner, 1987) and bread-making quality of wheat (Huebner and Bietz, 1986; Marchylo *et al*, 1989). SE-HPLC was used to quantify glutenin and gliadin of durum endosperm by measuring the amount of the first or second peak ($P_1$ or $P_2$), which was found to be highly correlated with gluten firmness, and gluten elastic recovery (Feillet *et al*, 1989). Analysis of variance further showed that the variability in amounts of $P_1$, $P_2$, and $P_3$ was almost exclusively genetically determined, whereas growing location had no effect. On the basis of $P_1$ content, the durum wheats included in the study could be divided into two groups; most of those with a high $P_1$ content were of $\gamma$-45 gliadin type, whereas those with a low $P_1$ content belonged to $\gamma$-42 type.

These tests quantify specific quality related-polypeptides and can be applied in early generation screening for quality.

## 2.6 CORRELATION STUDIES BETWEEN ALLELIC VARIATION OF PROLAMINS OF TETRAPLOID WHEATS AND TECHNOLOGICAL PROPERTIES

It is only in the last 15 years that it has been possible to associate specific components of the seed storage proteins with the rheological properties and bread- and pasta-making quality of dough to increase our understanding of the biochemical nature of quality, and for early-generation screening. Electrophoretic patterns of wheat cultivars of diverse quality properties have been subjected to statistical examination, to determine which of these protein components were related to flour quality attributes (Orth and Bushuk, 1972; Payne *et al*, 1979; Kosmolak *et al*, 1980; du Cros *et al*, 1982). In durum wheat, gliadin components have been studied extensively because of a strong correlation was found between certain γ-gliadin bands and gluten strength (eg. Damidaux *et al*, 1978; Kosmolak *et al*, 1980; du Cros *et al*, 1982).

### 2.6.1 Gliadins

#### *2.6.1.1 Durum wheats*

Damidaux *et al* (1978) analysed the gliadin proteins of 74 cultivars of durum wheat, covering a wide range of pasta cooking quality and from diverse sources, by Acid-PAGE (pH 3.1) and reported a close association between the presence of a particular γ-gliadin component and gluten strength. They found cultivars having gliadin band γ-45 always showed strong gluten in contrast to cultivars with band γ-42, which had weak gluten. Three classes were defined on the basis of the gluten elastic recovery as measured by the viscoelastograph:

(1) Poor, in which the absolute recovery of heat-moulded gluten (R) was less than 1 mm;

(2) Intermediate, $1 < R < 1.6$ mm;

(3) Superior, $R > 1.6$ mm.

Ten of the 47 cultivars containing band 45 were classified as intermediate and 34 superior, and 21 of the 23 cultivars containing band 42 were poor. Later work of Damidaux *et al* (1980b) confirmed this relationship. They found that 89% of the cultivars containing band 45 had R values above 1.2 mm and 98% of the 42 types below 1.2 mm. Subsequently, this result has been confirmed by many research groups around the world (Kosmolak *et al*, 1980; du Cros *et al*, 1982; Autran and Feillet, 1985; Taha and Sagi, 1987; Yupsanis and Moustakas, 1988) and now much attention is given to determining whether band 45 is present in breeding lines.

Bands 42 and 45 are γ-gliadins coded by alleles at the *Gli-B1* locus (Joppa *et al*, 1983a; du Cros *et al*, 1983; Lafiandra *et al*, 1983). Other γ-components, coded by *Gli-B1* alleles have also been correlated with technological properties. For example, cultivars with band γ-45 were better than γ-43 type (Damidaux *et al*, 1978; Kosmolak *et al*, 1980; du Cros *et al*, 1982) and plants with γ-43.5 were better than cultivars with γ-40 (Pogna *et al*, 1985b). Most of the reports have concentrated on one locus only (i. e. *Gli-B1*) (du Cros *et al*, 1982; Payne *et al*,

1984c) and the relationships between technological attributes and allelic variants at the other six loci (*Glu-1*, *Glu-A3*, *Gli-A1*, *Gli-2*) which encode the major seed storage proteins, were not well known. However recently, Autran and Galterio (1989b) observed that the β-60 or α-73 gliadin types correlated with higher values of gluten recovery and of gluten firmness in an extensive study.

Despite this close relationship between quality parameters and certain gliadin types, there were some exceptions to this pattern. For example, Damidaux *et al* (1978) found that gluten from three of the 47 cultivars with band 45 performed poorly (R < 1 mm) when measured by the viscoelastograph, and two of the 23 cultivars with band 42 had intermediate elastic recovery. Of all cultivars examined, only one contained both bands, and this particular type had superior viscoelastic properties. Four cultivars did not contain either band 42 or 45, and three of these performed poorly and one had intermediate viscoelasticity. An independent study in Australia found that eight of the 103 durum breeding lines investigated which contained band 42 had intermediate-to-strong dough instead of the weak dough expected (du Cros *et al*, 1982). Kosmolak *et al* (1980) examined 30 durum wheat cultivars grown at different locations in Canada and found that cultivars having band 45 possess much stronger gluten than the 42 types, but the association between gliadin pattern and cooking quality was less definite. Furthermore, in other studies it was reported that lines containing gliadin γ-42 all had weak gluten whereas those with gliadin γ-45 had gluten ranging from weak to strong, also indicating that the control of viscoelasticity involved the effects from other loci (Leisle *et al*, 1981; du Cros *et al*, 1982). Autran and Galterio (1989b) demonstrated that lines with γ-45 and α-73 bands gave a significantly higher average score of gluten elastic recovery (1.74 mm) than lines with γ-45 and α-76 (1.49 mm), and the average of all γ-45 types (1.61 mm), indicating a cumulative effect on quality from different loci.

Relationships between electrophoretic and morphological characters and quality attributes have also been reported (Wrigley *et al*, 1982b). Durum wheat biotypes with brown glumes usually possessed gliadin band 42 whereas those with white glume had gliadin band 45 (Leisle *et al*, 1981; Hare *et al*, 1986; Yupsanis and Moustakas, 1988). In another study, the β-60 gliadin band was found to be associated with high values for 1000 kernel weight, higher flour yield and susceptibility to black-point, and the α-76 gliadin band with higher semolina yield but poorer colour index of pasta (Autran and Galterio, 1989b). These agronomic characters could be easily assessed and Autran and Feillet (1985) recommended that they could be used for quality assessment or prediction at an early breeding stage.

### *2.6.1.2 Bread wheats*

Sozinov and Poperelya (1980, 1982) made an extensive study of the allelic variation of gliadin components and their association with bread-making quality parameters in bread wheat and showed some gliadin components had a strong influence on quality attributes. Allelic blocks of gliadin components were graded according to their effect on grain quality as follows: chromosome 1A controlled blocks; block *Gld-1A7* > 4 > 2 > 5 > 3 > 1 ≥ 6;. chromosome 1B

controlled, block *Gld-1B1* > 2 ≥ 7 > 5 > 4 > 3 > 6; *Gld-1D4* > 5 > 1 > 2 > 3; *Gld 6A3* > 1; *Gld-6B2* > 1 and *Gld-6D2* > 1 ± 3. The combined effect of these alleles was also recorded (Sozinov and Poperelya, 1982). Several other groups have reported the association of specific gliadin components with quality parameters, but no agreement was reached on specific gliadins from different studies (Doekes, 1968; Huebner and Rothfus, 1968; Wrigley, 1982b; Branlard and Dardevet, 1985, Sasek *et al*, 1986; Campbell *et al*, 1987).

### 2.6.1.3 *Are gliadins causally related to quality differences or just genetical markers?*

In bread wheat, glutenins have been shown to have a much greater effect on quality parameters rather than the gliadins, in contrast to the results described above with durum wheat. Despite the observed associations between gliadin and quality (Damidaux *et al*, 1978), the monomeric structure of gliadins did not indicate how they might play such a role in imparting gluten viscoelasticity. Two possible reasons for the observed correlation have been proposed (Damidaux *et al*, 1978). Either they were only genetic markers for other linked genes affecting quality or the gliadin proteins had a functional role in the determination of gluten quality. Jeanjean *et al* (1980) showed that some gliadin fractions could participate in the formation of an insoluble protein network upon heating of gluten, probably through disulphide bonding, and this appeared to support the second hypothesis. Cottenet *et al* (1983) found that bands 42 and 45 had a similar content of cysteine whereas du Cros (1987b) reported that gliadin 42 contained about 1% more cysteine residue than band 45. Since these proteins had a cysteine content of only 2-3%, this difference was quite significant. Kaczkowski and Mieleszko (1980) showed that the amount of disulphide bonds in the gliadin proteins, when subjected to reducing agents, varied with quality type in bread wheat. Gliadins from low-quality wheat could be reduced more rapidly than those from good-quality wheat and this might help to explain why the γ-42 gliadin type appeared to have a higher cysteine content but was poorer in quality than the type with gliadin band γ-45. However, no conclusions could be drawn about which of the cysteine residues in gliadins 42 and 45 might participate in disulphide bonding. The finding of positional differences of these residues introduced the possibility that structural differences between the two proteins could explain functional differences (Review by Lasztity, 1984). Cottenet *et al* (1983) found that gliadin bands 42 and 45 had identical MW and similar amino acid composition, and the observed difference in their overall hydrophobicity was not sufficient to account for the differences in gluten quality. Furthermore, differences in the quantity of these proteins could not explain the different gluten characteristics, since the proportion of gliadin γ-42 was only slightly higher (3.9 %) than that of gliadin γ-45 (2.6%) (Cottenet *et al*, 1983, 1984). The question was then raised whether the strong gluten properties of cultivars carrying γ-45 (Damidaux *et al*, 1978) were due to the closely linked genes coding for ω-gliadin 35 or for LMW-2 (Payne *et al*, 1984c).

It was suggested that the strong gluten associated with band 45 type might be due to the occurrence of large aggregates from linked functional proteins (Feillet, 1980; Autran, 1981).

The LMWGS, with their ability to form large aggregates, were more likely the causal factors of increased gluten strength rather than the gliadin component (Autran and Berrier, 1984; Payne *et al*, 1984c), since the genes controlling them were known to be closely linked (Payne *et al*, 1984d). Furthermore, the observed higher total amount of LMW glutenins (27%) in the 45 type compared to the 42 type (14%), supported the argument that LMW glutenin aggregating proteins were the direct causal agents of gluten viscoelasticity and firmness (Autran *et al*, 1987). The discovery of the cultivar, Berillo, which contained gliadin γ-42 together with LMW-2 instead of the usual LMW-1 and showed high gluten elastic recovery, supported the argument that gliadin γ-42 was only a genetic marker for poor quality (Pogna *et al*, 1988). This observation, however, was based on one sample only and needs further confirmation, since it has been shown previously that the 42 type durums with LMW-1 group could also have intermediate to strong gluten (Damidaux *et al*, 1978, 1980b). Moreover, since the physicochemical basis of the aggregative behaviour of LMWGS has not been fully explained (Autran *et al*, 1987), it cannot be decided whether the different effects of the allelic proteins on quality were due to qualitative or quantitative differences.

## 2.6.2 HMW glutenin subunits

### *2.6.2.1 Durum wheats*

It has long been known that the glutenin proteins of bread wheat imparted strength or elasticity to a dough (Ewart, 1968, 1979; Payne, 1984c) and it was expected that the glutenin of durums might also have an equivalent influence on gluten strength, since *Triticum turgidum* (AABB) shares two similar genomes with common wheat (AABBDD). However, no strong correlations have been found between specific durum wheat HMWGS and quality. Wasik and Bushuk (1975b) reported that the amount of certain glutenin polypeptides appeared to be related to spaghetti-making quality, but these polypeptides were later recognised to be gliadins (Autran *et al*, 1982). Autran *et al* (1982) found no relationship between HMWGS and gluten viscoelastic properties, although intrinsic cooking quality was associated with some unusual glutenin patterns. Vallega (1986) also found no strong relationship between the HMW glutenin composition and spaghetti cooking quality. Those results mentioned might have been due to the narrow range of genetic materials used in the previous two studies, since a weak relationship was observed in later studies. It was shown that certain alleles at the *Glu-B1* locus (coding for bands 6+8, and novel bands 6+17) and the *Glu-A1* locus (band 2*) were found to be correlated with both gluten rheological properties and surface properties of cooked pasta (Autran and Feillet, 1985; du Cros, 1987a; Boggini and Pogna, 1989). Also γ-42 gliadin type plants possessing band 2* coded by the *Glu-A1* allele showed greater gluten strength (du Cros, 1987a). Boggini and Pogna (1989) found that certain HMWGS of durum wheat controlled by *Glu-B1* affected bread-making quality in the order 7+8 >> 20 > 6+8, similar to the effect observed in hexaploid wheats (Payne *et al*, 1984b).

Overall the relationship of the HMW glutenin subunit variants of durum wheat with quality parameters seemed to be less pronounced than with the bread wheats (du Cros, 1987a;

Boggini and Pogna, 1989). In bread wheat, the technological effect of the *Glu-D1* variants (and *Glu-A1* alleles as well) was significantly greater than the *Glu-B1* variants. In durum wheat most of major HMWGS present were coded by the *Glu-B1* locus since they lacked the *Glu-D1* locus and were usually null for 1A-coding subunits, therefore, it was not surprising that the relationship between the HMWGS and quality was not so strong (Autran, 1981; Autran *et al*, 1982; Autran and Berrier 1984; Vallega, 1986; du Cros, 1987a). This might also be the reason most durum wheats had poor dough strength (Finney *et al*, 1987).

#### 2.6.2.2 *Other tetraploids*

Although the wild tetraploid wheats have been shown to possess many novel HMWGS, no studies have been conducted to associate this allelic variation with quality differences. Vallega and Waines (1987) suggested that tetraploid material with individual 'null' *Glu-B1* or *Glu-A1* alleles, or both 'null' alleles might be useful in the study of HMWGS dosage effects on the quality of wheats. Levy *et al* (1988) suggested that the large number of HMWGS genes found in *T. dicoccoides* but not in cultivated durums should be evaluated for their effect on nutritional as well as on technological qualities.

#### 2.6.2.3 *Bread wheats*

The relationship between the presence of certain HMWGS and the bread-making quality of bread wheats has been well documented (Payne *et al*, 1979, 1981a, 1983, 1984b; Payne and Lawrence, 1983; Burnouf and Bouriquet, 1980; Moonen *et al*, 1983). The relative importance of the different bands controlled by the *Glu-1* loci on flour quality have been summarised in the following order: *Glu-D1* locus (5+10 > 2+12 = 3+12 > 4+12) > *Glu-A1* locus (1 > 2*> 0) > *Glu-B1* locus (17+18 = 13+16 = 7+8 > 7+9 > 7 > 6+8) (Payne *et al*, 1984b, 1987a, 1987c; Payne, 1987c) and quality scores were assigned to each of the *Glu-1* alleles based on SDSS values (Payne *et al*, 1987a).

### 2.6.3 LMW glutenin subunits

Until recently the variation, genetic control and functional properties of the LMWGS have not been examined in any detail (Payne *et al*, 1984c), even though they account for more than 80% of the total glutenin fraction in bread wheat (Payne *et al*, 1983).

#### 2.6.3.1 *Durum wheats*

Earlier work showed that the gluten proteins appeared to be important in influencing the quality of pasta products (Matsuo and Irvine, 1970; Walsh and Gilles, 1971; Matsuo *et al*, 1972; Wasik and Bushuk, 1975a). Wasik and Bushuk (1975b) observed that the glutenins conferring excellent spaghetti-making quality contained more of subunit 6 (MW 53 KD) than subunit 5 (MW 60 KD), and those with mediocre quality appeared to have less subunit 6 relative to subunit 5, indicating that the LMW subunits might have a greater effect than the

HMW subunits, while the discovery of a close association of γ-gliadin components with quality attributes shifted attention to studies on the gliadin fraction. The association of LMW-2 with gluten strength and LMW-1 gluten weakness has refocussed attention on the LMWGS (Payne *et al*, 1984c; Autran and Berrier, 1984).

The occurrence of many allelic types at the *Glu-A1*, *Glu-B1* and *Glu-D1* loci might explain the continuous range of gluten quality in bread wheat cultivars (Autran *et al*, 1987). Autran *et al* (1987) assumed that though HMWGS in total have little effect in durums, all aggregative protein fractions in durum wheat could contribute to quality with LMWGS being the major functional markers of genetic differences. However, the contributions to the gluten functional properties of the different alleles located at the 1AS locus (i. e. *Glu-A3*) and at the 6AS and 6BS loci (tentatively Glu-A4 and Glu-B4) have not yet been determined.

### 2.6.3.2 *Bread wheats*

Payne *et al* (1987b, 1987c) were the first workers to associate LMWGS with quality characters of hexaploid wheat by normal SDS-PAGE techniques. A detailed study of such association was made later by Gupta *et al* (1989) and a particular LMWGS (*Glu-A3m*) was associated with a greater improvement in quality than the HMW glutenin band 2* (*Glu-A1b*) (Gupta *et al*, 1989). It was reported that the combined role of LMWGS and HMWGS with positive effects was more effective and important than individual glutenin bands in improving the bread-making quality of wheat (Gupta, 1989). The relative effects of various alleles at the *Glu-A3* locus for dough extensibility in bread wheats were found to be: *Glu-A3a* > *Glu-A3e* (Payne *et al*, 1987b), *Glu-A3b* > *Glu-A3c* > *Glu-A3e* (Gupta and Shepherd, 1988; Gupta *et al*, 1989). Information on the functional properties of other LMW glutenin alleles at the *Glu-B3* and *Glu-D3* was not available. The LMWGS and HMWGS were found to be mainly responsible for the difference of quality characters in two bread wheat crosses (Gupta *et al*, 1989), although quality was largely influenced by *Glu-A1* and *Gli-A1* genes (Payne *et al*, 1984c, 1987b). Moreover, the close genetic linkage between the LMWGS and gliadin loci made it difficult to interpret whether the observed effects on quality were due to the effect of the LMWGS or the gliadin components.

## 2.7 GENETIC MANIPULATION FOR DURUM WHEAT QUALITY IMPROVEMENT

A major objective in durum breeding is to produce varieties with a strong, elastic gluten, which were satisfactory for bread-making quality, as well as acceptable pasta-making quality. Such a dual purpose durum would have alternative markets in years of high production and might also be used instead of bread wheat as a filler wheat in high quality flour blends (Boggini and Pogna, 1989). However, gluten strength of semolina from durums was generally low (very weak to medium strong), as compared to flour milled from comparably grown Hard Red Spring (HRS) bread wheats (Finney *et al*, 1987). Durum semolina had a shorter mixing time

and less stability than those for the HRS on the farinograph (review by Finney *et al*, 1987). The poor bread-making quality of durum wheats has restricted their use for this purpose (Boggini and Pogna, 1989), although durum wheats with good leavened pan bread baking quality have been reported in Italy (Boggini and Pogna, 1989; Lafiandra, pers. comm.), India (Prabhavathi *et al*, 1976), Mexico (CIMMYT, 1979), the USSR and other countries (Quick and Crawford, 1983), suggesting that genetic variation in bread-making quality might not reside solely within the D genome (Payne *et al*, 1979, 1984b, 1987c; Moonen *et al*, 1983). Selection for improved bread-making quality based on prolamin genes from different sources in the durum background could be direct and efficient through the identification by electrophoresis of their HMWGS and LMW glutenin subunit composition.

### 2.7.1 Genes on chromosomes 1B and 1A

It has been shown that the genes on chromosome 1A had a negligible effect on quality parameters compared to genes on chromosome 1B (Josephides *et al*, 1987). This was most likely due to the frequent absence of functional HMWGS alleles i. e. *Glu-A1a* and *Glu-A1b*. Du Cros (1987a) observed an exceptional line which contained a γ-42 type but had intermediate dough strength. This line was found to have the HMWGS band 1 coded by *Glu-A1* genes on chromosome 1A, which endowed it with improved strength in the poor quality background. Chromosome 1B has been shown to be especially important in determining gluten strength of durum wheat cultivars. For example, when the 1B chromosomes from two strong gluten cultivars Kharkof-5 and Edmore, were substituted into Langdon, in all cases, the 1B substitution lines had gluten strength equivalent to the donor parents rather than the recurrent parent Langdon (Josephides *et al*, 1987). This has been shown to be due mainly to the allelic differences of LMWGS coded by *Glu-B3* genes on chromosome 1B (Autran *et al*, 1987; Pogna *et al*, 1988), although there were also some effects of the HMWGS (Pogna *et al*, 1990).

### 2.7.2 Genes on chromosome 1D of hexaploid wheat

The influence of *Glu-D1* alleles on bread-making quality has been conspicuous in bread wheat (review by Payne, 1987c) but the effect of these alleles in a durum background has not been tested in any detail.

Addition of D-genome chromosomes from different D genome cultivars of *Triticum tauschii* to the tetraploid generally improved dough quality (Welsh and Hehn, 1964; Morris *et al*, 1966; Kerber and Tipples, 1969; Orth and Bushuk, 1973a; Lagudah *et al*, 1987). Furthermore, the synthetic hexaploid plants which possessed *Glu-D1d* subunits 5+10 had longer peak dough development times and higher loaf volumes than those with *Glu-D1a* (2+12) (Lagudah *et al*, 1987). The specific HMW glutenin bands coded by *Glu-D1d* (5+10) (Payne *et al*, 1979, 1984b; Burnouf and Bouriquet, 1980; Moonen *et al*, 1983) have been shown to favourably affect the baking characteristics of common wheat.

'Null' alleles of HMWGS have been observed to have deleterious effects on quality, possibly because of the decreased percentage of HMW subunits to total endosperm protein (Lawrence *et al*, 1988). Even a single null involving the *Glu-D1* locus, as in bread wheats Ottawa and Nap Hal, resulted in a large reduction in quality properties (Lawrence *et al*, 1988; Payne *et al*, 1987a, 1988c).

At the tetraploid level, Kaltsikes *et al* (1968a, b) found that the extracted AABB tetraploid from Prelude had equivalent quality to the hexaploid parent Prelude, possibly due to the presence of a 1DL translocation in the AABB line, whereas two other extracted AABB tetraploids in Thatcher and Rescue had relatively poorer quality than their respective hexaploid parents. Joppa *et al* (1983b) reported that substitution of chromosome 1B of durum Langdon by 1D of hexaploid wheat Chinese Spring gave improved gluten quality, suggesting that chromosome 1D had a larger influence on quality than 1B (see section 2.7.1.1), even when carrying the allele, *Glu-D1a* (2+12) of Chinese Spring, which has generally shown poorer quality for bread-making (Payne *et al*, 1984b; Moonen *et al*, 1983).

### 2.7.3 Durum wheat quality improvement

Improvement of durum dough quality is possibly best approached through the genetic modification of the protein composition of the grain. Several alternative methods were available, such as substituting a pair of hexaploid wheat chromosomes for tetraploid wheat chromosomes (Joppa *et al*, 1978), translocating a segment of hexaploid chromosome (Kaltsikes *et al*, 1968a, 1968b) or intercrossing within the durum collection and selecting for stronger gluten and bread-making quality (Quick, *et al*, 1979). Although the last procedure has been used successfully at the North Dakota State University (Quick and Crawford, 1983), it appeared that introduction of *Glu-D1d* or *Glu-D1a* genes from bread wheats into durum wheats, coupled with modification of the other three glutenin loci (*Glu-A1, Glu-A3* and *Glu-D3*, or *Glu-B1, Glu-B3* and *Glu-D3*) might result in higher gluten quality because the strongest durum wheat currently available were still poorer than HRS bread wheats in gluten strength (Quick and Crawford, 1983; Finney *et al*, 1987).

Substitution of the 1D gene *Glu-1Dd* (band 5+10) into durum wheats, should have a larger effect on bread-making properties compared to substitution with *Glu-D1a* (band 2+12) because of the known relative values of these alleles in hexaploid wheat. Also the 1D(1A) substitutions could be further improved by combining more effective *Glu-B1* and *Glu-B3* alleles with the substitution. For example, incorporating *Glu-B1i*, (i. e. 17+18) into durums, which was known to have a strong influence on bread-making quality and did not currently occur in durum wheats, could result in improved quality (Lawrence *et al*, 1988). Furthermore with the 1D(1B) substitution type, introduction of active genes such as *Glu-A1a*, *Glu-A1b* onto chromosome 1A is expected to have a larger effect on technological quality than the null gene *Glu-A1c* (du Cros, 1987a) which was present in most current durum wheats. Replacing the existing alleles from the accessions of diploid wheat, *T. urartu* and *T. dicoccoides* at the

*Glu-A1* locus by equivalent genes (Waines and Payne, 1987; Levy *et al*, 1988) which had active genes coded for both x and y HMWGS, might result in further improvement of quality.

The effect of translocating part of the 1D chromosome into the tetraploid wheat showed that tetraploid wheats had equivalent quality potential to that of hexaploid wheats (Kaltsikes *et al*, 1968a). Therefore, homoeologous recombination technique could be used to introduce these particular genes into durum wheat while retaining most of the durum wheat genome. This might overcome the drastic effects on yield which accompany the substitution of a complete chromosome 1D into tetraploid wheat (Joppa and Williams, 1988).

The identification of the major storage protein components controlled by these three genomes could be easily screened by electrophoretic and HPLC analyses, especially in the early generation of breeding program, hence facilitating the selection of improved quality types. It is expected that durum wheat containing the 1D storage protein genes with high yield potential would have wide application in the future food markets of the world, especially in the developing countries. Carleton (1901) predicted that durum wheat could be widely used for bread-making since it has desirable flavour, texture, and shelf-life properties. Considerable improvement in yielding ability of durum wheat has already been made throughout the world in the past ten years (Quick and Crawford, 1983). The recent development of high yielding semidwarf durums has again shown that durum wheat yields may be equal or superior to hexaploid wheats in many areas (Breth, 1984).

In summary, the current literature review identified two major areas in which research work should be directed. One area is an investigation of the variability of the LMWGS of durum wheat as well as other tetraploid wheats, which would facilitate future genetic and breeding work for crop quality improvement. Secondly, a comparison will be made on quality character differences between durum and bread wheats in terms of the dough strength. In particular, a study will be made on the quality effects of chromosome 1D of hexaploid wheat in durum wheats. The ultimate aim of the present research is to assist plant breeder in developing new durum cultivars combining good spaghetti-cooking and bread-making quality with high yield.

# CHAPTER 3. GENERAL ELECTROPHORETIC METHODS FOR ENDOSPERM PROTEIN SEPARATION

## 3.1 INTRODUCTION

The development of new methods of protein separation has been a major factor contributing to recent advances in understanding of the genetic basis of wheat quality. In this chapter, the general procedures used in the present study for extracting seed storage proteins and separating for these proteins by gel electrophoresis will be described in detail.

## 3.2 EXTRACTION OF SEED ENDOSPERM STORAGE PROTEINS

Single seeds were cut in half and the brush ends were crushed with a hammer and prolamins were extracted as a group (the first paragraph of sections 3.2.1 to 3.2.4) or sequentially (the second paragraph) and separated by electrophoresis as described below (section 3.3). Alternatively, the same procedures were used with flour rather than grain samples.

### 3.2.1 Extraction of unreduced prolamins for normal Acid-PAGE

The extraction procedure of Bushuk and Zillman (1978) was used with the following modifications. Gliadins were extracted in 100 µl of 70% (v/v) aqueous ethanol [or 50% (v/v) n-propanol] from one fifth of the kernel or ten mg of flour samples for at least one hour at 60°C or three to 18 hours at room temperature. Three drops of a dye solution (10 ml glycerol and 4 ml 10% methyl violet) were added and mixed thoroughly. This extract was centrifuged in a Beckman Microfuge-II at speed ten for 3 min and 20 µl of supernatant solution was transferred to a slab gel of the Acid-PAGE system (see section 3.3.1).

If the above samples were to be used for further extraction of glutenins, half-endosperm or 20 mg flour sample was extracted in 300 µl of 50% n-propanol (or 70% aqueous ethanol) for one hour at 60°C with repeated vortexing for gliadin extraction. After centrifugation for two min, 100 µl of the supernatant was transferred to another tube and mixed with three or four drops of the dye stock solution and loaded onto the gel.

### 3.2.2 Extraction of unreduced prolamins for normal SDS-PAGE

The extraction procedure was similar to that described by Lawrence and Shepherd (1980). Crushed endosperm or flour was treated with 100 to 150 µl of 40 mM Tris-HCl buffer containing 4% (w/v) SDS without reducing agent at 60°C for at least 30 min to extract gliadins for SDS-PAGE. The extracts were centrifuged in a Beckman Microfuge-11 for 2 min at speed ten and 8-10 µl supernatant loaded onto a slab gel of SDS-PAGE as described in section 3.3.2.

If the above samples were to be used for further sequential extraction, 300 µl of extractants (50% n-propanol or 70% ethanol) were used to extract gliadins as described in the second paragraph of section 3.2.1, and 100 µl supernatant of the extracts (sections 3.2.1 and 3.2.4) was transferred to a new tube, evaporated at 60°C until 20 to 30 µl remained. Then 100 µl 2X SDS sample buffer (containing 80 mM Tris-HCl buffer and 8% SDS) was added without reducing agent, and a subsample of 7 µl loaded per well onto SDS-PAGE slab gel.

### 3.2.3 Extraction of unreduced prolamins for two-step 1-D SDS-PAGE

Unreduced prolamins were extracted (Gupta and Shepherd, 1990a) from a half seed (or 20 mg of flour) in 1.0 ml of 70% aqueous ethanol (pH. 5.9) at 60°C for at least 2 hours, being vortexed briefly once or twice. The top portion of the supernatant (about 700 µl) was transferred to another tube, centrifuged for 3 minutes in a Beckman Microfuge-11 at speed ten and evaporated at 60-65°C in an oven. The residue was re-dissolved in 80 µl of 40 mM Tris-HCl buffer containing 4% (w/v) SDS and 35-50 µl of the extract loaded for the first gel slab electrophoresis of the two-step 1-D procedure (Gupta, 1989) described in section 3.3.3.

This high temperature extraction procedure was later replaced by the sonification extraction procedure described by Singh *et al* (1990b). The latter method was quicker and more efficient since the samples could be extracted directly by sonication for only 20 to 30 seconds at scale five by using a micro-probe of Branson Sonifier (model B-12 cell disrupter, Branson Sonic Power Company, Danbury, CT). The top portion of the supernatant (700 µl) after centrifugation was transferred to a new tube, treated as described above. A much larger amount of proteins was extracted by this technique compared to the high temperature treatment (Singh *et al*, 1990b).

### 3.2.4 Extraction of glutenins for one-step SDS-PAGE

The new simplified procedure developed by Singh *et al* (1991b) to separate total glutenins from seed endosperm or flour did not require the first-step of the SDS-PAGE procedure described in section 3.3.3. Only one gel run was required.

Half endosperm or 20 mg of flour samples were extracted twice in 1.0 ml of 50% (v/v) n-propanol at 60°C by repeated vortexing (at least twice) for 30 min, centrifuging in a Beckman Microfuge-11 for 2 min at speed ten and discarding the supernatant both times. The residue was washed with 0.5 ml of 50% n-propanol and the liquid removed by a syringe or vacuum. Then 100 µl of 80 mM Tris-HCl (pH 8.0) containing 1% dithiothreitol (DTT) in 50% n-propanol was added to the residue and incubated for 30 min at 60°C. To this was added 100 µl of 80 mM Tris-HCl (pH 8.0) plus 0.14 M 4-vinylpyridine (4-VP) in 50% n-propanol and the samples further incubated for 15 min at 60°C. After centrifugation for 2 min, 25 µl of the supernatant was transferred to another tube containing 50 µl of sample buffer [containing 80 mM Tris-HCl (pH 8.0), 40% glycerol (w/v), 2% SDS (w/v) and 0.02% bromophenol blue],

allowed to stand for 15 min at 60°C, centrifuged for 2 min and 10-15 µl subsample loaded onto the slab gel (section 3.3.4).

If gliadin extraction was also required from the same sample, the procedure described earlier in sections 3.2.1 or 3.2.2 were followed. Hence, the same seed sample could be used for analysis of both gliadins and glutenins.

## 3.3 ELECTROPHORESIS

All the following procedures are based on the discontinuous-buffer system of Laemmli (1970) except for Acid-PAGE where a continuous buffer system was employed as first developed by Jones *et al* (1959).

### 3.3.1 Acid-PAGE for gliadin separations

The acrylamide gel systems of Bushuk and Zillman (1978) and Khan *et al* (1985) were modified as follows. The continuous gel system containing 7% (w/v) acrylamide, 0.35% (w/v) bisacrylamide, 0.25% (w/v) aluminium lactate, 0.24 % (w/v) ascorbic acid and 0.02 % (w/v) $FeSO_4$, was adjusted to pH 3.1 with lactic acid. After 20-30 sec aspiration, the gel solution was polymerized with 3% hydrogen peroxide in a Hoefer slab former (180 x 160 x 1.5-3.0 mm). The electrode buffer, for both lower and upper tanks contained 0.125% (w/v) aluminium lactate adjusted to pH 3.1 with lactic acid. Twenty microlitres of samples (section 3.2.1) were loaded into wells at the anodal end and were electrophoresed at a constant voltages (400) at temperature (12-14°C) until the tracking dye front reached the bottom of the gel. The band separation was not affected even when the pH reading of the electrode buffer was below 3.0.

### 3.3.2 Uniform SDS-PAGE for gliadin separations

The method of Lawrence and Shepherd (1980) was adopted. The separating gel was a Hoefer unit (180 x 160 x 1.0 mm), containing 10% (w/v) acrylamide, 0.1% (w/v) bisacrylamide (Bis), 0.1% SDS and 0.375M Tris adjusted to pH 8.8 with HCl. The stacking gel (20 mm) contained 3.5% (w/v) acrylamide, 0.08% Bis, 0.1% (w/v) SDS and 0.125M Tris adjusted to pH 6.8 with HCl. Both gels were polymerized with N, N, N', N'-tetramethyl-ethylenediamine (TEMED) and ammonium persulphate. The electrode buffer, for both upper (cathodal) and lower (anodal) tanks, contained 0.1% (w/v) SDS and 0.025M Tris adjusted to pH 8.3 with glycine. Ten microlitres of the protein samples (section 3.2.2) were loaded into wells at the cathodal end and were electrophoresed at a constant current of 40 mA per gel.

### 3.3.3 Two-step one-dimensional SDS-PAGE for glutenin separations

The two-step one-dimensional SDS-PAGE procedure of Singh *et al* (1990b) was adopted to achieve a clear resolution of LMW glutenin subunits. In the first step, the polyacrylamide gel was cast in the same gel unit as described in section 3.3.2 except a lower acrylamide concentration (usually 8-8.5%) was used. Electrophoresis was stopped once the dye reached the bottom of the gel, the top 0.8-1.0 cm portion of the separating gel was then cut off and transferred into 50 ml of an equilibration solution [consisting of 10 % (w/v) glycerol, 0.07M Tris and 2.4% (w/v) SDS adjusted to pH 6.8 with HCl]. A small quantity of bromophenol blue and 10 mM DTT were added to the equilibration solution and incubated for 1 hour at about 60°C and then 1.7 % (v/v) 4-VP was added for another hour to induce alkylation.

The second step of electrophoresis consisted of loading the reduced gel strip onto the top of a vertical slab gel of greater thickness (1.5 mm). The separating gel in this step usually had a linear gradient of acrylamide concentration from 7.5 to 13 % with 1.5 % cross-linking. Electrophoresis was carried out at a constant current of 40 mA/gel for about 3-4 hours with tap water circulating as coolant.

### 3.3.4 One-step one-dimensional SDS-PAGE for glutenin separations

After a new extraction procedure was developed by Singh *et al* (1991b), the reduced and alkylated glutenin extracts (section 3.2.4) were loaded directly onto a gradient gel as described above from the second step of electrophoresis (section 3.3.3). The glutenin banding patterns obtained by this method were qualitatively similar (Singh *et al*, 1991b; Gupta and MacRitchie, 1991) to those obtained from the two-step 1-D procedure used by Gupta and Shepherd (1988).

### 3.3.5 Staining and destaining

Gels were stained for at least 3 hours in a solution consisting of one part of 1% (w/v) Coomassie Brilliant Blue R mixed with 40 parts of 6% (w/v) trichloroacetic acid in water: methanol : glacial acetic acid (80 : 20 : 7) as described by Lawrence and Shepherd (1980). The stained gel was placed in deionized water for one day with several changes of water for destaining. The stain solution without the dye was used to obtain a rapid destaining of the gel when required.

## 3.4 SUMMARY

The earliest electrophoretic approach employing a 2-D system (1st-D, IEF/NEPGE; 2nd-D, SDS-PAGE) to separate the whole seed endosperm proteins, including the LMW glutenin subunits, was based on the principle that the protein components separated by charge in the first dimension and by size in the second. Thus, Jackson *et al* (1983) were able to locate the

genes controlling all the B and some C subunits on the short arms of group 1 chromosomes in Chinese Spring wheat. However, this technique could not be used routinely for a large number of samples because only one sample could be analysed per gel.

The introduction of a two-step 1-D SDS-PAGE system by Singh and Shepherd (1988a) provided a more rapid method for analysing a large number of samples in a single gel in a gliadin-free background, which was largely based on the finding that native gliadins are monomeric and native glutenins are large aggregates so the first-step gel 'filtered' through the monomeric part. Again, this procedure was later modified by using 70% ethanol as extractant instead of SDS buffer (Gupta and Shepherd, 1990a, 1990b) giving much clearer separation of the LMW glutenin subunits, free from contamination with non-prolamins which were present in the former procedure (Singh and Shepherd, 1988a). Using this approach, Gupta and Shepherd (1990a, 1990b) were able to survey a large number of hexaploid wheats and their relatives for the variation of LMW glutenin subunits.

However, this procedure has two obvious problems.

Firstly, the extraction rate of the ethanol-soluble glutenin was low even at high temperature (~60-65°C), therefore, very large amount of samples were required in the first-step gel in order to visualise subunits. Originally, gels gave either poor separation for HMW or LMW or both subunits, and a high level of skill was required to achieve reproducible results (Gupta, 1989). This was partially overcome by using a sonication technique to extract more ethanol-soluble prolamins (Singh *et al*, 1990b).

Secondly, the LMW glutenin subunits appeared to be actively involved in a re-oxidization processes after being reduced, resulting in fuzzy bands which were difficult to score. The method was improved by using an alkylating agent to block the active -SH group of the subunits for re-oxidization, making the patterns more reproducible (Singh *et al*, 1990b). The two-step procedure, however, was laborious and time-consuming.

The advent of one-step 1-D SDS-PAGE systems (Singh *et al*, 1991b; Gupta and MacRitchie, 1991; Zhen *et al*, 1993) was based on the differential solubility properties of gliadins and native LMW glutenins in aqueous alcohol, which allowed the gliadins to be removed by exhaustive extraction. This, left the highly aggregated glutenin in the residue so that it become possible to manipulate these separately. The new glutenin subunit separation system was efficient and reliable and applied in all present experiments after it had been developed in 1990 (Singh *et al*, 1991b).

# CHAPTER 4. VARIATION OF LMW GLUTENIN SUBUNITS AND GLIADINS IN DURUM AND RELATED TETRAPLOID WHEATS

## 4.1 INTRODUCTION

Wheat storage proteins played a key role in food processing such as in the manufacture of bread, biscuits, breakfast cereals, and pasta products (Payne and Rhodes, 1982), and the chief functional components were the LMW and HMW glutenin subunits (review by MacRitchie, 1990). Identification of allelic patterns of wheat prolamins and their association with functional properties would facilitate breeding programs aimed at improving flour end-use quality. However, the analysis of LMW glutenin subunit patterns has only recently been made possible, through the development of suitable gel separation systems (Brown *et al*, 1981; Jackson *et al*, 1983; Singh and Shepherd, 1984; Singh *et al*, 1991b).

In durum wheats, two major types of LMW glutenin patterns known as LMW-1 and LMW-2 were formerly reported by Payne *et al* (1984c) to be associated with gluten strength. Carrillo *et al* (1990a, 1990b) described three other types of LMW glutenin patterns from 139 Spanish durum landraces, which were also associated with quality. However, there has been no systematic study on the extent of genetic variation in durum wheat banding patterns for LMWGS. Also there were no reports on the variation of LMWGS among other cultivated tetraploid landraces and lines nor in the wild relatives of tetraploid wheat. In the first part of this chapter, an extensive survey of the variation of LMWGS among different durum wheat cultivars from a world collection has been conducted at the band pattern level and an attempt has been made to deduce the linkage blocks controlling these B subunits of glutenin. Since genes controlling LMWGS and ω- and γ-gliadins were known to be closely linked (Payne *et al*, 1984c), the variation of these gliadin banding patterns was also investigated.

The wild tetraploids and the less-widely cultivated tetraploid landraces and lines were potential sources of novel storage proteins, which might give rise to new cultivars with improved bread-/pasta-making quality. Furthermore, the ancestral wild and cultivated relatives contained rich genetic diversity for other important economic traits such as multiple disease resistances and physiological characters such as seed protein quality and resistance to diverse ecological stresses (Nevo, 1983, 1987; Nevo *et al*, 1986, 1988). The latter part of the chapter describes a survey aimed at identifying sources of variation of LMWGS and gliadins at the band pattern level in a large number of different tetraploid wheat species including 25 lines of *T. dicoccum*, 21 lines of *T. polonicum* and 235 lines of *T. dicoccoides*. This survey complements earlier studies on the variation HMWGS in similar materials (Nevo and Payne, 1987; Levy *et al*, 1988).

## 4.2 MATERIALS AND METHODS

### 4.2.1 Plant materials

#### *4.2.1.1 Durum (Triticum turgidum var. durum)*

A detailed list of the durum genotypes and their country of origin is given in Appendix A and is summarised below.

Seeds of 195 lines of durum wheat from North Africa [including Egypt (i. e. UAR), Algeria, Tunisia, Morocco and Libya], CIMMYT (i. e. Mexico), Syria and Italy, were kindly provided by Dr A. J. Rathjen of the Department of Plant Science, Waite Agricultural Research Institute (Waite Institute). Sixteen Italian cultivars were obtained from Dr G. Boggini (Germplasm Institute, Bari, Italy), fourteen North American cultivars were provided by Dr N. K. Howes (Agriculture Canada Research Section, Manitoba, Canada). Cultivars Coulter, Stewart and Vic were supplied by Mr M. C. Mackay, Australian Winter Cereal Collection (AWCC), Tamworth, N. S. W. The γ-gliadin reference cultivars of durum wheat Langdon (γ-42) and Edmore (γ-45) were used as frequent references, and provided by Dr K. W. Shepherd, Waite Institute; another three reference cultivars Capinera (γ-40), Drago (γ-43.5) and MG 2988 (γ-47) were obtained from Dr B. Margiotta, Germplasm Institute, Bari, Italy. Cultivars Durati, Dural, Duramba biotypes (A to D), Langdon substitution lines Langdon (Edmore-1B), Langdon (Kharkof-5-1B) were also obtained from the collection of Dr K. W. Shepherd at Waite Institute. An entire set of D-genome disomic substitution lines [viz. Ldn 1D(1A), Ldn 1D(1B) to Ldn 7D(7B)] in the durum wheat cultivar Langdon background was kindly provided by Dr L. R. Joppa, North Dakota State University, Fargo, USA.

[Note: The designation of chromosomes 4A and 4B was changed in 1989 to 4B and 4A, respectively (7th Int. Wheat Genetics Symp., Miller, T. E. and Koebner, R. M. D. eds) and the new system is used in the present study]

#### *4.2.1.2 Dicoccum (Triticum turgidum var. dicoccum)*

Two collections of *T. dicoccum* were available. The first source (16 lines) was supplied by Mr M. C. Mackay, AWCC (see Appendix B). One line originated from Iran, two from the former USSR and the remaining cultivars were of unknown origin. The second source (nine lines) was obtained from Dr G. Waines, University of California, USA with a wide range of origins (including one line each from Bulgaria, Ethiopia, Germany, India, Iran, Morocco and three from the former USSR, Appendix B).

#### *4.2.1.3 Polonicum (Triticum turgidum var. polonicum)*

Twenty-one lines of *T. polonicum* were also supplied by Mr M. C. Mackay, AWCC (see Appendix C).

#### 4.2.1.4 *Dicoccoides (Triticum turgidum var. dicoccoides)*

Two groups of accessions of *T. dicoccoides* were available. The first collection of 16 lines, was collected in Israel by Kushnir *et al* (1984), and seeds were kindly provided by Mr M. C. Mackay, AWCC (see Appendix D).

The second collection also came from Israel and consisted of 219 lines from 23 populations, provided by Professor E. Nevo, Institute of Evolution, University of Haifa, Haifa, Israel via Dr A. Rathjen, Waite Institute. These lines were collected at different geographic sites in Israel (Nevo and Payne, 1987; Nevo and Beiles, 1989) with about ten lines from each location (see Appendix E).

### 4.2.2 Extraction of gliadins and glutenins and fractionation by electrophoresis

The extraction of prolamins and their electrophoretic separation was accomplished by the methods described previously (Chapter 3). The two-step 1-D SDS-PAGE was initially used for the survey of the durum wheat LMW glutenin subunit banding patterns but was replaced by the one-step procedure when it became available in 1990 (Singh *et al*, 1991b). Acid-PAGE was used to separate ω-γ-gliadin bands, and SDS-PAGE ω-gliadin bands.

### 4.2.3 Gene symbols used for prolamins of wheats

The locus and allele symbols for HMW glutenin subunits corresponded to those of Payne *et al*, (1982b) and Payne and Lawrence (1983), respectively, and for the gliadins those of Payne *et al*, (1982b) and Bushuk and Zillman (1978), were used. The novel alleles detected in durum wheats were assigned symbols using the system of Vallega (1986, 1988b).

The terms LMW-1 and LMW-2 were originally introduced by Payne and co-workers (1984c) to indicate a 1BS-controlled block of LMWGS (i. e. gliadin 42/45-linked LMW glutenin banding patterns), but these terms were used by other workers to describe the whole B subunit patterns (Margiotta *et al*, 1987; Pogna *et al*, 1988, 1990). In the present study, the term "LMW pattern" was used to apply to the whole B subunits of glutenin. To prevent further confusion in terminology, the complete LMW glutenin B subunit banding phenotype was assigned a different capital letter in alphabetical order to denote different patterns. For example, the two durum wheat reference cultivars Langdon and Edmore were denoted to the "A" and "B" type patterns of the whole B subunits of glutenin (i. e. patterns LMW-A and LMW-B), respectively.

Among previous reports, only Gupta and Shepherd (1988) and Margiotta *et al*, (1990) mentioned the 1AS-coded bands in the B subunit region. This was due to the difficulty of differentiating the glutenin bands in the earlier studies from the gliadin bands when they were present together in the gel. However, the *Glu-A3* variants were also ignored in a recent study of Carrillo *et al* (1990a, 1990b), using the two-step 1-D SDS-PAGE procedure of Singh and Shepherd (1988a). Since the B subunits of glutenin in tetraploid wheat were known to be

coded by both *Glu-B3* and *Glu-A3* loci, obviously, it was inappropriate to describe only one of the loci.

In the present study, the interspecific D-genome substitution lines and two intervarietal substitution lines were used to determine the chromosomal control of the LMWGS. The nomenclature suggested by Singh and Shepherd (1988a) for the locus coding for LMWGS was adopted but with modified block symbols, i. e. the locus symbols *Glu-A3* and *Glu-B3* were used plus an allele (block) letter, with a prime. For example, *Glu-A3a'* was used to describe a block of B subunits of glutenin in durum wheat, and the prime differentiated it from the symbol used in bread wheat to describe the unrelated block *Glu-A3a* described earlier (Gupta, 1989). Therefore, the symbol LMW-A refers to the whole B subunit pattern of Langdon and *Glu-A3a'* and *Glu-B3a'* refers to its allelic components.

With the Nevo collection of *T. dicoccoides* from Israel (Nevo and Payne, 1987; Nevo and Beiles, 1989), emphasis was given to pattern variation at phenotypic level and no attempt was made to categorise the banding patterns into linkage blocks, because of the vast range of variation detected and the very limited information on their segregational behaviour. In the initial screening, the lines were compared for their variability of LMW and HMW glutenin subunit patterns within each population. Each different pattern identified within a population was given a different letter in alphabetical order (A, B, C etc). These patterns were compared with the patterns of Langdon and Edmore but they were not compared in detail with the known LMW glutenin subunit patterns of hexaploid and durum wheats (Gupta, 1989 and first part of this Chapter).

### 4.2.4 Methods of pattern analysis

Since the LMWGS had a narrow range of MW, they were usually clustered in a small region of the gel after SDS-PAGE separation. All the genotypes (cultivars and lines) included in the survey were examined at least five times in separate runs to check their banding patterns in detail. Those lines having recognisably similar patterns were rechecked by loading them in adjacent lanes in a new gel to detect minor differences in the banding patterns.

After the different banding patterns had been established (in durum wheat only) an attempt was made to define the allelic blocks making these patterns. A series of γ-gliadin reference bands γ-40, γ-42, γ-43.5, γ-45 and γ-47 all believed to be controlled by genes on chromosome 1BS (Dal Belin Peruffo *et al*, 1981, 1985; Joppa *et al*, 1983a; Payne *et al*, 1984c; du Cros and Hare, 1985; Autran and Galterio, 1989a; Pogna *et al*, 1990), were used for this purpose.

The approach used was based on knowledge that the *Gli-1* and *Glu-3* loci were closely linked (Sozinov and Poperelya, 1980; Payne *et al*, 1984d; Payne, 1987c; Singh and Shepherd, 1988a) and therefore these protein bands should be co-inherited. For example, gliadin band γ-42 was usually associated with the LMW-1 bands and band γ-45 with LMW-2 bands (Payne *et al*, 1984c). However, it should be emphasised that although this was correct

in theory in segregating population or cultivars produced in breeding programs, it did not necessarily apply to landraces or wild populations. In these latter examples, it was likely that given enough time they would reach linkage equilibrium and the gliadin and LMW glutenin subunit components would appear to be unassociated, even though their genes were highly linked.

The analytical procedure used was as follows:

If the gliadin patterns of two cultivars possessed the same major 1BS-controlled γ-gliadin band but differed in other major ω-γ-gliadin bands, it was assumed that the common gliadin banding pattern was controlled by the same *Gli-B1* gene (allele) and the different bands of two cultivars by different alleles of *Gli-A1* genes. Similarly, if a similar group of 1BS-controlled B subunits was recognized but differed in other major B subunits, it was assumed that the common bands were controlled by the same *Glu-B3* gene (allele) and the other different bands were controlled by different alleles of *Glu-A3* genes.

Similarly, if two out of three bands of a block of B subunits were present in lines with common γ-gliadin components, it was assumed that these B subunits represented different 1BS-controlled linkage blocks.

The limitation of using gliadin markers as markers for the identification and deduction of LMW glutenin subunit allelic types is acknowledged, but it at least provided an opportunity to determine the possible linkage blocks, and these must be confirmed by inheritance studies.

## 4.3 RESULTS

### 4.3.1 Genetic variation of LMW glutenin subunits and gliadins in *T. durum*

#### *4.3.1.1 Characterization of LMW glutenin subunits using two-step or one-step 1-D SDS-PAGE*

Initially, the glutenins were extracted without alkylation and they usually separated into fuzzy bands, which were difficult to score and had poor reproducibility. Later, this problem was overcome by alkylating the reduced LMWGS (Chapter 3). The two-step and the one-step procedures gave qualitatively similar results (cf. Gupta and Shepherd, 1990a, 1990b with Fig. 4.1A to 4.1C). Since the one-step procedure was much faster, more simple and reliable than the two-step procedure, it was adopted as the standard procedure for this work after it became available in 1990 (Singh *et al*, 1991b).

A total of 240 durum wheat cultivars and advanced lines from 12 countries were included in the survey for the LMW glutenin subunit patterns. The reduced and alkylated glutenin extracts from these wheats were separated into three groups of bands (A, B and C) by the gradient SDS-PAGE (Fig. 4.1A to 4.1C), corresponding to the HMW (A) and LMW (B and C) glutenin subunits (Payne and Corfield, 1979). Extensive pattern variation was observed and detailed results were given in Appendix A. Altogether 41 different LMW glutenin subunit

**Figure 4.1** SDS-PAGE patterns of durum wheat glutenin subunits (A, B and C) of different phenotypes, and controls.

**A** $C_1$, Edmore (LMW-B/γ-45 control); $C_2$ and $C_4$, Langdon (LMW-A/γ-42 control); (a-h), LMW-A variant patterns; $C_3$, Capinera (LMW-E/γ-40 control).

(a), T. d 10254 (LMW-AD); (b), Zaraa (LMW-AE); (c), Beladi Bouhi (LMW-AA); (d), BD-1588 (LMW-AF); (e), Langdon (LMW-A/γ-42 control); (f), Beladi Dakar (LMW-AG); (g), Riehi (LMW-AB); (h), Marouani (LMW-AC).

**B** $C_1$, Langdon (LMW-A/γ-42 control); (a-s), LMW-B variant patterns; $C_2$, Drago (LMW-D/γ-43.5 control).

(a), Touggourt (LMW-BD); (b), Gionp 1954 (LMW-BG); (c), T. d 13106 (LMW-BJ); (d), T. d 10257 (LMW-BL); (e), T. d 10264 (LMW-BN); (f), Morocco C 10897 (LMW-BM); (g), Edmore (LMW-B/γ-45 control); (h), Ble Dur 250 (LMW-BP); (i), T. d 13103 (LMW-BQ); (j), T. d 13142 (LMW-BH); (k), Medea (LMW-BE); (l), Piceno (LMW-BK); (m), T. d 13117 (LMW-BK); (n), Flemeen (LMW-BK); (o), Mahoudi Glabre AP 3 (LMW-BB); (p), P-66/270 (LMW-BF); (q), Coulter (LMW-BO); (r), Kharkof-5 (LMW-BI); (s), Ble Dur Noh 866 (LMW-BO).

**C** $C_1$, $C_4$, Langdon (LMW-A/γ-42 control); (a-d), LMW-E variant patterns; (e-k), LMW-D variant patterns; (l-p), LMW-C variant patterns; $C_2$, Edmore (LMW-B/γ-45 control); $C_3$, Capinera (LMW-E/γ-40 control).

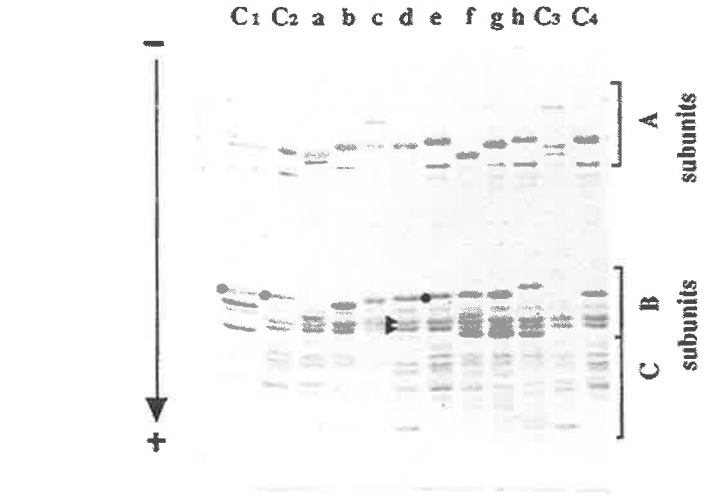
(a), Capinera (LMW-E/γ-40 control); (b), Surgul (LMW-EA); (c), Girgeh (LMW-EB); (d), Darkar 52 (LMW-EC); (e), T. d 10267 (LMW-DD); (f), Biancu Sicilianu (LMW-DB); (g), Drago (LMW-D/γ-43.5 control); (h), T. d 10256 (LMW-DC); (i), Durum H2 (LMW-DA); (j), J G Dickson 377 (LMW-DA); (k), T. d 13115 (LMW-DA); (l, o), MG 2988 (LMW-C/γ-47 control); (m), T. d 13140 (LMW-CA); (n), T. d 13062 (LMW-CB); (p), Kameshli (LMW-C).

**Symbols used in figures:**

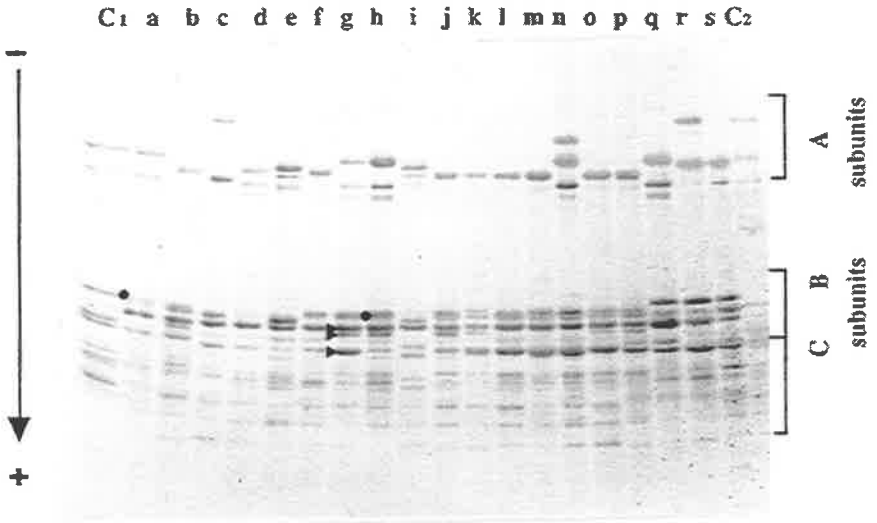
$C_1$ to $C_4$ = Control cultivars;

▶ = 1BS-controlled bands;

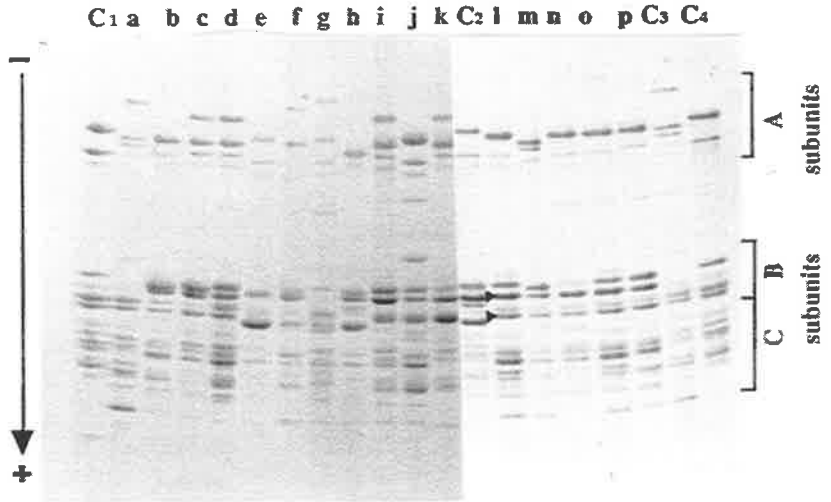
● = 1AS-controlled bands.


C1 C2 a b c d e f g h C3 C4
A subunits
C B subunits

A


C1 a b c d e f g h i j k l m n o p q r s C2
A subunits
C B subunits

B


C1 a b c d e f g h i j k C2 l m n o p C3 C4
A subunits
C B subunits

C

banding patterns were identified when only the B subunits were considered. Each genotype tested had two to six B subunit bands and three to seven C bands, and they varied in staining intensity with the former group being generally dark and the latter relatively faint (Fig. 4.1A to 4.1C). All of these B subunit variant patterns were further checked for their γ-gliadin bands according to five γ-gliadin reference cultivars with bands γ-42, γ-45, γ-47, γ-43.5 and γ-40 (Margiotta *et al*, 1987). These five γ-gliadin reference cultivars had five different LMW glutenin subunit patterns and the patterns were designated LMW-A, LMW-B, LMW-C, LMW-D and LMW-E, respectively. The 41 variant patterns were classified into five groups depending on their γ-gliadin markers and then assigned a second capital letter in alphabetical order to distinguish the different patterns within each group (Figs 4.1 and 4.2). These five groups included 8, 21, 3, 6 and 3 different patterns, respectively (Fig. 4.2).

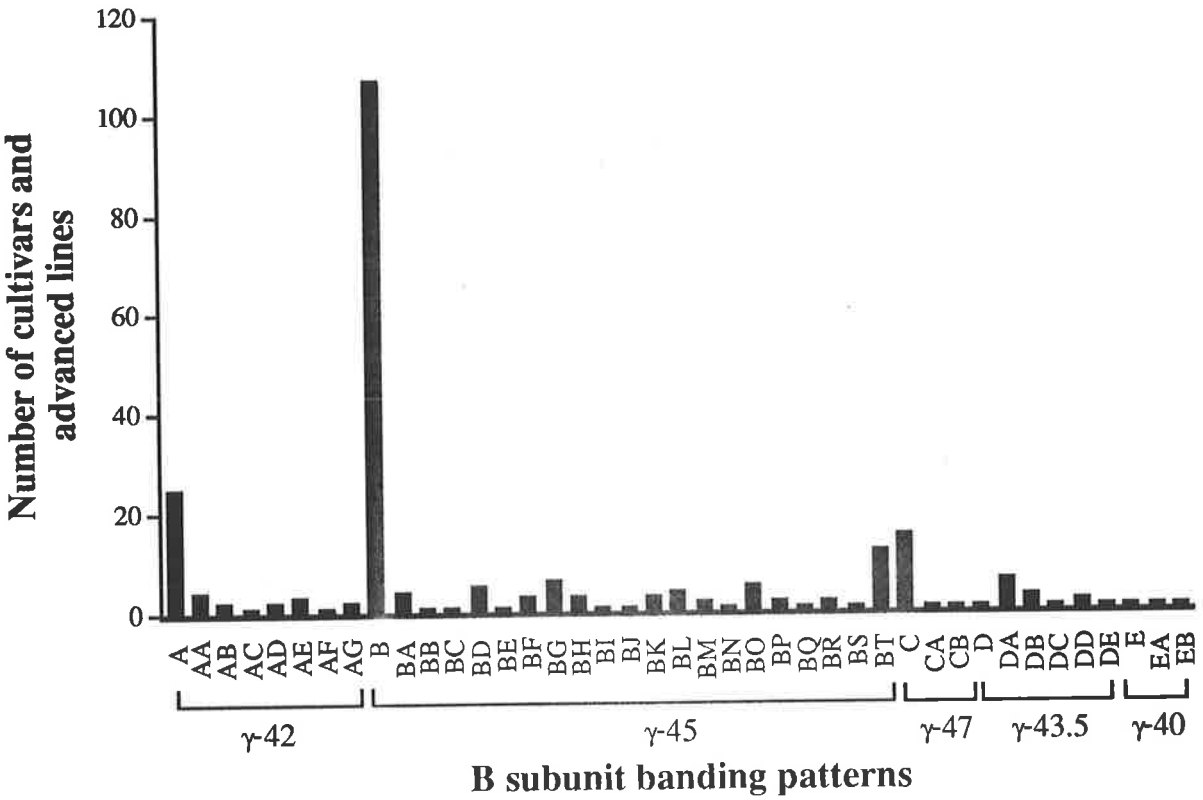



**Figure 4.2** LMW glutenin subunit banding patterns in 240 durum wheats and their frequency. The banding patterns A, B refereed to the patterns of Langdon (γ-42) and Edmore (γ-45), respectively, and corresponded to the former LMW-1 and LMW-2 types of Margiotta *et al* (1987). Patterns C, D and E corresponded to the other three γ-gliadin reference cultivars MG 2988 (γ-47), Drago (γ-43.5) and Capinera (γ-40) used in the survey. The different LMW banding patterns were grouped firstly by their γ-gliadin markers, then an extra capital letter was assigned in alphabetical order to each of the different patterns occurring within the group.

The frequency of the LMW-A pattern was quite low (10.4%) compared to that of the LMW-B type, which occurred in nearly 50% of all the samples analysed. The only other pattern which occurred with a reasonably high frequency (5.8%) was LMW-C. Altogether, these three types of patterns accounted for 60.0% of the total samples analysed. Most of the other new patterns occurred infrequently, usually once only (i. e. below 0.5%) (Fig. 4.2). The frequent pattern types LMW-A and LMW-B corresponded to durum wheat reference cultivars Langdon and Edmore, respectively, which previously had been referred to as patterns LMW-1 and LMW-2 in the papers of Margiotta *et al* (1987) and Pogna *et al* (1988) (Fig. 4.1A, lanes $C_2$, $C_1$). The third group, the LMW-C pattern (Fig. 4.1C, lane 1), had not been reported before, and differed from the LMW-B type in that its fastest band migrated more slowly than the fastest band of LMW-B and an intermediate faint band migrated faster than the second fast band of LMW-B type (cf. Fig. 4.1C, lanes 1, $C_2$). Most of the other new patterns differed in minor respects from one of the three common types, and would have been very difficult to identify by the traditional electrophoretic techniques, where gliadin and glutenin bands showed overlap when separated by 1-D SDS-PAGE (eg. Payne *et al*, 1984c; Margiotta *et al*, 1987).

Table 4.1 summarises the frequency of occurrence of the different LMW types from the various sources grouped by γ-42, γ-45 and other types. The frequency of LMW-B-related types (i. e. γ-45 typed LMW-B variants) was generally higher than any other types from most growing areas, especially in regions where cultivars have been bred and widely grown such as in Australia, CIMMYT (Mexico), USA and Italy. However, in samples from Egypt (i. e. UAR) and Morocco, previously undescribed types predominated (35.3% and 27.3%) with the LMW-B-related type accounting for only a low proportion (32.4% and 70.5%). Generally, the new types occurred more frequently in the North African and Mediterranean regions (Table 4.1).

### *4.3.1.2 Characterization of ω- and γ-gliadins in durum wheats*

### (A) Acid-PAGE

The genes coding for ω- and γ-gliadin bands were known to be tightly linked to genes coding for LMWGS (Payne *et al*, 1984c). Therefore, it is of interest to study the co-variation of these two classes of proteins.

Extensive variation was observed for gliadin banding patterns in the α-, β-, γ- and ω-regions (Fig. 4.3). However, variation of the α- and β-gliadin bands was not analysed further since these bands were known to be controlled by genes at loci on group 6 chromosomes (Wrigley and Shepherd, 1973; Payne *et al*, 1982a; Lafiandra *et al*, 1983).

The γ-gliadins were classified into five groups corresponding to the five gliadin reference cultivars used as controls (Fig. 4.3A to 4.3C) and their possible ω-gliadin blocks are shown in the diagram, Fig. 4.4A.

**Figure 4.3** Acid-PAGE patterns of durum wheat gliadin bands, and controls.

**A** $C_1$, Edmore (γ-45 control); (a-h), γ-42 variant patterns; $C_2$, Drago (γ-43.5 control).

(a), T. d 10254; (b), Zaraa; (c), Beladi Bouhi; (d), BD-1588; (e), Langdon (γ-42 control); (f), Beladi Dakar; (g), Riehi; (h), Marouani.

**B** $C_1$, Langdon (γ-42 control); (a-s), γ-45 variant patterns.

(a), Touggourt; (b), Gionp 1954; (c), T. d 13106; (d), T. d 10257; (e), T. d 10264; (f), Morocco C 10897; (g), Edmore (γ-45 control); (h), Ble Dur 250; (i), T. d 13103; (j), T. d 13142; (k), Medea; (l), Piceno; (m), T. d 13117; (n), Flemeen; (o), Mahoudi Glabre AP 3; (p), P-66/270; (q), Coulter; (r), Kharkof-5; (s), Ble Dur Noh 866.

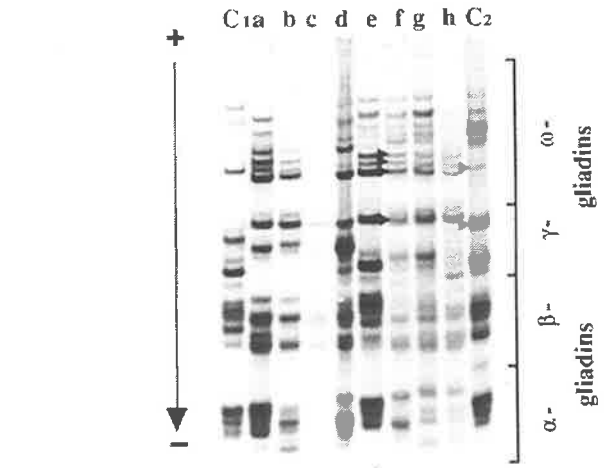
**C** $C_1$, $C_4$, Langdon (γ-42 control); $C_2$, MG 2988 (γ-47 control); (a-d), γ-40 variant patterns; (e-k), γ-43.5 variant patterns; (l-o), γ-47 variant patterns; $C_3$, Edmore (γ-45 control).

(a), Capinera (γ-40 control); (b), Surgul; (c), Girgeh; (d), Darkar 52; (e), T. d 10267; (f), Biancu Sicilianu; (g), Drago (γ-43.5 control); (h), T. d 10256; (i), Durum H2; (j), J G Dickson 377; (k), T. d 13115; (l), T. d 13140; (m), T. d 13062; (n), MG 2988 (γ-47 control); (o), Kameshli.

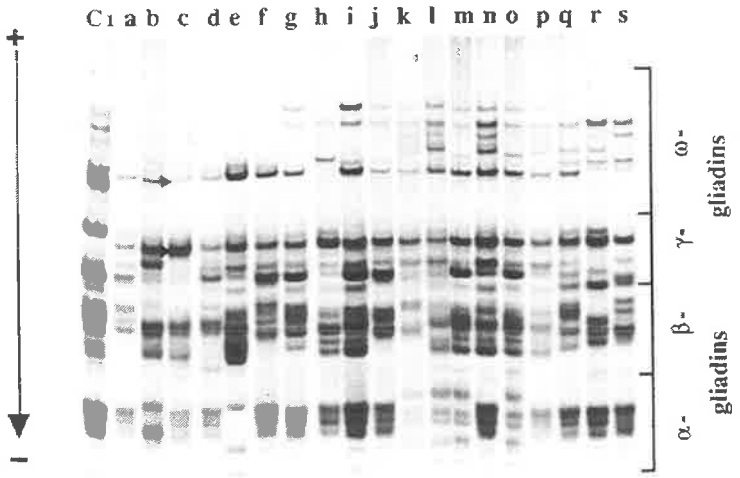
**Symbols used in figures:**

$C_1$ to $C_3$ = Control cultivars;

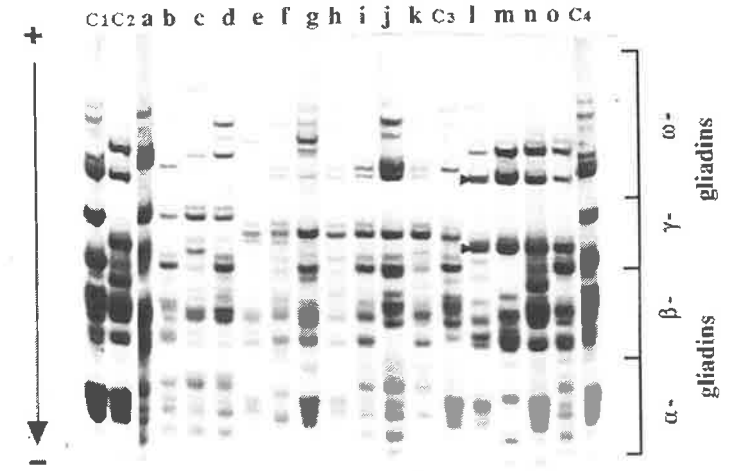
► = 1BS-controlled bands.


C1 a b c d e f g h C2
+
−
α- gliadins
β- γ- ω- gliadins

A


C1 a b c de f g h i j k l mn o p q r s
+
−
α- gliadins
β- γ- ω- gliadins

B


C1C2 a b c d e f g h i j k C3 l m n o C4
+
−
α- gliadins
β- γ- ω- gliadins

C

**Table 4.1** Frequency of LMW glutenin banding patterns of durum wheat cultivars from different sources

| | No. of samples analysed | **No. of different patterns** | LMW-B-related (γ-45) | | LMW-A-related (γ-42) | | Other patterns | |
|---|---|---|---|---|---|---|---|---|
| | | | No. | % | No. | % | No. | % |
| **Total** | **240** | **41** | **164** | **68.3** | **39** | **16.3** | **37** | **15.4** |
| Country | | | | | | | | |
| Algeria | 28 | **11** | 22 | 78.6 | 5 | 17.9 | 1 | 3.6 |
| Australia | 16 | **4** | 14 | 87.5 | 2 | 12.5 | 0 | 0.0 |
| Canada | 9 | **6** | 6 | 66.7 | 3 | 33.3 | 0 | 0.0 |
| France | 1 | **1** | 1 | 100.0 | 0 | 0.0 | 0 | 0.0 |
| Italy | 27 | **9** | 21 | 77.8 | 2 | 7.4 | 4 | 14.8 |
| Libya | 1 | **1** | 0 | 0.0 | 1 | 100.0 | 0 | 0.0 |
| CIMMYT | 16 | **2** | 16 | 100.0 | 0 | 0.0 | 0 | 0.0 |
| Morocco | 44 | **19** | 31 | 70.5 | 1 | 2.3 | 12 | 27.3 |
| Syria | 22 | **7** | 14 | 63.6 | 3 | 13.6 | 5 | 22.7 |
| Tunisia | 29 | **10** | 23 | 79.3 | 3 | 10.3 | 3 | 10.3 |
| UAR | 34 | **17** | 11 | 32.4 | 11 | 32.4 | 12 | 35.3 |
| USA | 13 | **5** | 5 | 38.5 | 8 | 61.5 | 0 | 0.0 |

Among the cultivars with a γ-42 band (Fig. 4.3A, lanes a to h), except one, all of them had the triplet of ω-gliadin bands 33-35-38. The exception BD-1588 (lane d) had only a single ω-38 band in that region. The γ-42 gliadin variants mostly possessed a weak γ-40 band as well. The ω-33-35-38-γ-42 bands (Fig. 4.3A, lane f, denoted as ►) had been shown earlier to be a block of bands controlled by a *Gli-B1* allele (Pogna *et al*, 1990). Altogether among all the 42 lines having the γ-42 band, only two lines were observed to have ω-bands different from the ω-33-35-38 block (see Appendix A).

Among the cultivars with a γ-45 band (Fig. 4.3B, lanes a to s), all of them had a single ω-35 band in this region except for Ble Dur 250 (lane h, ω-33 band), Kharkof-5 (lane r, a strong ω-32 and a weak ω-34 band) and Ble Dur Noh 866 (lane s, a strong ω-32 and two weak ω-34-35 bands). In the γ-gliadin region, most of these variants also had a weak γ-43 band (Fig. 4.4A). The ω-35-γ-45 band combination (Fig. 4.3B, lane c, denoted as ►) had been shown earlier to be a block controlled by another *Gli-B1* allele (Pogna *et al*, 1990). Among all the 171 lines having band the γ-45, only 15 lines were observed to have different ω-bands other than the ω-35-γ-45 linkage block, and γ-44.5 types were also included in this group (see Appendix A).

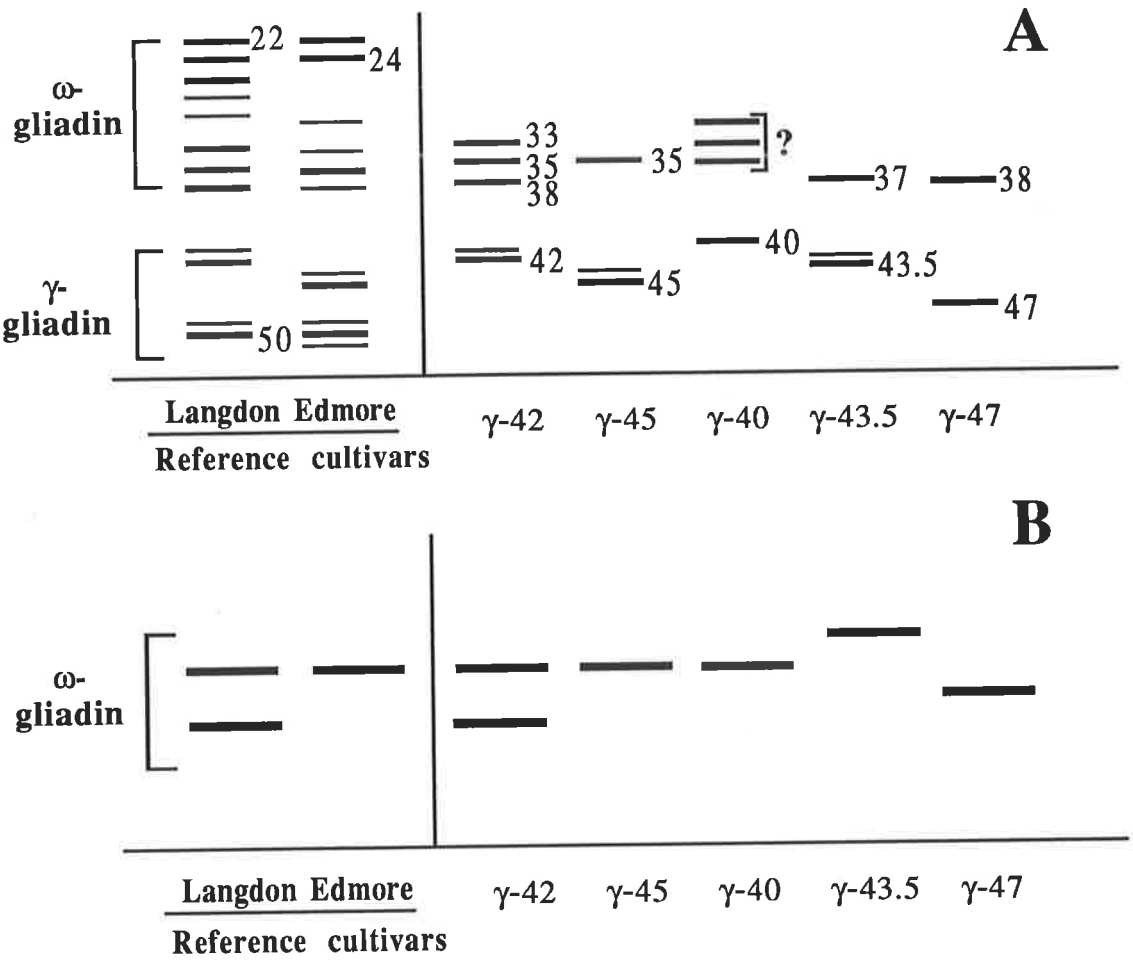



**Figure 4.4** Different possible ω- and γ-gliadin blocks of durum wheat cultivars deduced from (A) Acid-PAGE and (B) SDS-PAGE patterns, to be controlled by chromosome 1B, from 240 durum wheat cultivars and lines. The diagram was based on the five γ-gliadin band references used.

Among the γ-40 variants (Fig. 4.3C, lanes a to d), all possessed a weak band 39 and a strong band 40 but no consistent association with any particular ω-gliadin bands. The γ-40 reference (Fig. 4.3C, lane a) showed a triplet of ω-bands in the fast-moving region (bands 31-33-35), the second type had a single ω-34 band (lane b) while another type had ω-33 band (lanes c and d). Thus it was not clear whether there were any particular ω-bands associated with the γ-40 band as a linkage block (Fig. 4.4A).

All the γ-43.5 variants possessed an ω-37 band (Fig. 4.3C, lanes e to k). This block was also described by Carrillo *et al* (1990a) as the 34-37-44 block. Also, most of these types had a weak band 42 as well (Fig. 4.4A).

The γ-47 variants (Fig. 4.3C, lanes l to o) were all associated with a ω-38 gliadin band, assumed to be another inheritance block (Fig. 4.3C, lane l, denoted as ►, and Fig. 4.4A).

(B) SDS-PAGE

The gliadin bands were also separated by SDS-PAGE (Fig. 4.5A to 4.5C), since this separation technique was more simple to survey for the slowest ω-gliadins, provided partial information for the 1BS-controlled bands.

The γ-42 reference cultivar Langdon had two ω-gliadin bands (Fig. 4.5A, lane e, and Fig. 4.4B) and most of the other variants with γ-42 also had these two bands (Fig. 4.5A, lanes a to h, and denoted as ► in lane f) except for BD-1588 (lane d), which had two bands moving slightly faster than normal. [Note BD-1588 also gave a different ω-gliadin pattern when separated by Acid-PAGE (Fig. 4.3A, lane d)].

The γ-45 reference cultivar Edmore had one band (Fig. 4.5B, lane g and Fig. 4.4B), having the same mobility as the slower ω-band of the γ-42 control (Fig. 4.5A, lanes $C_1$ and $C_2$). The γ-45 types (lanes a to s) all had a single slow ω-gliadin band (lane g, denoted as ►) except for Kharkof-5 (lane r) and Ble Dur Noh 866 (lane s), which had a slightly faster faint band (Fig. 4.5B).

The γ-40 reference cultivar Capinera (Fig. 4.5C, lane a) and all the other γ-40 types (Fig. 4.5C, lanes a to d) possessed the same ω-band as the γ-45 control (Fig. 4.4B) except Surgul (lane b) which had a single faster band.

The γ-43.5 cultivars (Fig. 4.5C, lanes e to k) all possessed a single slow-moving ω-band (Fig. 4.4B).

Cultivars with the γ-47 type (Fig. 4.5C, lanes l to p) all had one or two ω-bands with mobility intermediate between that of two bands present in γ-42 control (Fig. 4.4B).

### *4.3.1.3 Identification of 1AS- and 1BS-controlled B subunit linkage blocks in durum wheat cultivars*

(A) Chromosomal control of LMW glutenin subunits in cultivar Langdon

The chromosomes controlling the LMWGS (B and C subunits) in Langdon were investigated by analysing the D-genome disomic substitution lines available. The only lines which gave different band patterns of B subunits were the 1D(1A) and 1D(1B) substitution lines (Fig. 4.6, lanes q, r). The slowest B subunit band was absent from Langdon 1D(1A). In Langdon 1D(1B), the faint slower band was missing but the two faster dark bands remained with reduced band staining intensity. It was difficult to be certain that the two fastest bands of Langdon were absent in the substitution line, since these bands overlapped with the bands coded by *Glu-D3* of chromosome 1D in substitution line. Convincing evidence for this was obtained later when it was found that these three faster bands were present in a Langdon 1AL.1RS translocation line but absent in the Langdon 1BL.1RS line (see Chapter 5 section

**Figure 4.5** SDS-PAGE patterns of durum wheat gliadin bands, and controls.

**A** $C_1$, Edmore (γ-45 control); $C_2$ and $C_4$, Langdon (γ-42 control); (a-h), γ-42 variant patterns; $C_3$, Capinera (γ-40 control).

(a), T. d 10254; (b), Zaraa; (c), Beladi Bouhi; (d), BD-1588; (e), Langdon (γ-42 control); (f), Beladi Dakar; (g), Riehi; (h), Marouani.

**B** $C_1$, Langdon (γ-42 control); (a-s), γ-45 variant patterns; $C_2$, Capinera (γ-40 control); $C_3$, Drago (γ-43.5 control).

(a), Touggourt; (b), Gionp 1954; (c), T. d 13106; (d), T. d 10257; (e), T. d 10264; (f), Morocco C 10897; (g), Edmore (γ-45 control); (h), Ble Dur 250; (i), T. d 13103; (j), T. d 13142; (k), Medea; (l), Piceno; (m), T. d 13117; (n), Flemeen; (o), Mahoudi Glabre AP 3; (p), P-66/270; (q), Coulter; (r), Kharkof-5; (s), Ble Dur Noh 866.

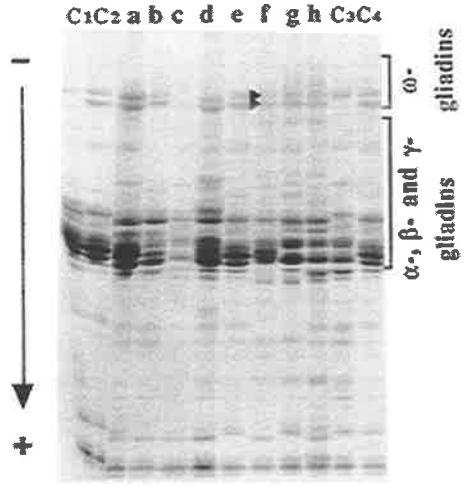
**C** $C_1$, Langdon (γ-42 control); (a-d), γ-40 variant patterns; (e-k), γ-43.5 variant patterns; (l-p), γ-47 variant patterns; $C_2$, Edmore (γ-45 control).

(a), Capinera (γ-40 control); (b), Surgul; (c), Girgeh; (d), Darkar 52; (e), T. d 10267; (f), Biancu Sicilianu; (g), Drago (γ-43.5 control); (h), T. d 10256; (i), Durum H2; (j), J G Dickson 377; (k), T. d 13115; (l), T. d 13140; (m), T. d 13062; (n), MG 2988 (γ-47 control); (o), Kameshli; (p), T. d 13168.

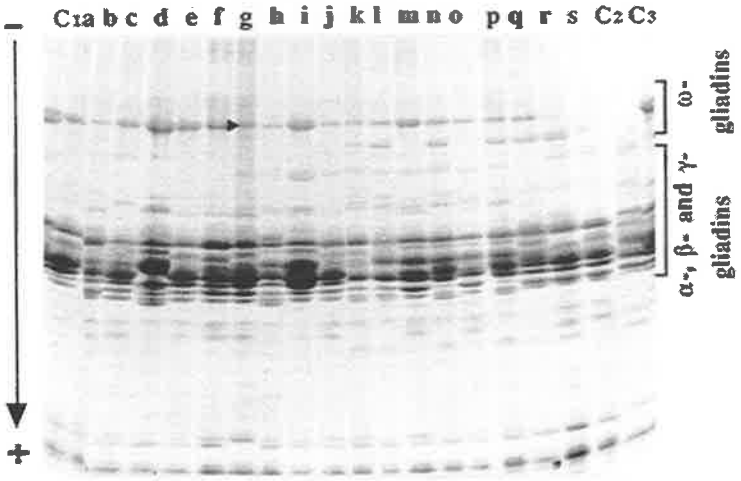
**Symbols used in figures:**

$C_1$ to $C_4$ = Control cultivars;

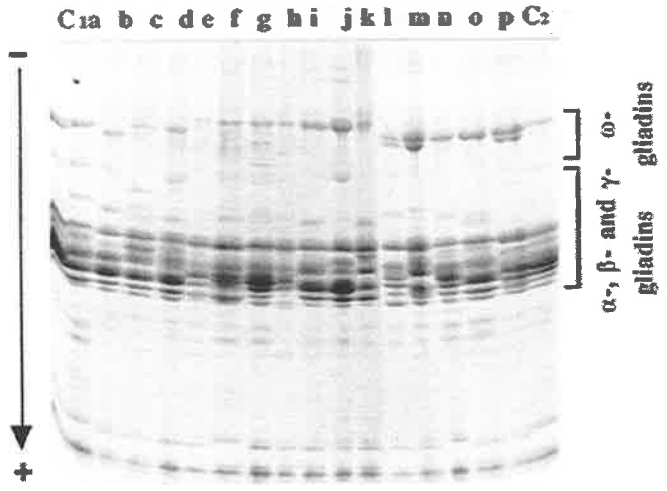
► = 1BS-controlled bands.


C1C2 a b c d e f g h C3C4
ω-gliadins
α-, β- and γ-gliadins
–
+

A


C1a b c d e f g h i j k l m n o p q r s C2C3
ω-gliadins
α-, β- and γ-gliadins
–
+

B


C1a b c de f g hi jkl mn o p C2
ω-gliadins
α-, β- and γ-gliadins
–
+

C

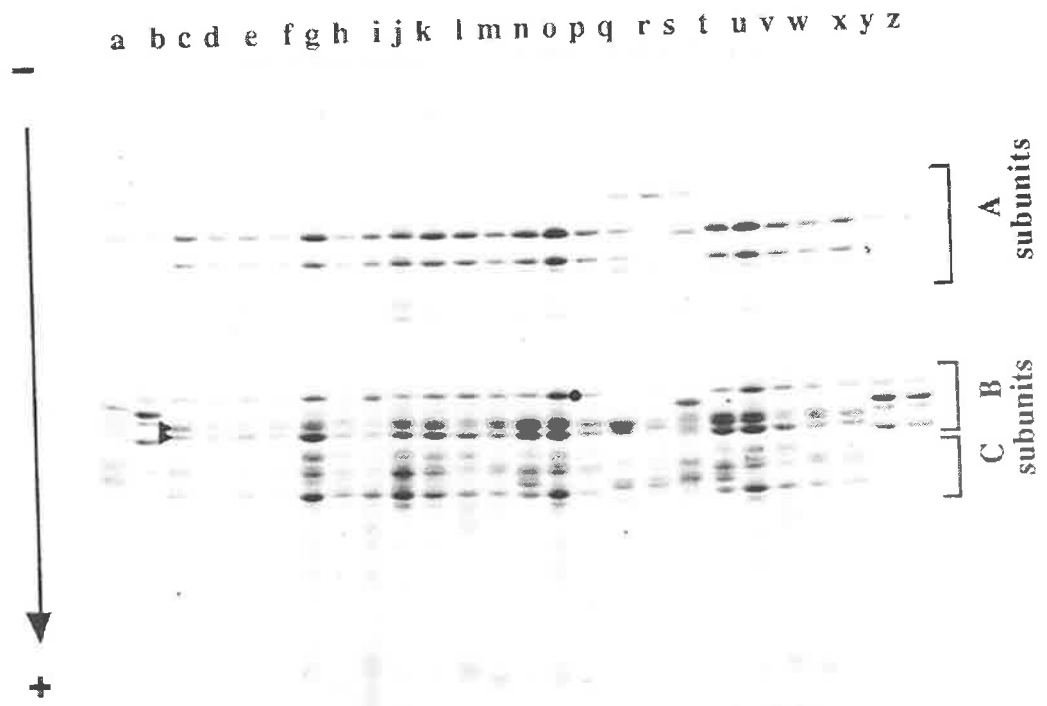
**Figure 4.6** SDS-PAGE patterns of glutenin subunits of durum wheat cultivar Langdon (Ldn) D-genome disomic substitution lines and controls.

(a, s), C. S.; (b, y, z), Edmore; (c, p, x), Langdon; (d), Ldn 2D(2A); (e), Ldn 2D(2B); (f), Ldn 3D(3A); (g), Ldn 3D(3B); (h), Ldn 4D(4B); (i), Ldn 4D(4A); (j), Ldn 5D(5A); (k), Ldn 5D(5B); (l, v), Ldn 7D(7A); (m, w), Ldn 7D(7B); (n, t), Ldn 6D(6A); (o, u), Ldn 6D(6B); (q), Ldn 1D(1A); (r), Ldn 1D(1B).

**Symbols used in figure:**

► = 1BS-controlled bands;

● = 1AS-controlled bands.


a b c d e f g h i j k l m n o p q r s t u v w x y z
−
+
A subunits
C B subunits

5.3.1 for details). These conclusions were consistent with those deduced earlier by Gupta and Shepherd (1988) using the Langdon (Edmore-1B) and Langdon (Kharkof-5-1B) substitution lines, confirming that the B subunits of Langdon durum were controlled by genes on the short arms of homoeologous group 1 chromosomes (Fig. 4.6).

There appeared to be many overlapping bands in the C subunit region and it was difficult to deduce the genetic control of these bands. However, some major differences were observed in the substitution lines. Substitution 7D(7B) (Fig. 4.6, lanes m and w) did not have the slowest C band and 6D(6A) (lanes n and t) was lacking the fastest C band but appeared to have gained two new bands. Langdon 3D(3B) to 7D(7B) (lanes g to o) appeared to have extra dark bands with high mobility at the bottom of the gel. Langdon 3D(3B), 5D(5A), 5D(5B), 7D(7A), 6D(6A) and 6D(6B) (lanes g, j to l, n and o) all had quantitative differences in the staining intensity of the slowest C subunits. The C subunits of hexaploid wheat were earlier reported to be controlled by genes on both homoeologous group 1 and 6 chromosomes (Gupta, 1989), but the present survey indicated that homoeologous group 5 and 7 chromosomes might also be involved in synthesizing some of these C subunits, and possibly group 3 chromosomes.

Because of the apparent inconsistency and complexity of the expression of the C subunits, the current study focused only on the variation and the genetic control of the B subunits.

## (B) Identification of linkage blocks of B subunits in durum wheats

The banding patterns of B subunits were divisible into two independent groups based on the knowledge that there were only two loci coding for the major B bands in tetraploid wheat (Payne *et al*, 1984c; Gupta and Shepherd, 1988; Autran and Galterio, 1989a). The determination of which band patterns were inherited as a block can only be properly determined in inheritance studies (see Chapter 5), but a clue to the different types of blocks present could be obtained by a combined analysis of gliadin and LMW glutenin subunit band patterns. In this section, attempts are made to deduce the linkage blocks among the B subunits from the presence of similar patterns in the different cultivars having the same gliadin markers. The LMW glutenin loci have been shown to be tightly linked to those controlling the ω- and γ-gliadin bands so they were usually co-inherited (Payne *et al*, 1984c; Gupta and Shepherd, 1990a; Pogna *et al*, 1990). Therefore, the gliadin bands were used as markers to deduce the inherited blocks of LMWGS.

All of the five γ-gliadin reference cultivars analysed gave different LMW glutenin subunit banding patterns (Fig. 4.1, cf. lanes marked with "C" and Fig. 4.1C, lane l). All of the different LMW patterns detected in the cultivar survey were arranged firstly by their common γ-gliadin type and then by LMW banding patterns showing the closest similarity in patterns. To allow direct comparisons of γ-ω-gliadin and LMW glutenin subunit patterns and to facilitate the detection of associated patterns, the cultivar protein extracts were all arranged in the same

loading sequence except for the controls, as illustrated in Figs 4.1, 4.3 and 4.5. That is, the γ-42 types were loaded in the same sequence in lanes a to h, Fig. 4.3A for Acid-PAGE gliadins, Fig. 4.5A for SDS-PAGE gliadins and Fig. 4.1A for LMWGS. The γ-45 types (lanes a to s, Fig. 4.3B, Fig. 4.5B and Fig. 4.1B, respectively), γ-40 types (lanes a to d), γ-43.5 types (lanes e to k) and γ-47 types (lanes l to p) in Fig. 4.3C, Fig. 4.5C and Fig. 4.1C, respectively, were arranged similarly.

The common 1AS-controlled B subunit in reference cultivars Langdon and Edmore was assigned symbol *a'* (Fig. 4.1A, lanes $C_1$ and $C_2$, Fig. 4.6, lane p, denoted as ●), and the different 1BS-controlled B subunits were assigned symbols *a'* and *b'*, respectively. The 1AS- and 1BS-controlled linkage blocks of Langdon (i. e. *Glu-A3a'* and *Glu-B3a'*) were deduced by comparing the band patterns of the 1D substitution lines (Fig. 4.6, lanes q, r) with the parental type (Fig. 4.6, lane p, denoted as ● and ► in lanes p and c). Confirmatory evidence for this classification was obtained in the inheritance study described later (see Chapter 5, section 5.3.2). So did for *Glu-B3b'* of reference cultivar Edmore (see Chapter 5, section 5.3.2). The symbols *c'* and *d'* were assigned to the presumed 1BS-controlled linkage blocks of the γ-43.5 and γ-47 reference cultivars (Drago and MG 2988), respectively (Fig. 4.1C, lanes g, l).

The γ-42 types with one exception, exhibited the block of gliadin bands ω-33-35-38-γ-42 in Acid-PAGE (Fig. 4.3A, lane f, denoted as ►) or 2 dark common ω-bands in SDS-PAGE (Fig. 4.5A, lane f, denoted as ►), all known to be controlled by chromosome arm 1BS (Fig. 4.4). These types all presented two common B subunit bands in SDS-PAGE (Fig. 4.1A, lane d, denoted as ►). Hence they were identified as having a *Glu-B3a'* linkage block (lanes b to e, g and h) except for lanes a and f, which were assigned symbol *Glu-B3g'* because the latter two lines appeared to have three common bands as a block. Since the slowest band of Langdon was known to be 1AS-controlled and the remaining bands 1BS-controlled (Gupta and Shepherd, 1988), it was assumed after accounting for the 1BS-controlled bands presented in all samples that the remaining B subunits were all 1AS-controlled (Fig. 4.1A, lanes a to h). The *Glu-A3* blocks, on this basis, were assigned the block symbols *h'*, *i'*, *g'*, *f'*, *a'*, *c'*, *c'* and *e'*, respectively (Fig. 4.1A, lanes a to h, and summarised in Fig 4.7).

The γ-45 gliadin types were associated with the ω-35 band in Acid-PAGE (Fig. 4.3B, lane c, denoted as ►) and one particular ω-gliadin band in SDS-PAGE (Fig. 4.5B, lane g, denoted as ►) but were associated with several slight different B subunit patterns (Fig. 4.1B, lanes a to s). However, the basic pattern of Edmore (three fast bands, denoted as ►, in Fig. 4.1B, lane g) was present among most of these variants with some minor differences and they were all assumed to be different *Glu-B3* blocks compared to the reference pattern *b'* (lane g) and the block symbols *f'*, *e'*, *h'*, *h'*, *n'*, *h'*, and *o'*, *p'*, *b'*, *b'*, *l'*, *l'*, *l'*, *b'*, *k'*, *f'*, *i'*, *f'* (Fig. 4.1B, lanes a to f and h to s and summarised in Fig. 4.7) were assigned accordingly.

These above deduced patterns were based on the assumption that the slowest bands with or without the fastest bands were 1AS-controlled and the remaining bands were 1BS-controlled. *Glu-A3 h'* was designated to a null form (eg. Fig. 4.1B, lane d) and the remaining

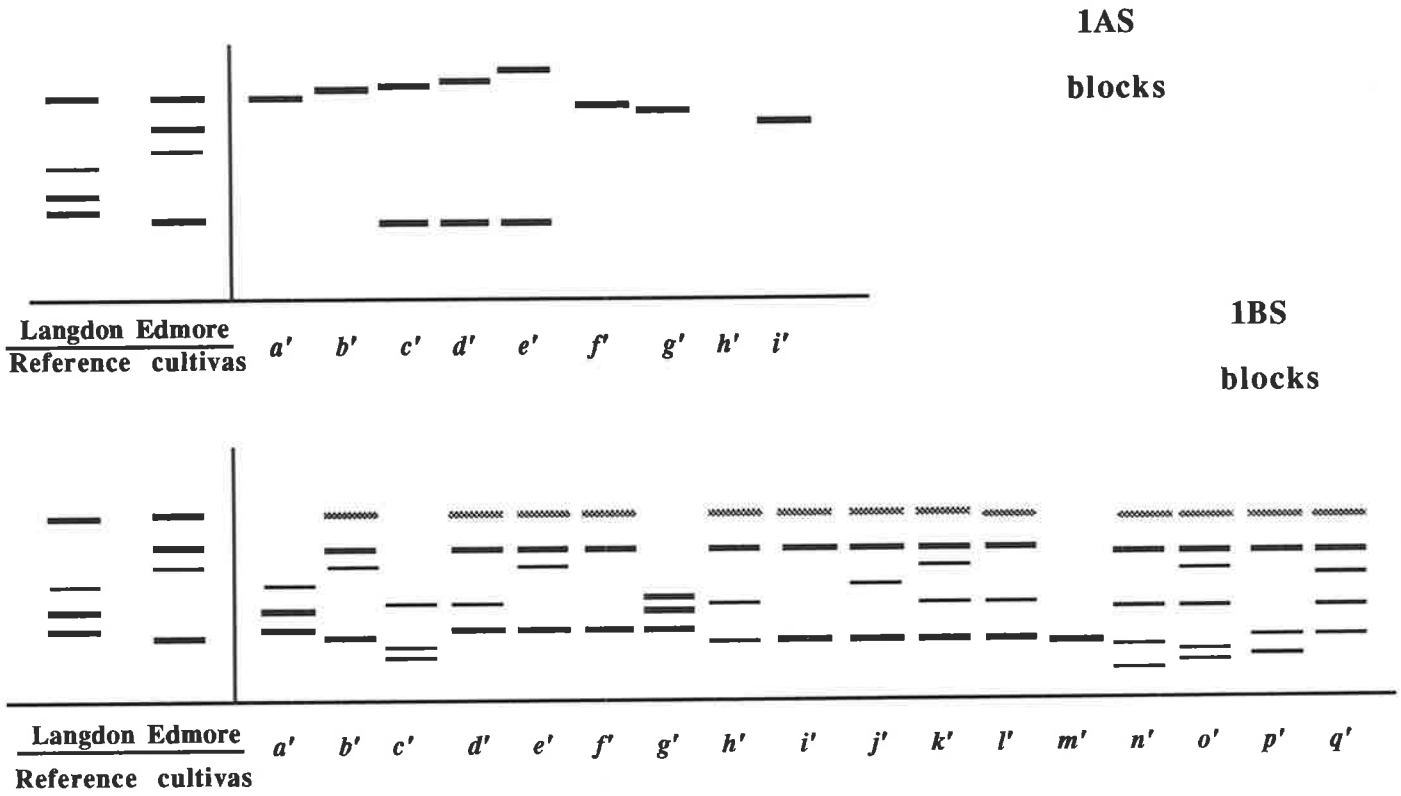



**Figure 4.7** Possible linkage blocks of B subunits of glutenin of durum wheat cultivars derived from SDS-PAGE patterns, controlled by chromosomes 1A and 1B, by analysis of 240 durum cultivars and lines. The diagram was deduced from the observed band patterns after firstly grouping them with particular γ-gliadin bands. The banding patterns of the reference cultivars Langdon and Edmore were determined by the intervarietal and interspecific substitution lines demonstrated earlier (Gupta, 1989) with few modifications. Patterns "*a'*" and "*b'*" in the 1BS blocks were based on references Langdon and Edmore, respectively.

*Glu-A3* and *Glu-B3* blocks were deduced by applying the same type of analysis to Figs 4.1B and 4.1C.

More detailed studies obviously need to be carried out to confirm these assumptions. However, as a working hypothesis, it was postulated that there were 17 different *Glu-B3* and nine *Glu-A3* blocks present in the surveying cultivars as shown in Fig. 4.7 with blocks *Glu-A3a'*, *Glu-A3f'*, *Glu-B3a'*, *Glu-B3b'*, *Glu-B3d'* and *Glu-B3f'* being the most commonly occurring types (Table 4.2 and see Appendix A for detail).

**Table 4.2** Summary of the allelic frequencies at *Glu-3* loci of durum wheat

| Locus | Alleles | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *Glu-A3* | *a'* | *b'* | *c'* | *d'* | *e'* | *f'* | *g'* | *h'* | *i'* | | | | | | | |
| Freq | 180 | 4 | 11 | 1 | 7 | 12 | 8 | 8 | 9 | | | | | | | |
| *Glu-B3* | *a'* | *b'* | *c'* | *d'* | *e'* | *f'* | *g'* | *h'* | *i'* | *j'* | *k'* | *l'* | *m'* | *n'* | *o'* | *p'* | *q'* |
| Freq | 37 | 115 | 1 | 17 | 6 | 18 | 4 | 7 | 8 | 1 | 15 | 3 | 2 | 1 | 3 | 1 | 1 |

### *4.3.1.4 Variation of HMW glutenin subunits*

Table 4.3 shows the frequency of the HMWGS among the durum wheat analysed. Most lines were null for *Glu-A1* (87.1%) whereas the functional alleles, 1 and 2*, occurred rarely (4.2%) (Fig. 4.1 and see Appendix A for detail). The *Glu-B1*-coded bands, bands 20, 6+8 and 7+8 occurred with frequencies of 31.7%, 23.3% and 14.6%, respectively (Fig. 4.1 and see Appendix A for detail). One new *Glu-A1* band (*Glu-A1$_V$*) and ten novel *Glu-B1* bands assigned letters *I* to *X* together were observed at a relatively high frequency (i. e. 8.8% and 22.1% respectively) in this survey. These novel HMW glutenin subunits did not compare with the published patterns and further investigation is needed to clarify them. The current results on the variation of HMWGS were, in general, consistent with those of previous studies (Vallega, 1986, 1988a, 1988b; Branlard *et al*, 1989; Pogna *et al*, 1990), representing a subsample of world collections.

**Table 4.3** Summary of the allelic frequencies at *Glu-1* loci of durum wheat[a]

| Locus | Alleles | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *Glu-A1* | *a* (1) | *b* (2*) | *c* (0) | *V* | | | | | | | | | | | | |
| Freq | 6 | 4 | 209 | 21 | | | | | | | | | | | | |
| *Glu-B1* | *a* (7) | *b* (7+8) | *d* (6+8) | *e* (20) | *f* (13+16) | *I* | *II* | *III* | *IV* | *V* | *VI* | *VII* | *VIII* | *IX* | *X* | ? |
| Freq | 1 | 35 | 56 | 76 | 11 | 1 | 4 | 3 | 1 | 3 | 1 | 1 | 8 | 15 | 16 | 8 |

[a] = The number designation of HMW glutenin subunit alleles was given in brackets and the nomenclature for novel alleles was based on Vallega (1986, 1988b).

### 4.3.2 Variation of LMW glutenin subunits and gliadins in *T. dicoccum*

Two sets of *T. dicoccum* lines were studied and a large amount of variation in LMWGS was detected (Figs 4.8.1A and 4.8.2A). Eight LMW glutenin subunit patterns were observed among the first set of 16 lines with four to six B subunits and three to ten C subunits, with variation in band mobility and staining intensity (Fig. 4.8.1A).

A similar high level of polymorphism was also observed among gliadins by Acid-PAGE (Fig. 4.8.1B). Altogether, nine different patterns were detected based solely on major ω-gliadin bands, ranging from two to five bands with a large degree of variability in band mobility. Aus 3717 (Fig. 4.8.1B, lane a) had two dark γ-gliadin bands 44 and 46, Aus 3446, Aus 17968 and Aus 3735 (lanes b to d) possessed a γ-gliadin band similar to γ-43.5, but it was likely that they were linked to ω-27-31 gliadin bands. Aus 3740 (lane e) exhibited band 45, linked to the usual band ω-35. Aus 3731 (lane f) possessed ω-36 and γ-46 gliadin bands. Aus 20222 (lane g) exhibited the typical γ-42 block with ω-gliadin bands. The last three lines (lanes h, i and j) gave no recognisable gliadin patterns. Similarly, a very large range of variation in the ω-gliadin bands separated by SDS-PAGE was also observed, ranging from one faint band to six bands (Fig. 4.8.1C).

Despite the large differences in ω-γ-gliadin bands overall, lanes b to d (Fig. 4.8.1A) appeared to possess a similar set of three 1BS-controlled bands, consistently associated with a similar ω-γ-gliadin block (Fig. 4.8.1B, lanes b to d).

The second set of nine *T. dicoccum* lines from the USA exhibited eight different B subunit patterns (Fig. 4.8.2A), seven different ω- and γ-gliadin patterns in Acid-PAGE (Fig. 4.8.2B) and seven different ω-gliadin patterns in SDS-PAGE (Fig. 4.8.2C). CI 2213 (lane a, Fig. 4.8.2B) had the same ω-gliadin block as the band γ-43.5 reference (i. e. cultivar Drago, lane $C_1$) and it showed a close resemblance to the LMW-DD band pattern (cf. Fig. 4.8.2A, lane a and Fig. 4.1C, lane e). Similarly, lanes c to e, and g to i all possessed γ-43 (Fig. 4.8.2B), associated with different ω-gliadin bands, however, they possessed a common fastest dark band to the LMW-DD type (Fig. 4.8.2A, lanes c, d, and f to i). Curiously, lane d (Fig. 4.8.2A) had only one dark band and one faint band, suggesting that it possessed a null *Glu-A3* gene. Lanes b and f (Fig. 4.8.2B) both had a band similar to γ-45 but it was associated with a ω-33 band and had a similar *Glu-B3b'* pattern (Fig. 4.8.2A, lanes b and e). In general, the data presented suggested that the normal ω-γ-gliadin-B-subunit linkage in durum wheat surveyed before (section 4.3.1) was not fully repeated here.

**Figure 4.8.1** Electrophoretic banding patterns of the *T. dicoccum* lines obtained from AWCC, Tamworth, and controls.

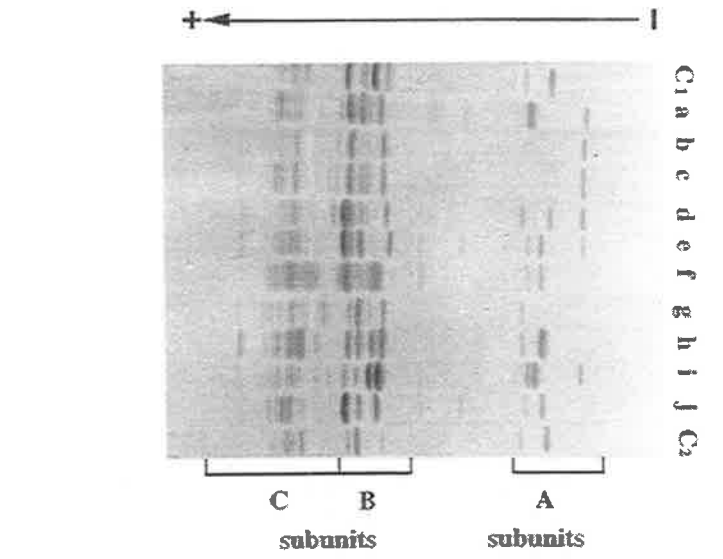
**A** SDS-PAGE patterns of glutenin subunits;

**B** Acid-PAGE patterns of gliadin bands;

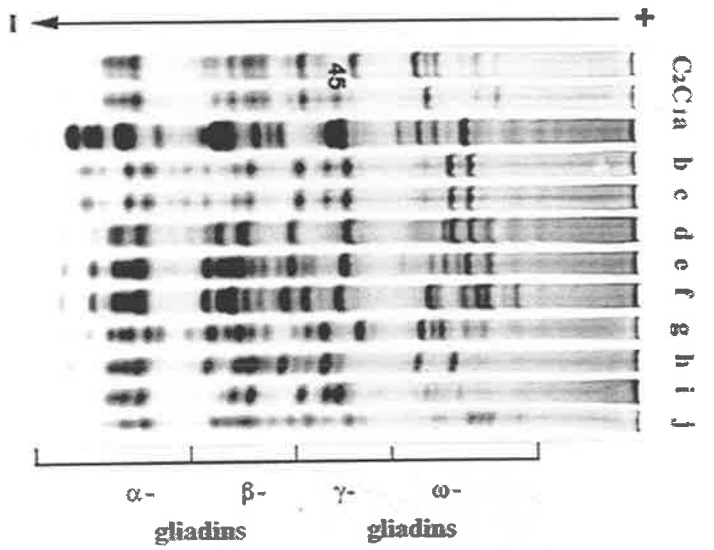
**C** SDS-PAGE patterns of gliadin bands.

($C_1$), Edmore (LMW-B/γ-45 control); ($C_2$), Langdon (LMW-A/γ-42 control); (a-j), *T. dicoccum* lines.

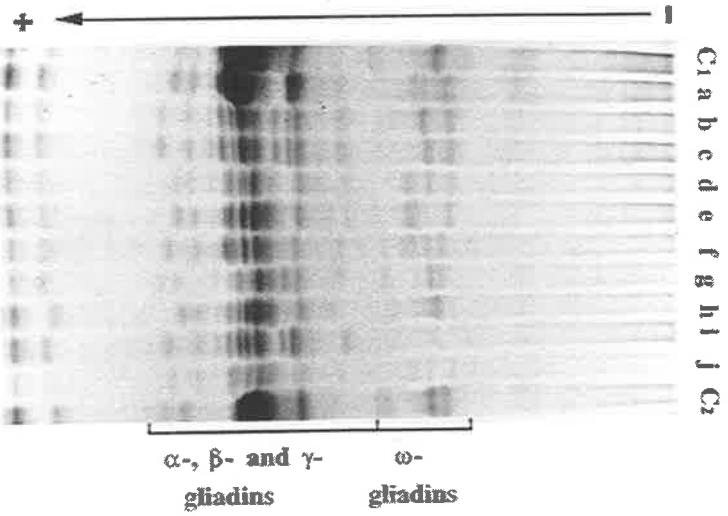
(a), Aus 3717; (b), Aus 3446; (c), Aus 17968; (d), Aus 3735; (e), Aus 3740; (f), Aus 3731; (g), Aus 20222; (h), Aus 4037; (i), Aus 19385; (j), Aus 3734.


+
-
C1 a b c d e f g h i j C2
C
B
A
subunits
subunits

A


-
+
45
C2C1a b c d e f g h i j
α-
β-
γ-
ω-
gliadins
gliadins

B


+
-
C1 a b c d e f g h i j C2
α-, β- and γ-
gliadins
ω-
gliadins

C

**Figure 4.8.2** Electrophoretic banding patterns of the *T. dicoccum* lines obtained from Dr G. Waines, USA, and controls.

**A** SDS-PAGE patterns of glutenin subunits;

($C_1$), Langdon (LMW-A/γ-42 control); ($C_2$), Edmore (LMW-B/γ-45 control); (a-i), *T. dicoccum* lines.

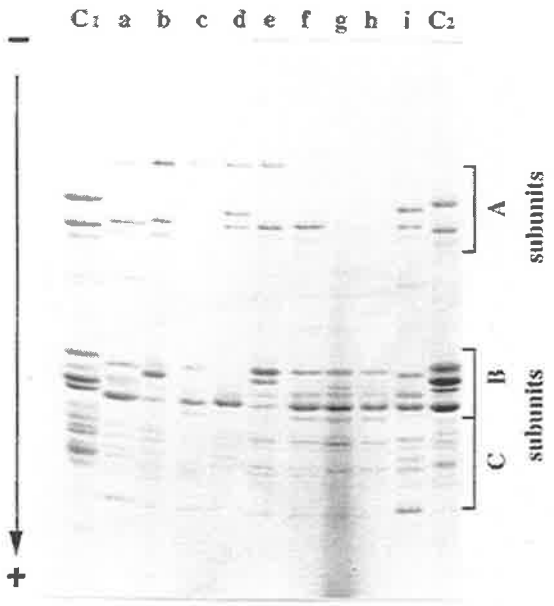
(a), CI 12213; (b), PI 94683; (c), PI 94640; (d), PI 355505; (e), PI 94633; (f), PI 94669; (g); PI 94668; (h), PI 349045; (i), PI 94665.

**B** Acid-PAGE patterns of gliadin bands;

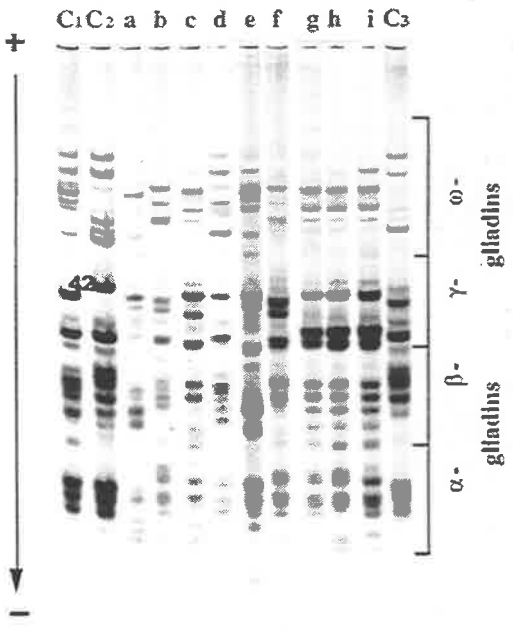
**C** SDS-PAGE patterns of gliadin bands.

($C_1$), Drago (γ-43.5 control); ($C_2$), Langdon (γ-42 control); (a-i), *T. dicoccum* lines; ($C_3$), Edmore (γ-45 control).

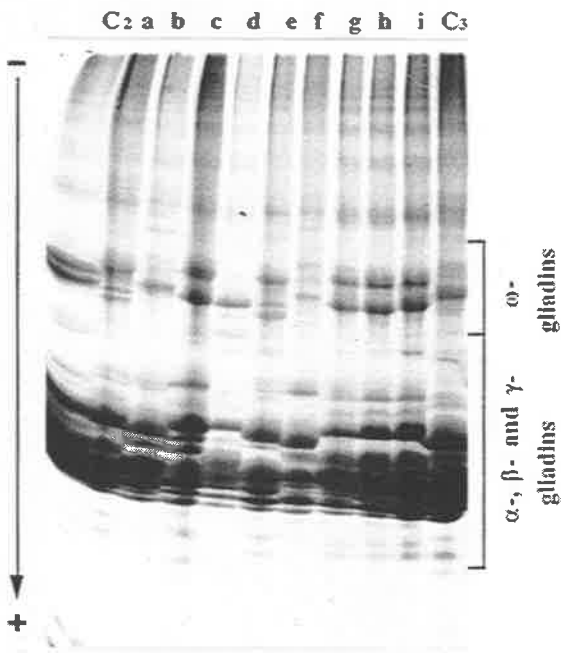
(a), CI 12213; (b), PI 94683; (c), PI 94640; (d), PI 355505; (e), PI 94665; (f), PI 94633; (g), PI 94669; (h); PI 94668; (i), PI 349045.


C1 a b c d e f g h i C2
A subunits
B subunits
C

A


C1C2 a b c d e f g h i C3
ω-gliadins
γ-gliadins
β-gliadins
α-

B


C2 a b c d e f g h i C3
ω-gliadins
α-, β- and γ-gliadins

C

### 4.3.3 Variation of LMW glutenin subunits and gliadins in *T. polonicum*

Twenty-one lines of *T. polonicum* were analysed and only seven different B subunit patterns were detected (Fig. 4.9A) with five different γ-ω-gliadin patterns detected by Acid-PAGE (Fig. 4.9B) and three ω-gliadin patterns in unreduced SDS-PAGE (Fig. 4.9C).
The samples in lanes a to f all possessed the ω-35 gliadin band associated with a γ-45 band, similar to Langdon (Edmore-1B) (Fig. 4.9B, lane $C_5$), suggesting that they had a common γ-45 block of bands. These lines also had a common ω-gliadin band in SDS-PAGE (Fig. 4.9C, lanes a to f), equivalent to the *Gli-B1* band of Edmore, consistent with the above evidence. Nevertheless, in several cases the LMW glutenin patterns of these samples were different (Fig. 4.9A, lanes a, c, e, f). Aus 3814 and Aus 3815 (lanes g and h) gave the same ω- and γ-block as Langdon in Acid-PAGE (Fig. 4.9B, lane $C_1$) and SDS-PAGE pattern (Fig. 4.9C, lane $C_1$) and they corresponded to the *Glu-B3a'* linkage block of Langdon (Fig. 4.9A, lane $C_1$). However, they had different *Glu-A3* linkage blocks. Aus 22343 (lane e, Fig. 4.9A) seemed to have the same 1AS-controlled block as Kharkof-5 (*Glu-A3e'*) since the gliadin pattern (Fig. 4.9C, lane e) was the same as that of Kharkof-5, in which a group of faster-moving ω-gliadin bands were present (refer to Chapter 5, section 5.3.2.3).

### 4.3.4 Variation of LMW glutenin subunits and gliadins in *T. dicoccoides*

#### *4.3.4.1 Collection of Dr U. Kushnir*

The set of 16 lines in this collection showed seven different LMW glutenin subunit patterns (Fig. 4.10A), with four or five B subunits and three to eight C subunits with variable staining intensity.

Four different ω-γ-gliadin patterns were also detected by Acid-PAGE (Fig. 4.10B). Each line had four to seven ω-gliadin bands, with several having six bands. All lines possessed a common γ-43.5 gliadin band, and a ω-37-γ-43.5 block as found earlier among durum wheats (section 4.3.1.2). Two different ω-gliadin patterns were observed with unreduced SDS-PAGE and there were highly to be 1BS-controlled (Fig. 4.10C).

By comparing the co-variation among the B subunits and the gliadin patterns, two types of 1BS-controlled B subunits could be distinguished (Fig. 4.10A). One type had two groups of similar MW bands and occurred in lanes a to d, f, and h (Fig. 4.10A). The other type included in lanes e and g (Fig. 4.10A), had *Glu-B3* bands similarly to the *Glu-B3d'* type of durum wheat (cf. Fig. 4.1C, lane l).

#### *4.3.4.2 Collection of Dr E. Nevo*

The other set of 219 lines of *T. dicoccoides* surveyed came from 23 populations (or growing locations) in Israel (see Appendix E). Only four seeds from each line were analysed separately because the seed samples were believed to be homogeneous within each line, since

**Figure 4.9** Electrophoretic banding patterns of *T. polonicum* lines obtained from AWCC, Tamworth, and controls.

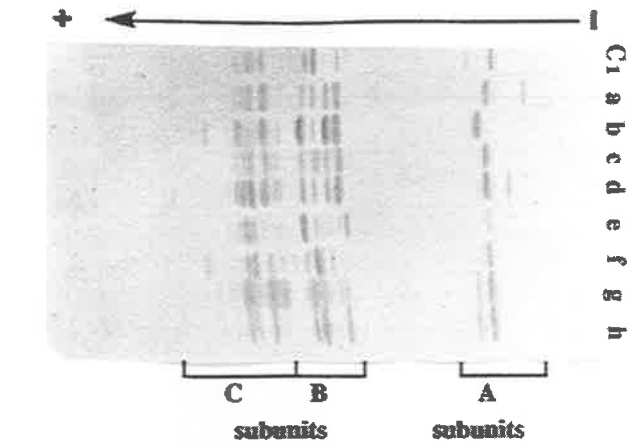
**A** SDS-PAGE patterns of glutenin subunits;

**B** Acid-PAGE patterns of gliadin bands;

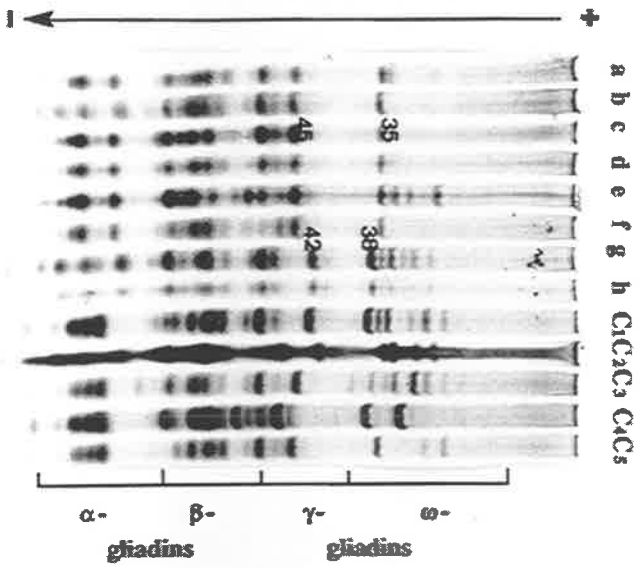
**C** SDS-PAGE patterns of gliadin bands.

($C_1$), Langdon (LMW-A/γ-42 control); ($C_2$), Capinera (LMW-E/γ-40 control); ($C_3$), Drago (LMW-D/γ-43.5 control); ($C_4$), MG 2988 (LMW-C/γ-47 control); ($C_5$), Langdon (Edmore-1B) (γ-45 control); (a-h), *T. polonium* lines.

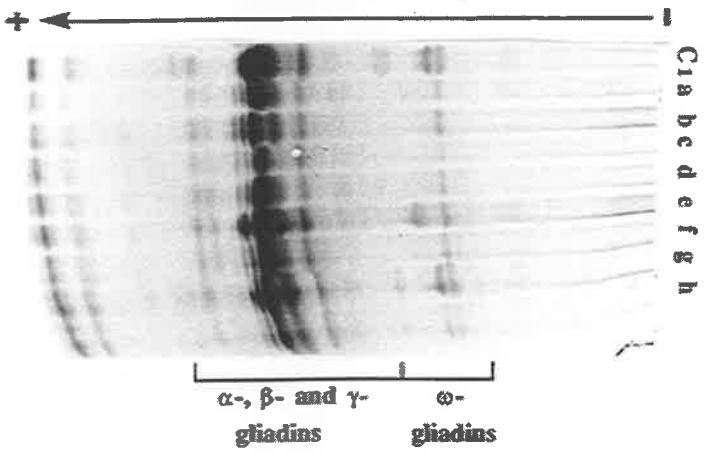
(a), Aus 22341; (b), Aus 3824; (c), Aus 3817; (d), Aus 3823; (e), Aus 22343; (f), Aus 17945; (g), Aus 3814; (h), Aus 3815.


+
-
C1 a b c d e f g h
C
B
A
subunits
subunits

A


-
+
a b c d e f g h C1C2C3 C4C5
45
35
42
36
α-
β-
γ-
ω-
gliadins
gliadins

B


+
-
C1a b c d e f g h
α-, β- and γ-
ω-
gliadins
gliadins

C

**Figure 4.10** Electrophoretic banding patterns of the *T. dicoccoides* lines obtained from AWCC, Tamworth, collected by Dr Kushnir, and controls.

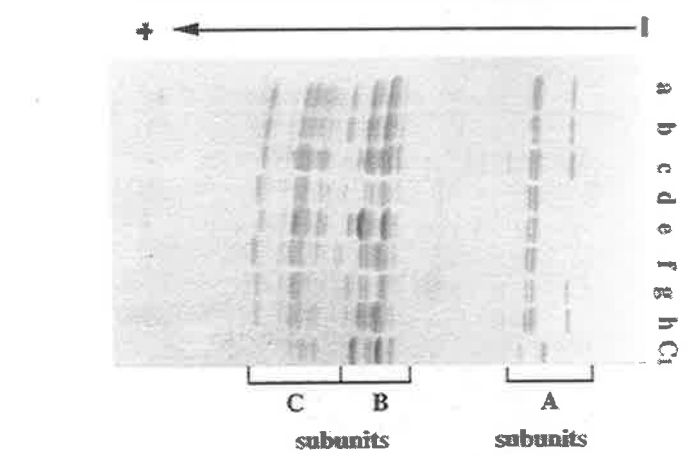
**A** SDS-PAGE patterns of glutenin subunits;

**B** Acid-PAGE patterns of gliadin bands;

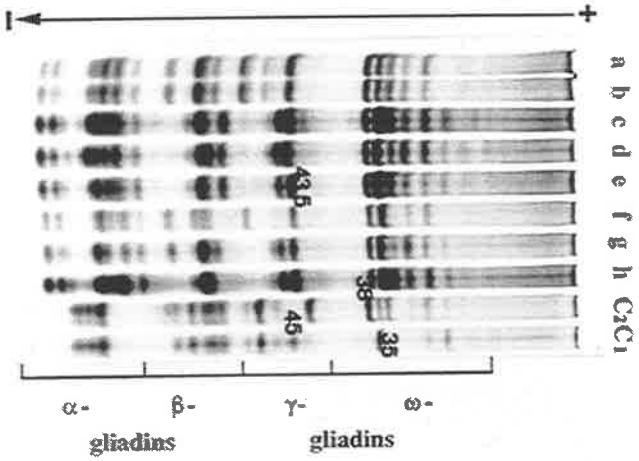
**C** SDS-PAGE patterns of gliadin bands.

(a-h), *T. dicoccoides* lines; ($C_1$), Edmore (LMW-B/γ-45 control); ($C_2$), Langdon (LMW-A/γ-42 control).

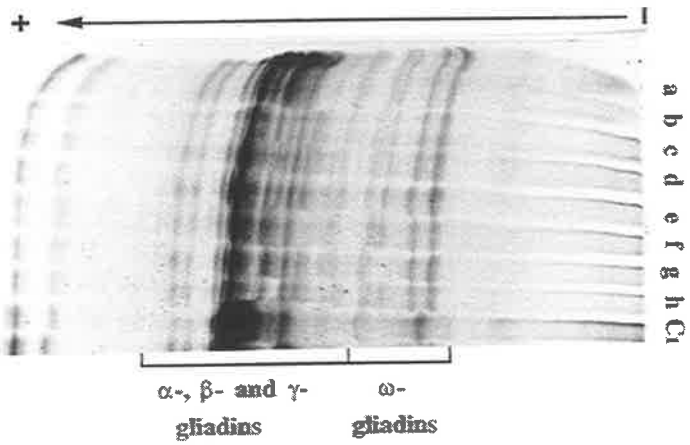
(a), Aus 21255; (b), Aus 21268; (c), Aus 21257; (d), Aus 21267; (e), Aus 21262; (f), Aus 21263; (g), Aus 21265; (h), Aus 21270.


+
-
a b c d e f g h Ci
C
B
A
subunits
subunits

A


-
+
a b c d e f g h C2C1
43.5
38
45
35
α-
β-
γ-
ω-
gliadins
gliadins

B


+
-
a b c d e f g h Ci
α-, β- and γ-
ω-
gliadins
gliadins

C

they were harvested from a single plant. At first, the variability of the LMW and HMW glutenin subunits between lines within each population was examined. They are summarised in Fig. 4.11A and Table 4.4. Only two populations (viz. population Daliyya and Givat-Koach) were homogeneous for both LMW and HMW glutenin subunits whereas the others were very heterogeneous for both types of subunits, giving from two to six different patterns within the population (Table 4.4). All the *T. dicoccoides* lines examined possessed two to five B subunit bands, similar to the extent of variation found in durum wheats (cf. Fig. 4.1 with Fig. 4.11A), although the C subunits were more variable both in band number, mobility and staining intensity. Due to their similar extent of variability in B subunits to that present in the durum wheats, detailed comparisons were not made to characterise these patterns fully. The similar LMW glutenin subunit patterns exhibited in 23 populations ran in the same gel to distinguish with each other identical patterns. This process was repeated twice and the results are summarised in Table 4.5. In general, more variability was observed among the HMWGS than the LMWGS.

Some problems were experienced with band mobility changes in the survey of *T. dicoccoides* patterns. For example, the slowest band of E-18 (Fig. 4.11A, Plate 3, lane v) was initially separated into two bands and conversely, the two fastest bands of M-41 (Fig. 4.11A, Plate 3, lane a) were initially separated as one single band. These irregularities in band pattern in different electrophoretic runs made it difficult to define a definite pattern for some samples. For example, J-30 (Fig. 4.11A, Plate 2, lane l) had three bands made up of two faster bands and one slower band. However, in another gel this pattern was found to be completely different, which made up of one dark faster band and two slower bands, probably due to the co-migration of the two faster bands and separation of the slower band into two bands.

The gliadins of these 219 lines were only surveyed by SDS-PAGE (Fig. 4.11B). As expected, many polymorphisms were observed among them. The number of ω-gliadin bands varied from one to four, with most having one or two, and they differed in staining intensity and band mobility. Because of their mobility, these ω-gliadin bands were likely to be coded by the *Gli-B1* genes but inheritance studies are required to confirm this. Much variation was also observed in the fast-moving region of gliadin bands but this needs to be analysed using Acid-PAGE to give better resolution of bands.

In summary, 72 different B subunit patterns were recorded from the 23 populations surveyed (Table 4.5), but some of these patterns were quite similar and differed only in band staining intensity or slight differences in mobility.

Variation in the HMW glutenin subunit patterns was only compared in detail within each population and because of the likelihood of repeated patterns in the different populations, the overall number of different patterns could be probably less than the total of 68 shown in Table 4.5 and more similar to Nevo and Payne (1987) and Levy *et al* (1988).

**Figure 4.11** Electrophoretic banding patterns of the *T. dicoccoides* lines obtained from Israel (Professor E. Nevo collection), and controls.

**A** SDS-PAGE patterns of glutenin subunits;

**B** SDS-PAGE patterns of gliadin bands.

($C_1$), Langdon (LMW-A/γ-42 control); ($C_2$), Edmore (LMW-B/γ-45 control).

Plate 1 (a), C-11; (b), C-09; (c), C-04; (d), C-24; (e), C-73; (f), 36-03; (g), 36-29; (h), 36-12; (i), 13-53; (j), 13-07; (k), 13-20; (l), 13-33; (m), 19-27; (n), 19-14; (o), 19-26; (p), 14-02; (q), 14-35; (r), 14-22; (s), 14-31; (t), 14-09; (u), 14-14; (v), 32-03; (w), 32-17;

Plate 2 (a), 18-21; (b), 18-35; (c), 18-43; (d), 18-56; (e), 18-52; (f), 318-28; (g), 18-46; (h), 20-31; (i), 20-22; (j), J-17; (k), J-37; (l), J-30; (m), J-11; (n), J-12; (o), L-10; (p), L-35; (q), L-61; (r), L-16; (s), 24-50; (t), 24-18; (u), 15-01;

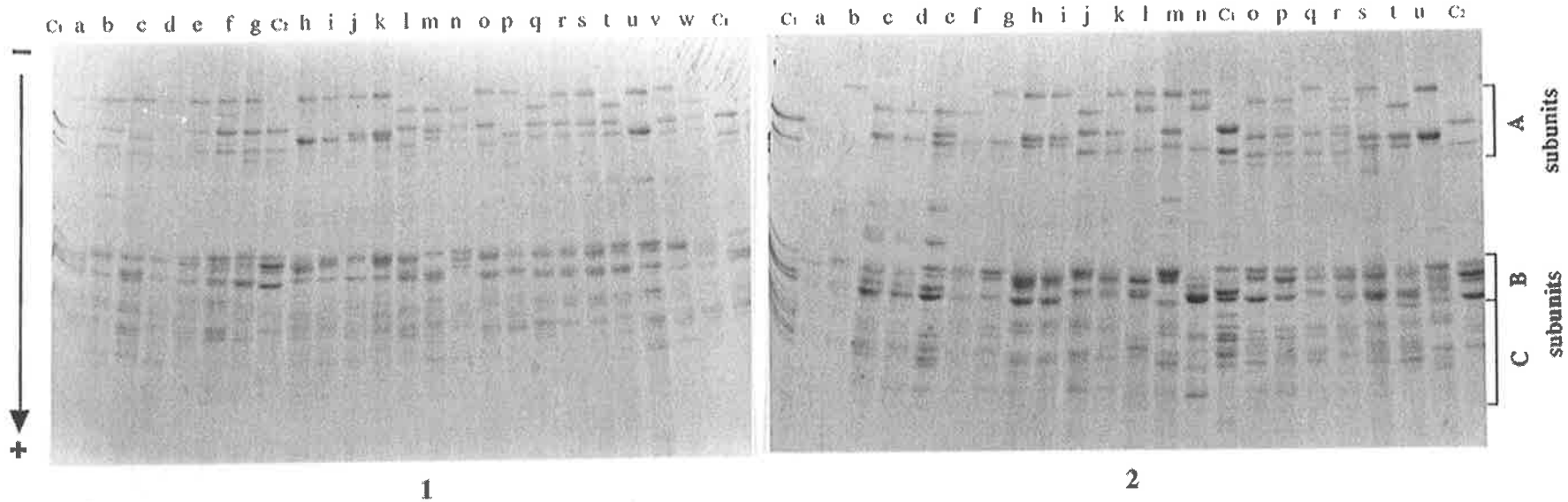
Plate 3 (a), M-41; (b), M-61; (c), M-71; (d), M-32; (e), M-12; (f), G-68; (g), G-46; (h), G-60; (i), G-61; (j), G-03; (k), A-82; (l), A-80; (m), A-37; (n), I-27; (o), I-02; (p), I-11; (q), I-07; (r), E-36; (s), E-43; (t), E-04; (u), E-42; (v), E-18;

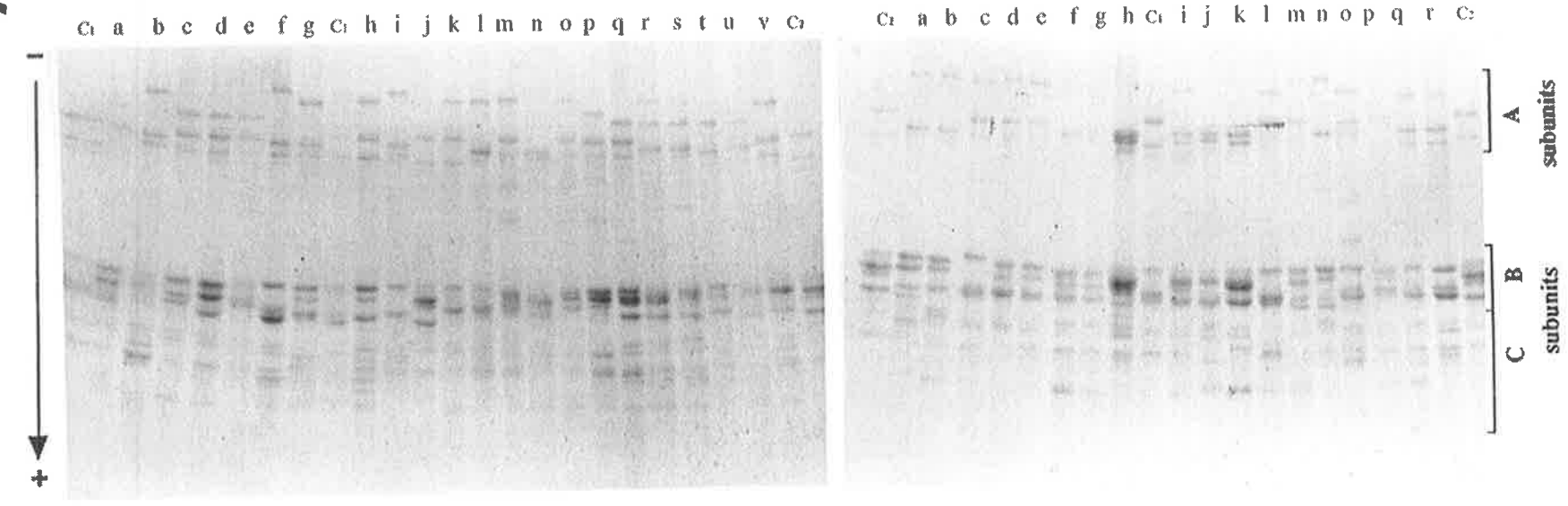
Plate 4 (a), K-14; (b), K-30; (c), K-45; (d), K-50; (e), K-46; (f), H-04; (g), H-02; (h), H-07; (i), B-14; (j), B-19; (k), B-06; (l), F-10; (m), F-25; (n), F-82; (o), 17-44; (p), 27-09; (q), 27-26; (r), 27-38.

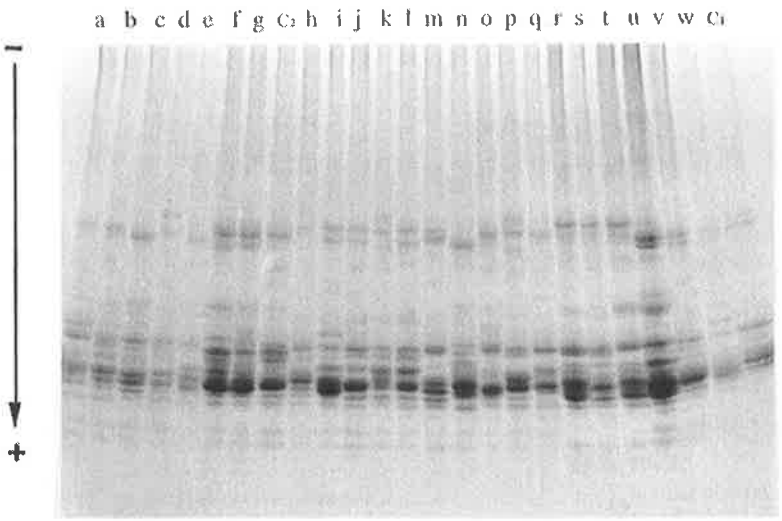
**Symbols used in figures:**

$C_1$ to $C_2$ = Control cultivars.

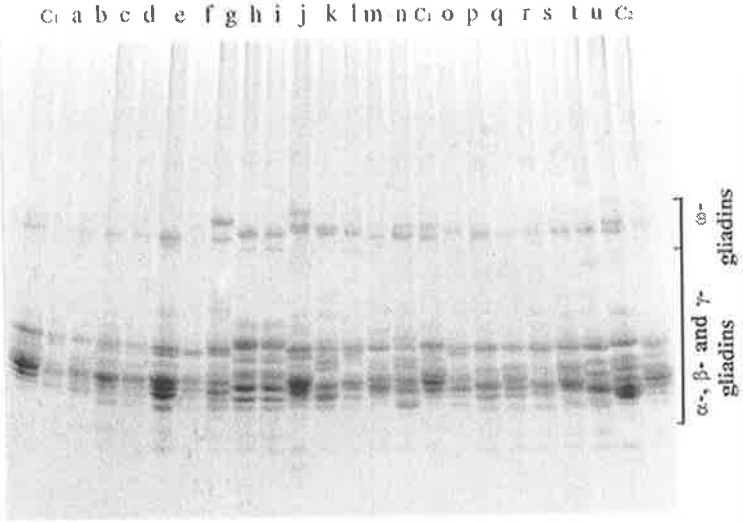
A


C1 a b c d e f g C2 h i j k l m n o p q r s t u v w C1
C1 a b c d e f g h i j k l m n C1 o p q r s t u C2
A subunits
B subunits
C subunits
1
2


C1 a b c d e f g h C2 i j k l m n o p q r s t u v C2
C1 a b c d e f g h C1 i j k l m n o p q r C2
A subunits
B subunits
C subunits
3
4

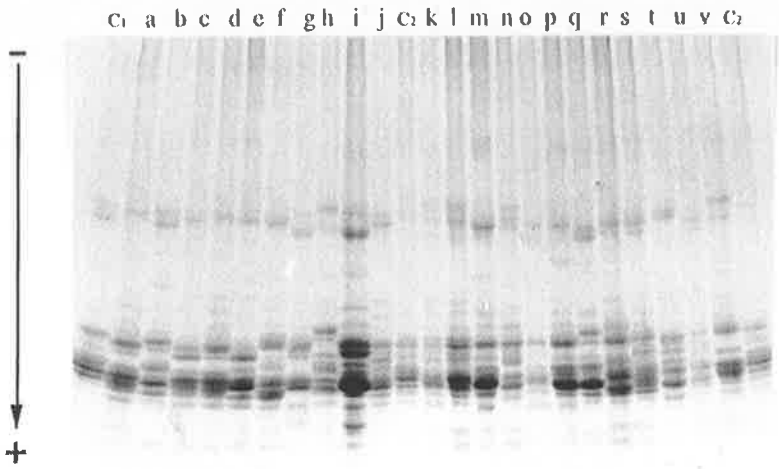

a b c d e f g C1 h i j k l m n o p q r s t u v w C1
−
+

1

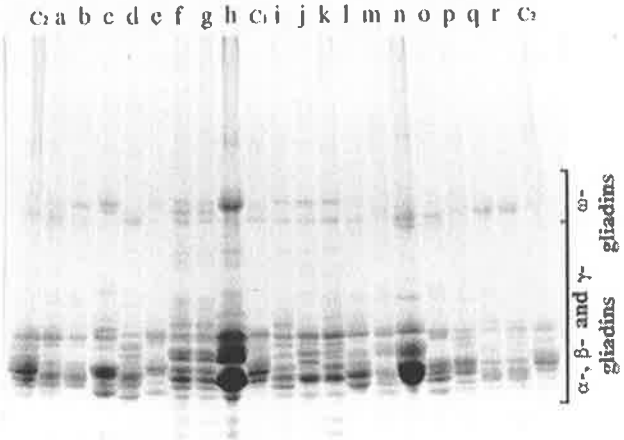

C1 a b c d e f g h i j k l m n C1 o p q r s t u C2
ω-gliadins
α-, β- and γ-gliadins

2

B


C1 a b c d e f gh i j C2 k l m n o p q r s t u v C2
−
+

3


C2 a b c d e f g h C1 i j k l m n o p q r C2
ω-gliadins
α-, β- and γ-gliadins

4

**Table 4.4** Frequency of glutenin subunit patterns from the second survey among the 23 populations of *T. dicoccoides* from Israel

| Population | Genotype | | Frequency | Line name |
|---|---|---|---|---|
| | LMWGS | HMWGS | | |
| Achihood | A | A | 4 | 13-12, 13-33, 13-34, 13-35 |
| | B | A | 4 | 13-17, 13-20, 13-25, 13-30 |
| | A | B | 1 | 13-07 |
| | C | C | 1 | 13-53 |
| Amirim | A | A | 2 | 14-22, 14-41 |
| | A | B | 2 | 14-31, 14-39 |
| | B | C | 2 | 14-02, 14-17 |
| | C | D | 1 | 14-35 |
| | D | A | 1 | 14-09 |
| | E | E | 1 | 14-14 |
| Bat Shelomo | A | A | 5 | I-07, I-08, I-14, I-17, I-22 |
| | B | B | 1 | I-11 |
| | C | C | 2 | I-02, I-04 |
| | D | D | 1 | I-27 |
| Beit-Oren | A | A | 9 | 15-01, 15-02, 15-04, 15-14, 15-17, 15-32, 15-37, 15-43, 15-44 |
| | A | B | 1 | 15-52 |
| Bet-Meir | A | A | 3 | F-37, F-82, F-54 |
| | B | B | 6 | F-03, F-07, F-18, F-25, F-28, F-67 |
| | C | C | 1 | F-10 |
| Daliyya | A | A | 9 | 20-04, 20-08, 20-11, 20-20, 20-31, 20-33, 20-34, 20-38, 20-42 |
| | A* | A | 1 | 20-22 |
| Gamla | A | A | 7 | 36-03, 36-25, 36-26, 36-28, 36-33, 36-39, 36-40 |
| | A* | A | 1 | 36-29 |
| | B | A | 1 | 36-12 |
| Gitit | A | A | 2 | 18-46, 18-48 |
| | B | B | 1 | 18-28 |
| | C | C | 1 | 18-56 |
| | D | D | 1 | 18-52 |
| | E | D | 2 | 18-21, 18-27 |
| | F | E | 1 | 18-35 |
| | G | D | 1 | 18-43 |
| Givat-Koach | A | A | 9 | 17-08, 17-09, 17-13, 17-14, 17-21, 17-32, 17-34, 17-39, 17-44 |
| Grizim | A | A | 1 | M-12 |
| | B | A | 1 | M-32 |
| | C | B | 5 | M-14, M-18, M-35, M-64, M-71 |
| | D | C | 2 | M-31, M-61 |
| | B | D | 1 | M-41 |
| J'aba | A | A | 7 | 19-05, 19-06, 19-13, 19-14, 19-36, 19-41, 19-46 |
| | B | B | 2 | 19-27, 19-40 |
| | C | C | 1 | 19-26 |
| Kokhav Hashohar | A | A | 2 | K-09, K-46 |
| | B | B | 2 | K-36, K-50 |
| | C | C | 3 | K-14, K-18, K-23 |
| | D | D | 1 | K-45 |
| | E | C | 1 | K-20 |
| | F | B | 1 | K-30 |

Table 4.4 (continued)

| Population | Genotype | | Frequency | Line name |
|---|---|---|---|---|
| | LMWGS | HMWGS | | |
| Mt. Dov | A | A | 2 | G-46, G-47 |
| | B | B | 4 | G-08, G-22, G-42, G-61 |
| | C | C | 1 | G-60 |
| | D | D | 2 | G-01, G-03 |
| | E | E | 1 | G-68 |
| Mt. Gilboa | A | A | 2 | L-1.6, L-40 |
| | B | A | 4 | L-10, L-17, L-21, L-42 |
| | C | A | 1 | L-43 |
| | D | A | 1 | L-35 |
| | E | B | 1 | L-61 |
| Nahef | A | A | 3 | 32-02, 32-03, 32-07 |
| | B | B | 6 | 32-17, 32-19, 32-31, 32-37, 32-44, 32-45 |
| | C | C | 1 | 32-46 |
| Nesher | A | A | 6 | 27-09, 27-11, 27-14, 27-15, 27-18, 27-33 |
| | B | B | 2 | 27-26, 27-31 |
| | C | C | 2 | 27-38, 27-41 |
| Qazrin | A | A | 6 | A-21, A-41, A-51, A-57, A-82, A-96 |
| | B | B | 2 | A-80, A-27 |
| | C | C | 1 | A-37 |
| Rosh-Pinna | A | A | 1 | C-11 |
| | B | B | 1 | C-09 |
| | C | C | 1 | C-04 |
| | B | C | 5 | C-24, C-29, C-34, C-57, C-74 |
| | B | D | 1 | C-73 |
| | D | A | 1 | C-12 |
| Sanhedriyya | A | A | 1 | E-18 |
| | B | B | 4 | E-12, E-19, E-30, E-42 |
| | C | C | 1 | E-04 |
| | ? | B | 1 | E-43 |
| | ? | B | 1 | E-36 |
| Tabigha | A | A | 5 | H-02, H-03, H-04, H-13, H-14 |
| | B | A | 2 | H-07, H-08 |
| | C | A | 3 | H-05, H-15, H-54 |
| Taiyiba | A | A | 1 | J-30 |
| | B | B | 1 | J-11 |
| | C | A | 2 | J-12, J-43 |
| | D | C | 3 | J-09, J-14, J-17 |
| | E | B | 1 | J-37 |
| Yabad | A | A | 7 | 24-02, 24-28, 24-31, 24-41, 24-48, 24-50, 24-61 |
| | B | B | 3 | 24-14, 24-18, 24-23 |
| Yehudiyya | A | A | 5 | B-37, B-06, B-41, B-32, B-08 |
| | B | B | 3 | B-19, B-15, B-16 |
| | B | A | 1 | B-40 |
| | C | C | 1 | B-14 |

* = Banding patterns appeared to be very similar.

**Table 4.5** Summary of glutenin subunit phenotypes among the 23 populations of *T. dicoccoides* lines from Israel

| Population (locations) | No. of lines analysed | No. of LMW glutenin patterns | No. of HMW glutenin patterns |
|---|---|---|---|
| Achihood | 10 | 3 | 2 |
| Amirim | 9 | 3 | 4 |
| Bat Shelomo | 9 | 4 | 4 |
| Beit–Oren | 10 | 1 | 1 |
| Bet-Meir | 10 | 3 | 3 |
| Daliyya | 10 | 1 | 1 |
| Gamla | 9 | 3 | 2 |
| Gitit | 9 | 6 | 5 |
| Givat–Koach | 9 | 1 | 1 |
| Grizim | 10 | 5 | 3 |
| J'aba | 10 | 3 | 3 |
| Kokhav Hashohar | 10 | 4 | 5 |
| Mt. Dov | 10 | 5 | 3 |
| Mt. Gilboa | 10 | 3 | 2 |
| Nahef | 10 | 2 | 3 |
| Nesher | 10 | 3 | 3 |
| Qazrin | 9 | 3 | 3 |
| Rosh–Pinna | 10 | 5 | 5 |
| Sanhedriyya | 8 | 4 | 4 |
| Tabigha | 10 | 2 | 2 |
| Taiyiba | 8 | 4 | 5 |
| Yabad | 10 | 2 | 2 |
| Yehudiyya | 10 | 2 | 2 |
| Total: 23 | 219 | 72 | 68 |

## 4.4 DISCUSSION

### 4.4.1 Polymorphism of LMW glutenin subunits in tetraploid wheats

#### *4.4.1.1 T. durum*

This study reports for the first time a large number of different LMW glutenin subunit patterns detected in a survey of 240 cultivars and lines from a world collection. Three common LMW patterns were observed, in contrast to the two major types of pattern previously reported

in durum wheat (Payne *et al*, 1984c; Margiotta *et al*, 1987). Several other new types were detected but they were infrequent and were more or less similar to one of the three major types and were difficult to identify using normal electrophoretic procedures.

Wide variation in LMW glutenin subunit patterns was found among durum wheats from North African and Mediterranean regions. Little variation was found among cultivars from Australia, CIMMYT (Mexico), USA and Italy, which was consistent with the conclusions drawn by Margiotta *et al* (1987). These differences were expected since the former two groups were mostly land races with a long history of growing durum wheats but having little modern breeding, compared to the other cultivars which originated in countries with a short history of durum cultivation and intensive breeding programs for high quality, tending to reduce genetic variability. For example, the frequency of the LMW-B pattern was nearly five times higher than that of the LMW-A type among the samples analysed, reflecting deliberate selection for better quality. The γ-45 related LMW-B patterns accounted for more than 80% of the cultivars and no or very few other new B subunits patterns were observed from areas with highly developed breeding programs, indicating that less genetic variability existed for physical dough properties among durums from these countries.

In the current study, 17 different *Glu-B3'* and nine *Glu-A3'* blocks of B bands were deduced from comparison of the presence or absence of bands in Langdon 1D(1A) and 1D(1B) disomic substitution lines as well as by band association using the ω- and γ-gliadin bands as markers. However, these linkage blocks deduced need to be treated as tentative until they have been confirmed in detailed inheritance studies. Recently, Spanish researchers have reported three other types of LMW glutenin patterns among 139 Spanish durum landraces using the same electrophoretic procedure as Singh and Shepherd (1988a) (Carrillo *et al*, 1990a, 1990b). They reported that LMW-1, $1^-$ patterns were generally associated with γ-42, LMW-2, $2^-$ with γ-45 and LMW-2* with γ-44. However, due to inter-laboratory differences in electrophoretic procedure, it was difficult to equate their patterns with the present ones.

It was difficult to follow the hexaploid wheat allele nomenclature as proposed by Gupta (1989), since all the durum wheat alleles assigned followed on from the study of hexaploid wheats (Gupta, 1989). For example, Gupta assigned a null *Glu-A3* allele reference cultivar Edmore but the present study proposed an active allele for it, same as the *Glu-A3* allele to another reference cultivar Langdon. This prevented its further use in the allele system of Gupta (1989). Moreover, similar banding patterns in hexaploid and durum wheat did not indicate that they possessed the same alleles since these two groups of wheat have separately evolved to a large extent. Hence, a new set of symbols was adopted to describe the linkage blocks in durum wheat.

The extent of variation in LMW glutenin subunit patterns controlled by chromosomes 1A and 1B was similar to that reported for gliadins and HMWGS (Sozinov and Poperelya, 1980; Payne and Lawrence, 1983; Galili and Feldman, 1983a, 1983b; Gupta and Shepherd, 1990a, 1990b). In all cases the seed proteins controlled by chromosome 1B have shown greatest

polymorphism, consistent with the hypothesis that the B genome in polyploid wheat was polyphyletic (Sarkar and Stebbins, 1956; Athwal and Kimber, 1972). However, Gupta (1989) suggested that genes on chromosome 1B coding for these storage proteins might have duplicated and diverged more extensively than the genes on chromosomes 1A and 1D. Therefore, a monophyletic origin of the B genome in polyploid wheats (Riley, 1965) cannot be rejected from these data.

The LMW glutenin subunit banding patterns described by Gupta (1989) were slightly different from those obtained in the present study. This was most likely due to differences in gel cross-linkage [cf. Fig. 4 in Gupta and Shepherd (1988) to Fig. 4.1]. Some LMWGS which appeared to be single in the earlier work, were separated into two bands using the present experimental conditions and vice versa. The LMW glutenin subunit patterns obtained by Ciaffi and Lafiandra (pers. comm.) were also different from those obtained in the present studies. For example, they found that the two slowest bands of Edmore in Gupta (1989) and the present study showed a reversal of mobility in their experiments. The degree of gel cross-linking used by Ciaffi, Gupta and the present study was 2.6%, 0.8% and 1%, respectively. Hence in order to obtain reproducible gel patterns, it is necessary to follow a common recipe for gel comparison.

The banding patterns from different electrophoretic runs showed small variations due to changes in the electrophoretic conditions. This was rather crucial for the analysis of those bands having only minor MW differences, particularly if they migrated together, thus causing confusion in identification, as observed with HMWGS for bands 2 and 2* (Payne *et al*, 1981a). For example, the *Glu-A3i'* band sometimes co-migrated with the second slowest band of *Glu-B3b'* and resulted in its false classification as a null *Glu-A3h'* type. This also occurred with *Glu-A3b'*, *Glu-A3c'* and *Glu-A3g'* bands. Conversely, the *Glu-A3a'* band sometimes separated into two bands. This added to the complexity of assigning LMWGS to particular linkage blocks. Several runs were required to confirm the band identification. Furthermore, bands having the same MW would always overlap in SDS-PAGE because this system separated protein bands on their size differences only. Because of this, it was likely that the extent of variation for the durum wheat B subunit composition was underestimated, contributing a lower number of band combinations in the LMWGS. Separation based on both size and electric charge differences using an appropriate 2-D system would maximise the detection of variation among these proteins.

Another source of potential variation was the occurrence of different biotypes within a cultivar. Canadian durum wheat cultivar Hercules was heterogeneous for γ-gliadin bands 42 and 45 (Howes, pers. comm.), and this study also revealed it to be heterogeneity for *Glu-B3a'* and *Glu-B3f'* bands as expected (see Appendix A). LMW glutenin subunit biotypes have also been reported in the Australian durum wheat cultivar Duramba (Payne *et al*, 1984c).

### 4.4.1.2 *Non-durum wheats*

The patterns of LMWGS observed in wild and cultivated tetraploid wheat accessions showed extensive variation, including the *T. dicoccoides* lines from Israel. Altogether, sixteen different LMW glutenin subunit patterns were identified among 25 samples of *T. dicoccum*, seven among 21 of *T. polonicum* and 79 among 235 lines of *T. dicoccoides*. The gliadin patterns separated by Acid-PAGE or SDS-PAGE also revealed a large range of pattern variation.

The B subunits of *T. dicoccum* in the first source had a similar level of variability to durum wheats while the second source of *T. dicoccum* had fewest bands for both HMW and LMW glutenin subunits of the groups of genetic material studied. One line was found to possess a null form for *Glu-A3*, with fewer bands than the durum wheat controls, whereas the gliadin patterns remained similar number of bands as the durum wheat controls. The latter materials should be interesting to study the effect of inactivation of the B subunits. Although the *T. polonicum* lines had several variable B and C subunits, the variation of the B subunits was similar to that of durum wheat. All of these lines possessed either γ-42 or γ-45 gliadin types, suggesting that they had less genetic variation based on γ-gliadin markers and more resemblance to durum wheat than the other tetraploid wheats.

In the Nevo (Nevo and Payne, 1987; Nevo and Beiles, 1989) collection of 219 *T. dicoccoides* lines from Israel, variability for HMWGS and LMWGS appeared to be similar. However, it seemed that the 1-D SDS-PAGE system separated better for the HMWGS than the LMWGS, since the latter fractions varied in a narrow MW range and could be easily overlapped and well underestimated. Most of these lines possessed a similar number of B subunits as found in durum wheat. No lines have been found having more than five or less than two darkly stained B subunits. The present result was consistent with a recent similar report on *T. dicoccoides* lines from Jordan and Turkey by Ciaffi *et al* (1993a), as they also observed enormous variation of LMW glutenin subunit banding patterns besides the usual ω-35-γ-45-LMW-2 pattern. The C subunits in these lines were highly variable, both with respect to band numbers, mobility and staining intensity over a relatively large MW range but they have not been subjected to detailed study at the present.

The variation of LMW glutenin subunit patterns in the *T. dicoccoides* lines within different locations appeared to be non-random. Some populations were monomorphic while others ranged from a low to very high level of polymorphism, consistent with previous studies on HMWGS and other traits such as isozymes (Nevo *et al*, 1986; Nevo, 1987; Ciaffi *et al*, 1993b). As reported previously, the genetic diversity of these *T. dicoccoides* lines appeared geographically structured and partly predictable by ecology and allozyme markers (Nevo *et al*, 1986, 1988; Nevo, 1987; Nevo and Payne, 1987; Levy *et al*, 1988; Levy and Feldman, 1988; Nevo and Beiles, 1989).

### 4.4.2 Evolution of genes controlling the LMW glutenin subunits

Substantial inactivation of the HMWGS has occurred during the transition from *T. dicoccoides* to durum (Galili *et al*, 1988) whereas the number of bands coded for by LMW glutenin subunit genes appeared to be similar in all the tetraploid wheats (Galili and Feldman, 1983c; Vallega and Waines, 1987; Levy and Feldman, 1988; Levy *et al*, 1988). The LMWGS occurred in a rather narrow MW region (36-45 KD) and the variability of the LMW bands seemed to be rather low at the tetraploid level. Gupta (loc cit) noted that the A genome in diploid wheat synthesised a larger number of LMWGS than tetraploid and hexaploid wheats, hence the inactivation for LMWGS appeared to start from diploid wheats (Gupta, 1989). It was thought that a non-random diploidization process (Galili and Feldman, 1983a, 1983b) might be due to the redundant genes going to a repression mechanism in HMWGS (Galili and Feldman, 1984, 1986) and presumably in LMWGS as well. It was not clear what extent the null *Glu-A3* genes have been inactivated like the HMW genes i. e. *Glu-A1*, since such changes were difficult to detect in the present study. One line of *T. dicoccum* obviously possessed a null for *Glu-A3* but surprisingly, no null *Glu-B3* types have ever been detected in tetraploids, either in this or other studies (section 4.3.1; Carrillo *et al*, 1990) nor in hexaploid wheats (Gupta and Shepherd, 1990a, 1990b). Why the null phenotype (both LMWGS and HMWGS) occurred only on chromosome 1A and not on the homoeologous counterparts was rather difficult to explain, since all the storage protein genes have been under weaker selection pressure and should exhibit a similar level of allelic variation for both loci (i. e. *Glu-1* and *Glu-3*). Deletion, spontaneous mutation or suppression of genes at both intra- and inter-genomic levels might be possible causes (Galili and Feldman, 1983c, 1984, 1986).

# CHAPTER 5. INHERITANCE OF LMW GLUTENIN SUBUNITS AND GLIADINS IN TETRAPLOID WHEATS

## 5.1 INTRODUCTION

It has been shown earlier that the B glutenin subunits in durum wheats were coded by genes at the *Glu-3* loci and that these were tightly linked with the *Gli-1* loci coding for ω- and γ-gliadins on the short arm of group 1 chromosomes (Payne *et al*, 1984c; Singh and Shepherd, 1988a; Gupta and Shepherd, 1990a). However, previous studies had only considered the inheritance of the LMW-1 and LMW-2 types, which were linked to the γ-gliadin bands 42 and 45, respectively (Payne *et al*, 1984c; Pogna *et al*, 1988, 1990). The extensive survey of the LMW glutenin composition of durum and related tetraploid wheats (Chapter 4) revealed a large amount of variation in B and C glutenin subunit patterns and provided the raw materials to extend the earlier inheritance studies.

In order to study the inheritance of different LMW glutenin subunit patterns utilising test-cross analyses, a parent lacking all or most of the LMW glutenin subunit bands was required, as shown in a previous study with hexaploid wheats (Gupta and Shepherd, 1993). Such a parent would allow the segregation of contrasting banding patterns in the $F_1$ to be studied with minimum ambiguity from band overlap in the test-cross progeny. However, a parent with such a phenotype was not identified in the cultivar survey.

An attempt was made to utilise the 'triple translocation stock' that was produced by Gupta and Shepherd (1993) and used successfully to study the inheritance of LMW glutenin subunits in hexaploid wheat (Gupta and Shepherd, 1993). However, it was found that it could not be used directly in tetraploid x hexaploid crosses because the progeny seeds were shrivelled and had low germination. Consequently, a breeding program was undertaken to derive a double-1AL.1BL.1RS translocation stock in a tetraploid background from the double-1AL.1BL.1RS hexaploid translocation stock. This tetraploid stock was used as the third test-cross (TC) parent in the inheritance studies.

## 5.2 METHODS AND MATERIALS

### 5.2.1 Parents and $F_1$ combinations

Four tetraploid wheats [Coulter, Beladi Bouhi and Kharkof-5 and a wild tetraploid line (*Triticum turgidum* var. *dicoccoides*) from Israel, 19-27] having LMW glutenin subunit banding patterns different from Langdon and Edmore were selected as parents from the survey population (see Chapter 4 for details). Each of these four parents was crossed with either Langdon or Edmore as reference parents, since the genetic control of the B subunits had already been determined in these latter two cultivars (Gupta, 1989). The actual parents were

chosen to maximise the parental band differences which could be scored in the TC progeny. $F_1$ combinations were produced as follows:

Coulter x Langdon
Beladi Bouhi x Edmore
Kharkof-5 x Langdon
Edmore x line 19-27
Langdon x line 19-27

### 5.2.2 Development of the test-cross parent — a double-1RS translocation stock in durum wheat

Crosses were made (Fig. 5.1) between the durum wheat reference parent Langdon and the hexaploid wheat line Chinese Spring-Gabo 1AL.1BL.1RS (Gupta and Shepherd, 1993) available from seed stocks held in the Department of Plant Science, Waite Institute. $F_2$ seeds from these crosses were screened to detect the two separate single-translocations and one double-translocation stock. To distinguish these 1RS translocation lines from those produced earlier in hexaploid wheat, they are referred to as the single-1RS translocation (tetraploid) and double-1RS translocation stocks (tetraploid), abbreviated to STr (4x) and DTr (4x), respectively.

### 5.2.3 Test-crosses

The $F_1$ hybrids of section 5.2.1 were test-crossed as female parents to the DTr (4x) stock to generate progeny to study the inheritance of LMW glutenin subunits and determine the allelic relationship of the genes controlling the LMW glutenin subunits (B subunits) among tetraploid wheat lines.

### 5.2.4 Back-crosses

In order to identify unambiguously all the LMW glutenin subunits present in the reference parents, Langdon and Edmore were crossed with the tetraploid double-1RS translocation stock and the $F_1$ backcrossed to this same stock to allow all the B subunits to be scored clearly. These crosses were as follows.

Langdon x $[DTr\ (4x)]^2$
Edmore x $[DTr\ (4x)]^2$

### 5.2.5 Protein extraction and electrophoresis

The procedure followed for the extraction of endosperm proteins, their separation by one-dimensional electrophoresis and for staining and destaining of gels was the same as described in Chapter 3.

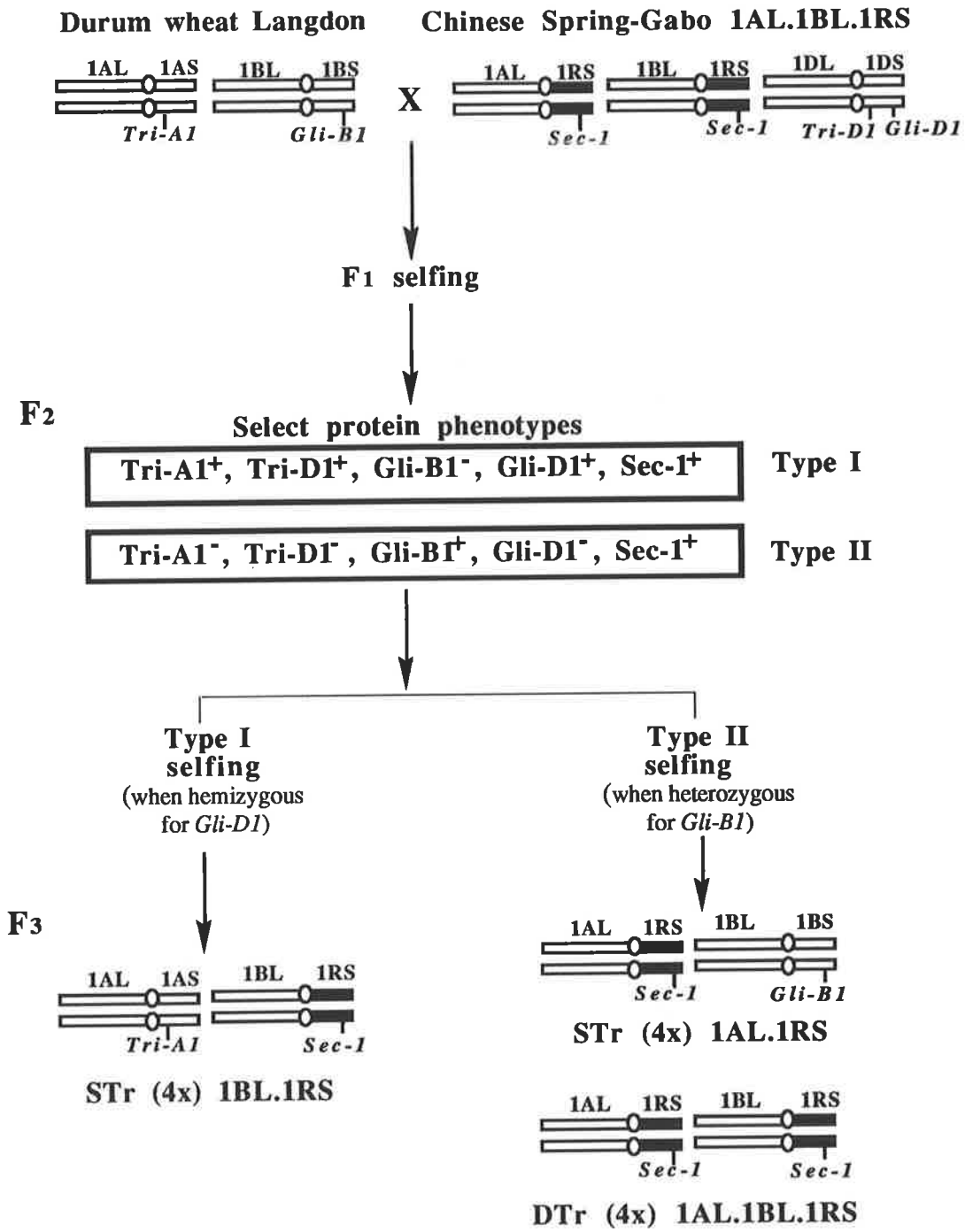



**Figure 5.1** Selection procedure for isolating tetraploid wheat single- and double-1RS translocation stocks from hexaploid double-1RS translocation stock Chinese Spring-Gabo 1AL.1BL.1RS. Presence or absence of protein markers located on the short arms of group 1 wheat chromosomes or the short arm of 1R chromosome were detected by electrophoresis. In $F_3$ generation, the selected STr (4x) and DTr (4x) lines were checked by protein markers (eg. negative for *Gli-D1*) and cytology (chromosome number) to ensure they were in fact tetraploid lines.

### 5.2.6 Cytology

The standard Feulgen technique was employed to analyse the chromosome configurations at metaphase-I of meiosis in pollen mother cells (pmcs) of the DTr (4x) stock at a later stage. The procedure involved selecting the required stage of pmcs, fixing them in ethanol : acetic acid = 3 : 1 overnight at 4°C, hydrolysing in 1N HCl at 60°C for 12 minutes and staining with Feulgen reagent for 1 hr. at room temperature. The stained pmcs were squashed in 45% acetic acid for microscopic examination.

### 5.2.7 Genetic analysis

Recombination fractions (p) were calculated directly by dividing the observed number of recombinants (R) by the total number of progeny (n) analysed, excluding any aneuploid progeny. The standard error (Sp) of the recombination fraction was calculated using the formula for a binomial distribution (Mather, 1951):

$$Sp = \sqrt{[p(1-p)/n]}$$

Map distances (cM) and their standard errors were calculated from recombination frequencies, using the Kosambi function (Kosambi, 1944) as follows:

$$cM \pm SE = 25 \times \ln\frac{100 + 2R}{100 - 2R} \pm \frac{2500S_R}{2500-R^2}$$

Where, R = recombination percentage; $S_R$ = standard deviation of R.

Where no recombination was detected between two protein markers in the TC progeny, the upper limit (95% confidence limit) of recombination was calculated using the method of Hanson (1959):

$$p = [1 - (0.05)^{1/n}]$$

Where n = number of euploid progeny analysed.

Since there were only two groups of data used in calculations, corrections for continuity were made for all $\chi^2$ tests. Furthermore, two by two (2 * 2) contingency tables were used to test for the independence of the segregation of individual bands or group of bands.

## 5.3 RESULTS

### 5.3.1 Production and characteristics of single- and double-1RS translocation lines in tetraploid wheat

#### *5.3.1.1 Isolation of lines*

Durum wheat cv. Langdon was crossed with the hexaploid double-1RS translocation stock Chinese Spring-Gabo 1AL.1BL.1RS, giving pentaploid $F_1$ seeds which were very

shrivelled. The $F_2$ seeds were screened for the presence and absence of protein markers located on the short arm of group 1 chromosomes (Fig. 5.1).

The identifiable protein markers for the double-translocation stock Chinese Spring-Gabo 1AL.1BL.1RS were Tri-D1$^+$, Gli-D1$^+$ and Sec-1$^+$, whereas those for Langdon were Tri-A1$^+$ and Gli-B1$^+$ (Fig. 5.1). The absence of chromosome arm 1BS of Langdon in the $F_2$ was indicated by the absence of the ω-gliadin bands coded by *Gli-B1* (i. e. Gli-B1$^-$). Similarly, the absence of arm 1AS was indicated by the absence of the *Tri-A1* band (i. e. Tri-A1$^-$). Seeds lacking both *Tri-A1* and *Gli-B1* bands were selected as the putative double-translocation stock (1AL.1BL.1RS) as they were expected to lack all LMW glutenin B subunits as well. Two seeds with a double-1RS translocation and three with a single-translocation chromosome 1AL.1RS were isolated from an $F_2$ individual with phenotype II [Tri-A1$^-$ Tri-D1$^-$ Gli-B1$^+$ Gli-D1$^-$ Sec-1$^+$] (Fig 5.1). Seven seeds with a single-1BL.1RS translocation were isolated from an $F_2$ individual with phenotype I [Tri-A1$^+$ Tri-D1$^+$ Gli-B1$^-$ Gli-D1$^+$ Sec-1$^+$].

$F_3$ seeds from these putative translocations were analysed again for gliadin and glutenin markers to confirm the identity of the original selection and also tested for homozygosity in the $F_4$. One of the confirmed double-translocation lines (1AL.1BL.1RS) was selected to be used as the third parent in test-crosses. The spike morphology of the DTr (4x) stock is shown in Fig. 5.2A.

It should be pointed out that during the selection procedure at $F_2$, eleven protein phenotypes were identified and they were planted for further segregation. Most $F_3$ plants were poorly developed or died and only three $F_3$ phenotypes survived. It was likely that many of the progeny seeds from the pentaploid $F_1$ still contained some D-genome chromosomes even though chromosome 1D protein markers were absent. Even after three generations of selfing, the selected single- and double-1RS translocation stocks still gave low seed set and some were self sterile. Despite possible cytological instability arising from the presence of extra D genome chromosomes, the line could be readily crossed with tetraploid wheat to give viable seeds and hence it was used as the third parent in the test-cross. In clarifying this, these stocks were checked cytologically at $F_3$ (see below). Moreover, one further backcross to Langdon was made at $F_3$ for the double-1RS translocation stock to ensure better seed yield for future work.

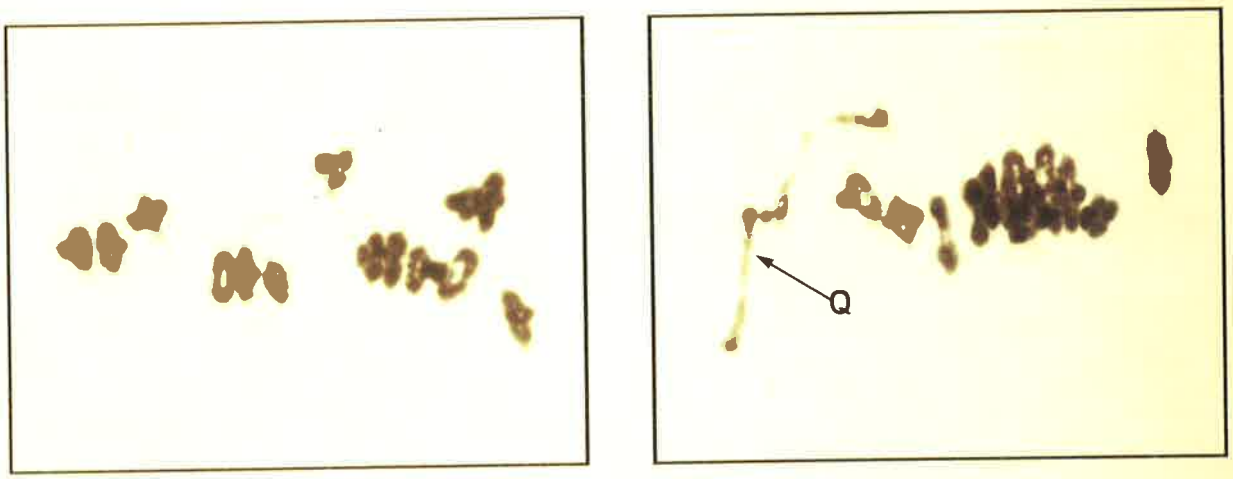
### *5.3.1.2 Cytology*

Fig. 5.2B shows chromosomal configurations in metaphase-I pmcs of DTr (4x) $F_3$ plants. As expected, a sample of pmcs analysed showed either 14 bivalents or 12 bivalents and 1 quadrivalent, due to the four doses of the 1RS chromosome present. The multivalents probably contributed to the instability and partial sterility of this stock.

### *5.3.1.3 LMW glutenin subunit (B and C subunits) composition*

Fig. 5.3A shows the glutenin subunit composition of STr (4x) and DTr (4x) lines separated by SDS-PAGE with durum wheats (Edmore, Kharkof-5), hexaploid wheat (Chinese


A




B1 B2

**Figure 5.2** Double-1RS translocation tetraploid line [DTr (4x)].

**A** Spike morphology of (left) Chinese Spring-Gabo 1AL.1BL.1RS translocation line, (middle) DTr (4x) and (right) durum wheat cv. Langdon;

**B** Chromosome configurations at metaphase-I in pmcs of homozygous DTr (4x) stock ($F_3$ plants) showing ($B_1$) $14^{II}$, ($B_2$), $12^{II} + 1^{IV}$.

**Symbol used in figure:**

Q = Quadrivalent.

**Figure 5.3** Tetraploid and hexaploid wheat 1RS translocation lines and controls.

**A** The SDS-PAGE patterns of glutenin subunits (A, B and C subunits);

**B** The Acid-PAGE patterns of gliadin bands;

**C** The SDS-PAGE patterns of gliadin bands.

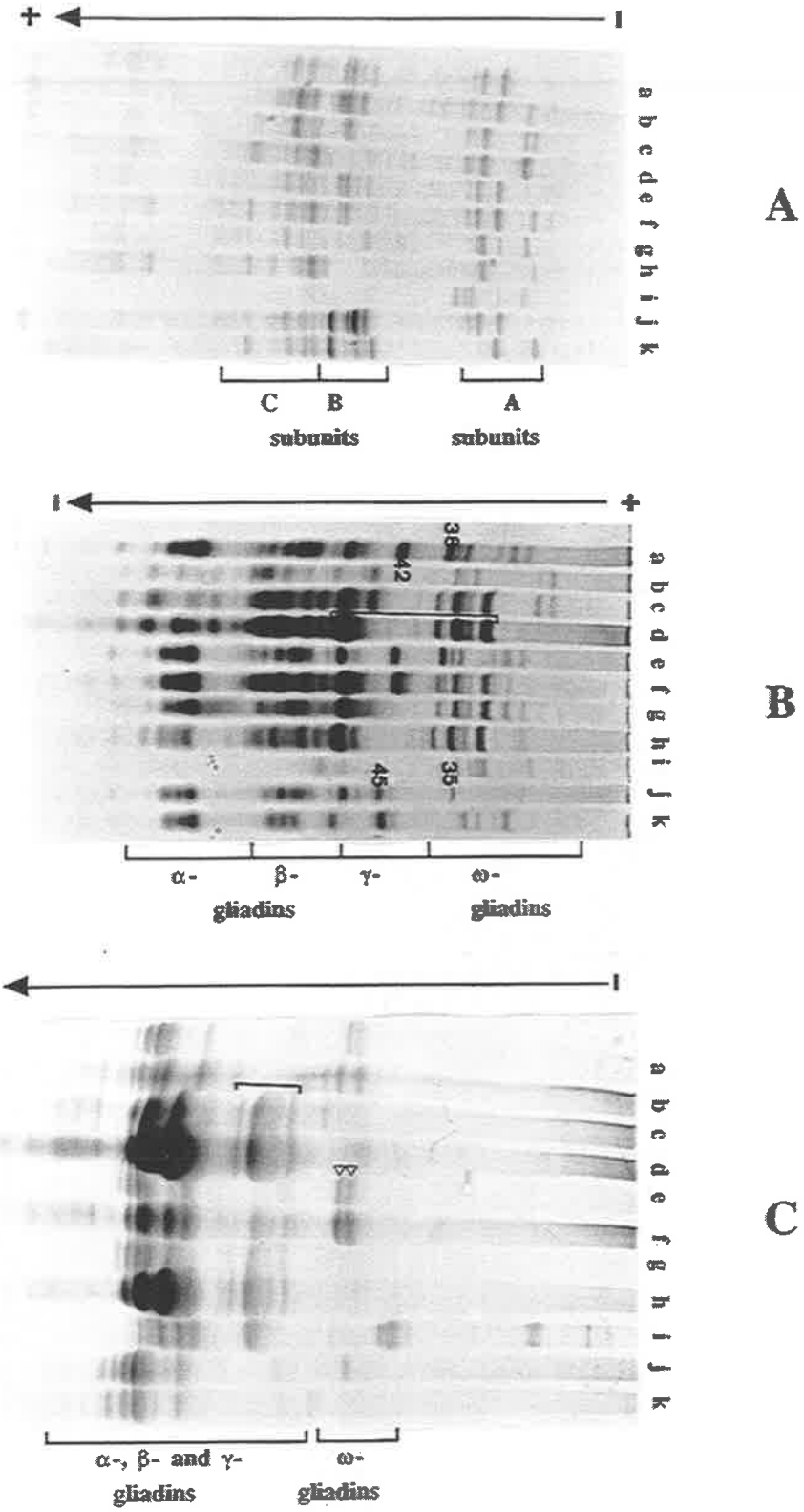
(a, e), Langdon (LMW-A/γ-42 control); (b), Chinese Spring (CS); (c), CS-Gabo 1AL.1BL.1RS; (d), CS-Gabo 1AL.1BL.1DL.1RS; (f), STr (4x) 1AL.1RS; (g), STr (4x) 1BL.1RS; (h), DTr (4x) 1AL.1BL.1RS; (i), Imperial rye; (j), Edmore (LMW-B/γ-45 control); (k), Kharkof-5.

**Symbols used in figures:**

▷ = bands controlled by 1BS;

[ = secalin bands controlled by 1RS;

Numbers denoted for bands in Acid-PAGE follow the nomenclature of Bushuk and Zillman (1978).


+
-
a b c d e f g h i j k
C
B
A
subunits
subunits
A
-
+
a b c d e f g h i j k
42
36
45
35
α-
β-
γ-
ω-
gliadins
gliadins
B
+
-
a b c d e f g h i j k
α-, β- and γ-
gliadins
ω-
gliadins
C

Spring-CS), Imperial rye and the hexaploid translocation lines CS-Gabo 1AL.1BL.1RS and CS-Gabo 1AL.1BL.1DL.1RS included as controls. The HMW glutenin subunit composition of Langdon was band combination 6+8 coded by *Glu-B1d* and a null allele (*c*) at *Glu-A1*. The LMW glutenin subunits of Langdon included four B subunits and at least seven C subunits (lanes a and e). The dark-staining slowest-moving B subunit was absent in the STr (4x) 1AL.1RS stock (lane f), indicating that it was controlled by a *Glu-A3* allele. The null form (*Glu-A1c*) of HMW glutenin subunits was also replaced by band 1 (*Glu-A1a*) from the CS-Gabo 1AL.1BL.1RS parent. In the STr (4x) 1BL.1RS line (lane g), the two fastest darkly-stained B subunits and one slower faint band of Langdon were absent while the 1AS-controlled band was present and strongly stained. HMW glutenin bands 2* and 17+18 of the CS-Gabo 1AL.1BL.1DL.1RS parent were present along with bands 6+8 of Langdon parent in this stock. As expected, all of the prominent B subunits were absent in the DTr (4x) line, but eight prominent C subunits remained (lane h). DTr (4x) also possessed the HMWGS band 1 and 17+18, which was from the hexaploid 1RS translocation parent (lane c). It was clear that no rye bands equivalent to the HMW glutenin subunits were substituted into the hexaploid and tetraploid 1RS stocks. In the C subunit region, rye showed very faint bands (lane i), but these bands were more strongly stained in the double-translocation (lane h), where the rye genes were present in extra dose, as already observed in the triple translocation stock (Gupta and Shepherd, 1993).

Since genes controlling B subunits of glutenin and ω-γ-gliadin bands were known to be tightly linked (Payne *et al*, 1984c), the gliadin composition of these translocation lines was also studied to investigate the co-inheritance of these bands. The composition of the ω- and γ-gliadin bands in the tetraploid 1RS translocation stocks and controls is shown in Fig. 5.3B after Acid-PAGE separation. The 1RS-controlled bands have separated into a strongly stained block comprising 6 bands within the wheat ω- and γ-gliadin region, as represented by CS-Gabo 1AL.1BL.1DL.1RS translocation lines in which all short arms of group 1 wheat chromosomes were absent (lane d). The slowest ω-gliadin band of Langdon (lanes a, e) was missing from the STr (4x) 1AL.1RS stock (lane f), confirming the previous report by Joppa *et al* (1983a) that this band was controlled by 1AS. Only band 38 of the triplet of ω-gliadin bands (block 33-35-38) in Langdon could be recognised in the translocations since some of the wheat bands co-migrated with the rye bands. As expected, the γ-42 gliadin band and the ω-38 gliadin band were missing in the STr (4x) 1BL.1RS stock (lane g). The two slowest ω-gliadin bands 16-18, band 38 and γ-gliadin band 42 were all absent in the DTr (4x) stock (lane h), and this stock resembled the CS-Gabo 1AL.1BL.1DL.1RS stock (lane d) except for some differences in the α-, β-gliadin bands, which were thought to be controlled by genes on homoeologous group 6 chromosomes (Payne *et al*, 1982a).

Gliadins were also separated under unreduced conditions in SDS-PAGE (Fig. 5.3C). The rye bands formed a very dark quadruplet of bands in the γ-gliadin region (Fig. 5.3C, lane c). The durum wheat parent Langdon (lanes a, e) had only faint bands in this area. The two slowest ω-gliadin bands of Langdon (denoted as ▷) were present in STr (4x) 1AL.1RS stock

(lane f) but absent in STr (4x) 1BL.1RS (lane g) and DTr 1AL.1BL.1RS stocks (4x) (lane h), indicating that these bands were controlled by 1BS. This simple electrophoretic procedure was useful for detecting the presence of 1RS bands and 1BS-controlled bands but the 1AS-controlled band(s) were difficult to identify, which precludes its wide use in inheritance studies.

## 5.3.2 Inheritance of LMW glutenin subunits and gliadins in durum wheats and a *T. dicoccoides* line

Since the DTr (4x) line possessed HMW glutenin subunits 1 and 17 + 18, coded by *Glu-A1* and *Glu-B1*, respectively, the correct parentage of all TC progeny could be confirmed by the presence of these HMW glutenin marker bands in reduced SDS-PAGE and by the presence of strongly stained secalin bands in unreduced SDS-PAGE and Acid-PAGE.

The DTr (4x) stock used as the third TC parent had one faint B subunit (Fig. 5.4A, lane o). This band was expected to be weakly stained in the TC progeny where only one dose of the relevant gene was present. Therefore, it should be facilitated to score the segregation of the B subunit blocks in the TC progeny without band overlap with bands from the recurrent parent. However, this parent possessed several C subunit bands as well as rye bands in the C subunit region, making it difficult to score the segregation of C subunits from the TC parents in that region.

### *5.3.2.1 [(Coulter x Langdon) $F_1$ hybrid x DTr (4x)] test-cross progeny*

Coulter and Langdon exhibited at least seven major differences with respect to the B subunits of glutenin (Fig. 5.4A). The B subunits of Coulter were designated "clt" for each of the 1BS- and 1AS-controlled groups whereas those from Langdon were designated "ldn". So the genes coding for these 1B- and 1A-controlled LMW glutenin subunits in Langdon were designated *Glu-B3*$_{ldn}$ and *Glu-A3*$_{ldn}$, respectively. Earlier work had shown that the slowest B band of Langdon was controlled by chromosome 1AS (Fig. 5.4A, lane h, denoted as ●) and the remaining three bands were controlled by 1BS (Fig. 5.4A, lane d, denoted as ▸ only for the two dark bands and Fig. 5.4'A') (Gupta, 1989). The top and bottom darkly-stained bands of Coulter were inherited as a unit but always as alternatives to the slowest band of Langdon indicating that they were a separate linkage block controlled by 1AS and the block has been designated *Glu-A3*$_{clt}$ for Coulter (Fig. 5.4A, lane i, denoted as ● and Fig. 5.4'A'). The middle bands of Coulter were also inherited as a unit and as alternatives to the *Glu-B3* bands of Langdon, indicating they were separate linkage blocks controlled by different alleles at the *Glu-B3* locus and the Coulter bands were assigned symbol *Glu-B3*$_{clt}$ (Fig. 5.4A, lane f, denoted as ▸ and Fig. 5.4'A'). The inheritance data (Fig. 5.4'A' and Table 5.1) showed that the banding patterns within both the 1AS- and 1BS-controlled groups fitted a segregation ratio of 1 : 1 ($P > 0.1$), whereas the banding patterns between the two groups were inherited independently ($P > 0.25$) (Table 5.2). The bands were inherited as intact blocks in all progeny (Fig. 5.4'A'), indicating that the controlling genes were allelic or very closely linked.

**Figure 5.4** Electrophoretic banding patterns of test-cross progeny [(Coulter x Langdon) x DTr (4x)], and the controls.

**A** SDS-PAGE patterns of glutenin subunits (A, B and C subunits);

**B** SDS-PAGE patterns of gliadin bands;

(a-l, p-u), test-cross seeds; (m), Coulter; (n), Langdon; (o), DTr (4x).

**C** Acid-PAGE patterns of gliadin bands.

(a-e, i-q), test-cross seeds; (f), Langdon; (g), Coulter; (h), DTr (4x); (r), Edmore (γ-45 control).

**Symbols used in figures:**

Clt = Coulter; Ldn = Langdon; Tr = DTr (4x);

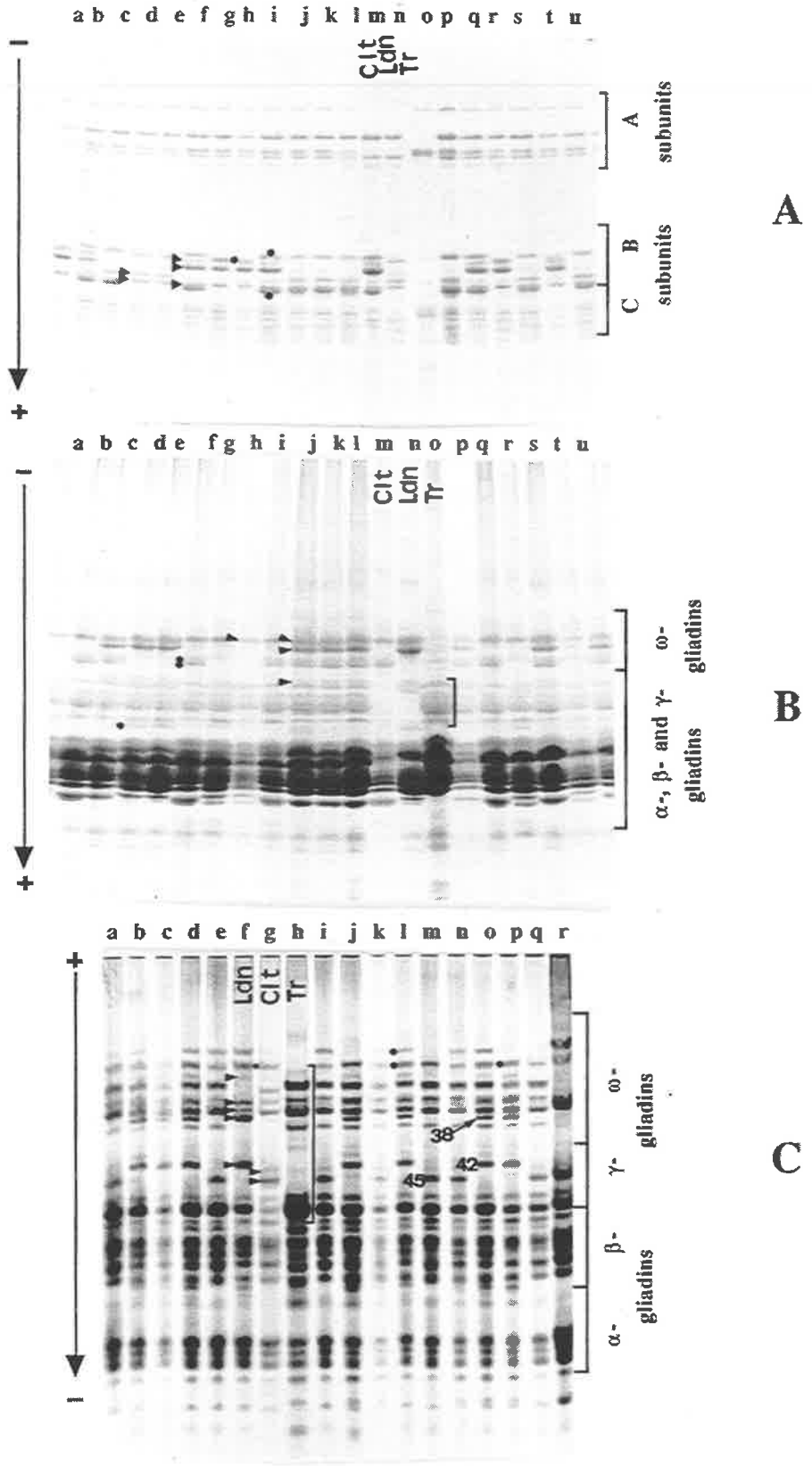
* = bands common in Coulter, Langdon or DTr (4x), and could not be scored in the test-cross progeny;

• = bands controlled by 1AS;

▸ = bands controlled by 1BS;

[ = secalin bands controlled by 1RS;

Numbers denoted for bands in Acid-PAGE follow the nomenclature of Bushuk and Zillman (1978).


a b c d e f g h i j k l m n o p q r s t u
Clt
Ldn
Tr
A subunits
B subunits
C subunits
A
a b c de fg h i j kl m no pq r s t u
Clt
Ldn
Tr
ω-gliadins
α-, β- and γ-gliadins
B
a b c d e f g h i j k l m n o p q r
Ldn
Clt
Tr
38
42
45
ω-gliadins
γ-gliadins
β-gliadins
α-gliadins
C

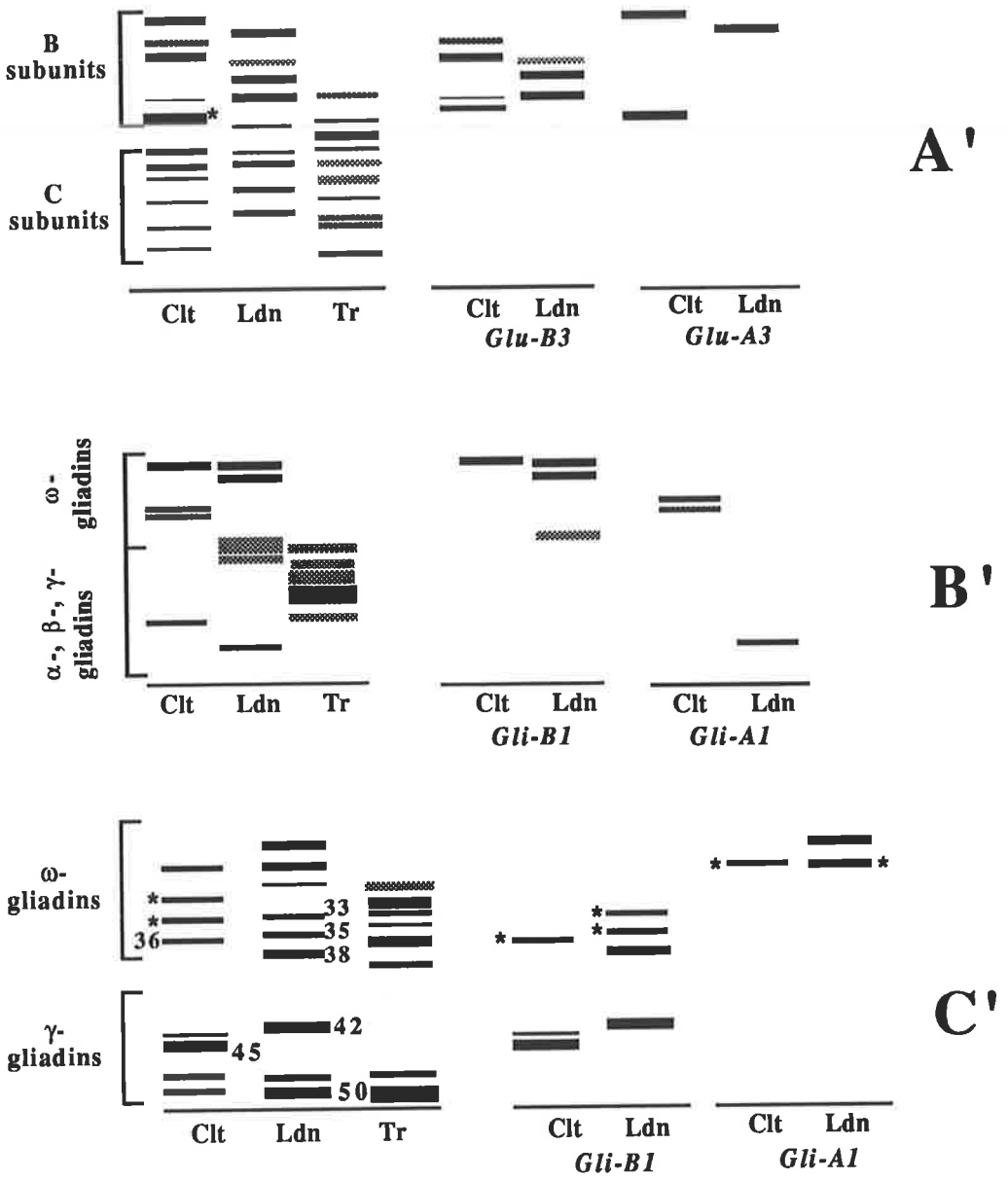



**Figure 5.4'** Diagrammatic representation of electrophoretic banding patterns of the test-cross progeny [(Coulter x Langdon) x DTr (4x)].

**A'** B subunit patterns by SDS-PAGE;

**B'** ω-gliadin patterns by SDS-PAGE;

**C'** ω- and γ-gliadin patterns by Acid-PAGE.

**Symbols used in figures:**

Same as in Fig. 5.4.

**Table 5.1** Individual segregation of B subunit blocks among 98 [(Coulter x Langdon ) x DTr (4x)] test-cross seeds

| LMW glutenin blocks (B subunits) | Observed frequency | | $\chi^2$ ($P_1 : P_2 = 1:1$) | Probability (df = 1) |
|---|---|---|---|---|
| | Parental types ($P_1 : P_2$) | Non-parental types | | |
| clt : ldn on 1BS | 56 : 41 | 0 | 2.02 | 0.11-0.25 |
| clt : ldn on 1AS | 49 : 48 | 0 | 0.00 | 1.00 |

**Table 5.2** Joint segregation of B subunit blocks among 98 [(Coulter x Langdon ) x DTr (4x)] test-cross seeds

| | *Glu-A3*$_{clt}$ | *Glu-A3*$_{ldn}$ | $\chi^2$ (2 X 2) | Probability (df = 1) |
|---|---|---|---|---|
| *Glu-B3*$_{clt}$ | 25 | 31 | | |
| | | | 1.31 | 0.25-0.50 |
| *Glu-B3*$_{ldn}$ | 24 | 17 | | |

The gliadin patterns of the TC progeny were examined in unreduced SDS-PAGE (Fig. 5.4B). The DTr (4x) line had no ω-gliadin bands whereas the Coulter and Langdon had one and two of the slowest ω-gliadin bands, respectively. The two slowest bands of Langdon (Fig. 5.4B, lane j, denoted as ►) were shown to be controlled by 1BS in the previous section (see section 5.3.1.3). Coulter had a single ω-gliadin band with the same mobility as the slowest ω-gliadin band of Langdon (Fig. 5.4B, lane h, denoted as ►). When the two 1BS-controlled bands of Langdon were absent, the slowest band from Coulter was always present, implying that this band was controlled by a different allele on 1BS. A block of ω-gliadin bands with fast mobility in Coulter and a γ-gliadin band of Langdon segregated as alternatives, but independently of the 1BS-controlled bands, suggesting that they were controlled by 1AS (Fig. 5.4B, lanes f and d, denoted as ●, respectively). Again, these gliadin blocks did not recombine, indicating that the genes involved were allelic or closely linked (Fig. 5.4'B').

The gliadin components were also fractionated by Acid-PAGE (Fig. 5.4C), because this system has been widely used in the past for the identification of gliadin bands (Bushuk and Zillman, 1978; du Cros and Wrigley, 1979; Sozinov and Poperelya, 1980, 1982; Dal Belin Peruffo *et al*, 1981; Khan *et al*, 1983). The parents Coulter and Langdon possessed γ-45 and γ-42 bands, respectively, and appeared to be controlled by 1BS (Pogna *et al*, 1990). The γ-42 band of Langdon was linked to the a triplet of ω-gliadin bands termed 33-35-38 whereas the band γ-45 of Coulter was linked to a ω-36 band (Fig. 5.4'C'). The gliadin genes (*Gli-B1*) were designated *Gli-B1*$_{clt}$ and *Gli-B1*$_{ldn}$, respectively (Fig. 5.4C, lanes g and f, denoted as ►). The two slowest ω-gliadin bands of Langdon segregated independently from the *Gli-B1* bands, indicating that they were *Gli-A1* bands (*Gli-A1*$_{ldn}$) (lane l, denoted as ●). The slowest band of

Langdon was absent when chromosome 1A was replaced by chromosome 1D (Joppa *et al*, 1983a). The slowest band of Coulter was probably coded for by 1AS, but it could not be scored because of overlap with the second slowest band of Langdon (Fig. 5.4C). However, when the *Gli-A1*$_{ldn}$ gene was absent, this band of Coulter always appeared to be present (lane g), suggesting that it was controlled by 1AS (Fig. 5.4'C').

The joint segregation patterns for the gliadin bands and the B glutenin subunits were also examined. In all cases, TC seeds with the Coulter 1AS- and 1BS-controlled gliadin patterns also had the respective B glutenin subunit pattern of Coulter, and similarly all the Langdon gliadin patterns were completely associated with the Langdon glutenin B subunit patterns (cf. Fig 5.4'A', 5.4'B' and 5.4'C'). Thus, no rare recombinants were observed between any of these different blocks (i. e. *Gli-1* and *Glu-3*), indicating that there was a close linkage between these genes on both chromosomes 1AS and 1BS in these two cultivars. Because of this co-inheritance, the genes controlling the gliadin patterns of Langdon have been designated *Gli-A1*$_{ldn}$ and *Gli-B1*$_{ldn}$, and symbols *Gli-A1*$_{clt}$ and *Gli-B1*$_{clt}$ were assigned to the genes controlling the gliadin patterns of Coulter. The absence of any recombinants among 98 TC seeds set an upper limit of 3.0% for the recombination rate between the *Glu-3* and *Gli-1* linkage blocks and within both blocks with a 95% confidence limit.

### *5.3.2.2 [(Beladi Bouhi x Edmore) $F_1$ hybrid x DTr (4x)] test-cross progeny*

#### (A) Screening of progeny

Altogether 114 TC progeny from this parental combination were analysed by SDS-PAGE and Acid-PAGE and from their banding patterns six seeds were found to be non test-cross seeds. The groups of B subunits in Edmore and Beladi Bouhi were assigned symbols "edm" and "bb", respectively. The genes coding for these respective B subunits were designated *Glu-B3*$_{edm}$, *Glu-B3*$_{bb}$, *Glu-A3*$_{edm}$ and *Glu-A3*$_{bb}$. There were four and three dark B subunit bands for Edmore and Beladi Bouhi, respectively (Fig. 5.5A and Fig. 5.5'A'). Gupta (1989) reported that all the B subunits of Edmore were controlled by chromosome 1BS but this was disproved in the present study, because the B subunits of Edmore segregated into three different linkage blocks. One block was composed of two dark bands (the fastest and third fastest) (Fig. 5.5A, lane j, denoted as ▶), one was the second fastest (lane r, denoted as ■) and the other was the slowest band (lane l, denoted as ●).

The two dark bands of Edmore (▶) had been shown earlier to be controlled by 1BS because of their presence in the Langdon (Edmore-1B) substitution line (Gupta and Shepherd, 1988), and since these segregated as alternatives to the two fastest bands of Beladi Bouhi (Fig. 5.5A, lane c, denoted as ▶), it was concluded that these two latter bands were also controlled by 1BS genes (Fig. 5.5'A'). The slowest bands of each parent were inherited mutually exclusively in the TC progeny and were therefore likely to be controlled by genes at a locus on 1AS (Fig. 5.5A, lanes k and l, denoted as ●).

**Figure 5.5** Electrophoretic banding patterns of test-cross progeny [(Beladi Bouhi x Edmore) x DTr (4x)], and the controls.

**A** SDS-PAGE patterns of glutenin subunits (A, B and C subunits);

(a-c, g-y), test-cross seeds [m, y, non test-cross seeds]; (d), Beladi Bouhi; (e), Edmore; (f), DTr (4x).

**B** Acid-PAGE patterns of gliadin bands.

(a-i, l-q), test-cross seeds; (j), Beladi Bouhi; (k), Edmore (γ-45 control); (r), DTr (4x).

**Symbols used in figures:**

Bb = Beladi Bouhi; Edm = Edmore; Tr = DTr (4x);

* = bands common in Beladi Bouhi, Edmore or DTr (4x), and could not be scored in the test-cross progeny;

● = bands controlled by 1AS;

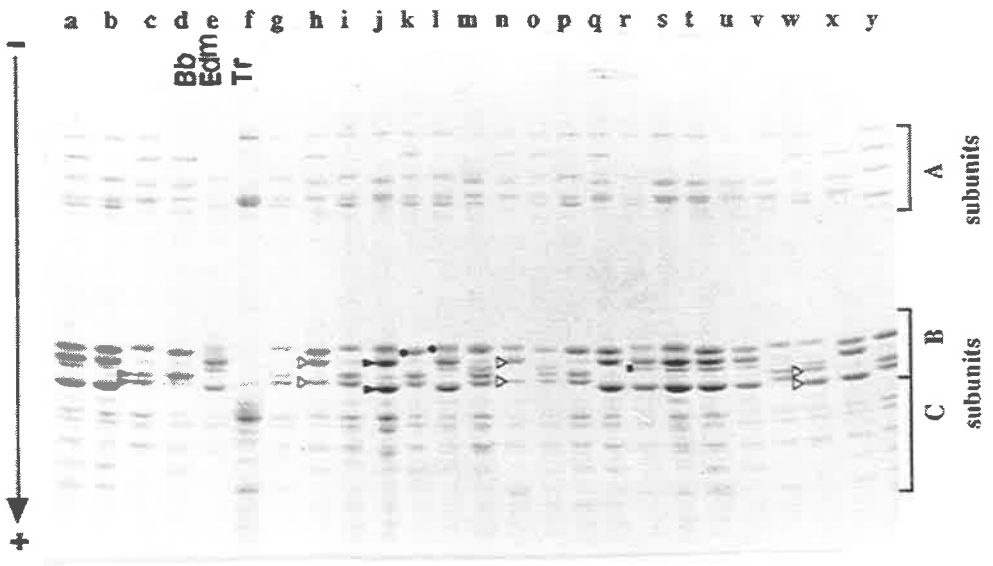
▸ = bands controlled by 1BS;

▹ = suspected recombinants;

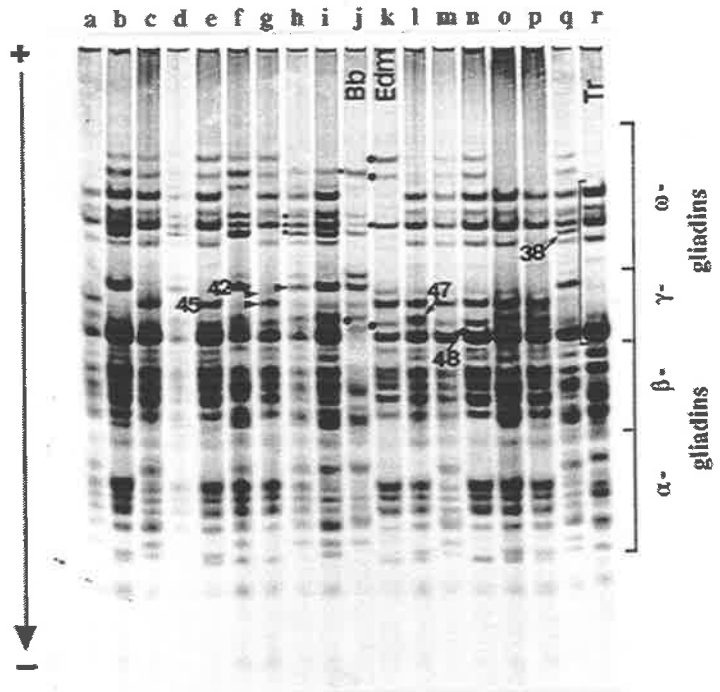
[ = secalin bands controlled by 1RS;

■ = band controlled by a gene on 1BS apparently loosely linked with that controlling other B subunits of Edmore;

Numbers denoted for bands in Acid-PAGE follow the nomenclature of Bushuk and Zillman (1978).


a b c d e f g h i j k l m n o p q r s t u v w x y
Bb
Edm
Tr
A subunits
B subunits
C subunits
-
+

A


a b c d e f g h i j k l m n o p q r
Bb
Edm
Tr
45
43
47
48
38
α- β- γ- ω- gliadins
+
-

B

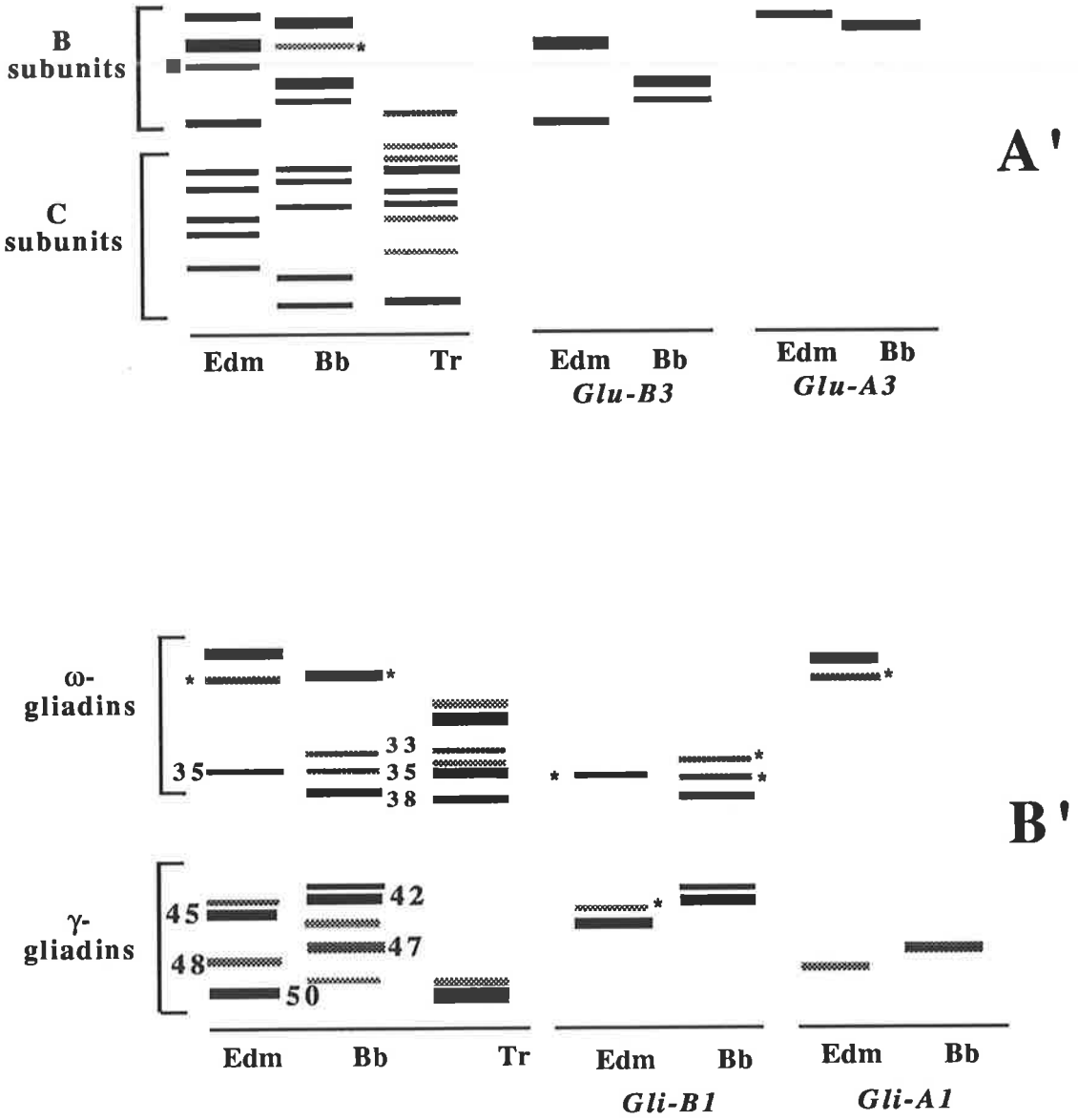



**Figure 5.5'** Diagrammatic representation of electrophoretic banding patterns of the test-cross progeny [(Beladi Bouhi x Edmore) x DTr (4x)].

**A'** B subunit patterns by SDS-PAGE;

**B'** ω- and γ-gliadin patterns by Acid-PAGE.

**Symbols used in figures:**

Same as in Fig. 5.5.

The ■ band of Edmore (Fig. 5.5A, lane r) was found to show loose linkage with the other 1BS-controlled bands (Fig. 5.5A, lane j, denoted as ►) such that about 90% of the progeny with ■ band possessed the Edmore 1BS-bands whereas the remainder had the Beladi Bouhi 1BS-bands, giving a recombination value estimate of 17.1 ± 3.7% (Table 5.3).

The B subunit patterns within each group of bands (1AS- and 1BS-controlled), excluding the ■ band described above, segregated in a 1 : 1 ratio (P > 0.9) (Table 5.4) and when tested in a 2x2 contingency table both groups of bands were found to segregate independently of each other (P > 0.25) (Table 5.5).

**Table 5.3** Joint segregation of the second fastest B subunit of Edmore (■ in Fig. 5.5A) with the *Glu-3* blocks among 105 [(Beladi Bouhi x Edmore) x DTr (4x)] test-cross seeds

| LMW glutenin blocks | ■ Band[a] | Observed frequency | | Comments |
|---|---|---|---|---|
| | | BB block | Edm block | |
| *Glu-B3* | + | 12 | 45 | Recombination value |
| | - | 42 | 6 | = 17.1 ± 3.7% |
| *Glu-A3* | + | 30 | 28 | No linkage |
| | - | 21 | 29 | detected |

[a] + = band present; - = band absent.

Two other types of apparent recombinants were detected involving the LMW glutenin bands controlled by the *Glu-B3* locus, but these were much rarer (3 plants, Table 5.4) than those described above. Two TC seeds (Nos 82 and 88) possessed the fastest B subunit of Beladi Bouhi and the third fastest band of Edmore (Fig. 5.5A, lanes h and n, denoted as ▷), representing bands from two different blocks. Another TC seed (No. 97) had the fastest B band of Edmore and the second fastest band of Beladi Bouhi (Fig. 5.5A, lane w, denoted as ▷), once again combining bands from two different *Glu-B3* blocks.

**Table 5.4** Individual segregation of B subunit blocks among 108 [(Beladi Bouhi x Edmore) x DTr (4x)] test-cross seeds

| LMW glutenin blocks (B subunits) | Observed frequency | | $\chi^2$ ($P_1 : P_2 = 1:1$) | Probability (df = 1) |
|---|---|---|---|---|
| | Parental types ($P_1 : P_2$) | Non-parental types | | |
| edm : bb on 1BS | 53 : 52 | 3 | 0.00 | 1.00 |
| edm : bb on 1AS | 53 : 55 | 0 | 0.01 | > 0.90 |

**Table 5.5** Joint segregation of B subunit blocks among 105 [(Beladi Bouhi x Edmore) x DTr (4x)] test-cross seeds[a]

| | *Glu-A3$_{edm}$* | *Glu-A3$_{bb}$* | $\chi^2$ (2 X 2) | Probability (df = 1) |
|---|---|---|---|---|
| *Glu-B3$_{edm}$* | 24 | 27 | | |
| | | | 0.75 | 0.25 - 0.50 |
| *Glu-B3$_{bb}$* | 31 | 23 | | |

[a] = Three non-parental types were excluded from the calculation.

The two parents showed gliadin band differences in both γ and ω components on Acid-PAGE (Fig. 5.5B). The 1BS-controlled blocks in Beladi Bouhi and Edmore were the same as described in a previous study (Pogna *et al*, 1988) because they contained the well-known bands 42 and 45 blocks (i. e. γ-42, ω-33-35-38 versus γ-45, ω-35) (Fig. 5.5B, lanes g and h, denoted as ►). The two slowest ω-gliadin bands and a γ-gliadin band 48 of Edmore segregated independently from these 1BS-bands, indicating that they were probably controlled by 1AS (Fig. 5.5B, lane k, denoted as ●). A γ-gliadin band 47 of Beladi Bouhi were thought also to be controlled by 1AS (Fig. 5.5B, lane j, denoted as ●), since they segregated independently of the 1BS-bands of Beladi Bouhi (band 42) and as alternatives to the 1AS-bands of Edmore. However, these γ-gliadin bands of both parents were difficult to score sometimes due to poor separation in the gel. Hence, when the 1AS-ω-bands of Edmore were absent, it was assumed that the band present was the 1AS-band of Beladi Bouhi (Fig. 5.5'B').

When the segregation patterns of the gliadins separated by Acid-PAGE were compared to those of the B subunits from 1-D SDS-PAGE, these two types of patterns co-segregated together as one unit except for the three putative recombinants involving 1BS-controlled B subunits already described (Table 5.4). Thus there was no evidence of recombination between the *Glu-3* and *Gli-1* linkage blocks in this TC progeny, providing an upper limit for the recombination value between them of 2.7% (with a 95% confidence limit).

## (B) Progeny tests of suspected recombinants

The TC progeny seeds 82, 88 and 97 had two unique protein banding patterns. However, only two of these seeds (Nos. 82 and 97) germinated and gave rise to subsequent progeny seeds. Fig. 5.6A shows the segregation patterns for glutenin subunits in these two progenies (lanes k to s and t to ac, respectively). For comparison, progeny from two normal TC plants, one with *Glu-A3$_{edm}$* and *Glu-B3$_{edm}$* linkage blocks (lanes a to d) and the other with *Glu-A3$_{bb}$* and *Glu-B3$_{edm}$* linkage blocks (lanes e to h) were included as controls (Fig. 5.6A, lanes c and d, denoted as ● and ►, respectively). These two plants segregated as expected in their progeny (Fig. 5.6A and 5.6B, lanes a to h).

**Figure 5.6** Electrophoretic banding patterns of progeny from two suspected recombinants (test-cross seed Nos 82 and 97) from the test-cross [(Beladi Bouhi x Edmore) x DTr (4x)], and the controls.

**A** SDS-PAGE patterns of glutenin subunits (A, B and C subunits);

**B** Acid-PAGE patterns of gliadin bands.

(a-d, e-h), two selfing progenies from two normal test-cross seeds; (i), Edmore; (j), Beladi Bouhi; (k-s), progeny from test-cross seed No. 82; (t-ac), progeny from test-cross seed No. 97; ($C_1$), Langdon ($\gamma$-42 control); ($C_2$, $C_3$), Edmore ($\gamma$-45 control).

**Symbols used in figures:**

EDM = Edmore; BB = Beladi Bouhi; $C_1$, $C_2$ and $C_3$ are cultivar controls;

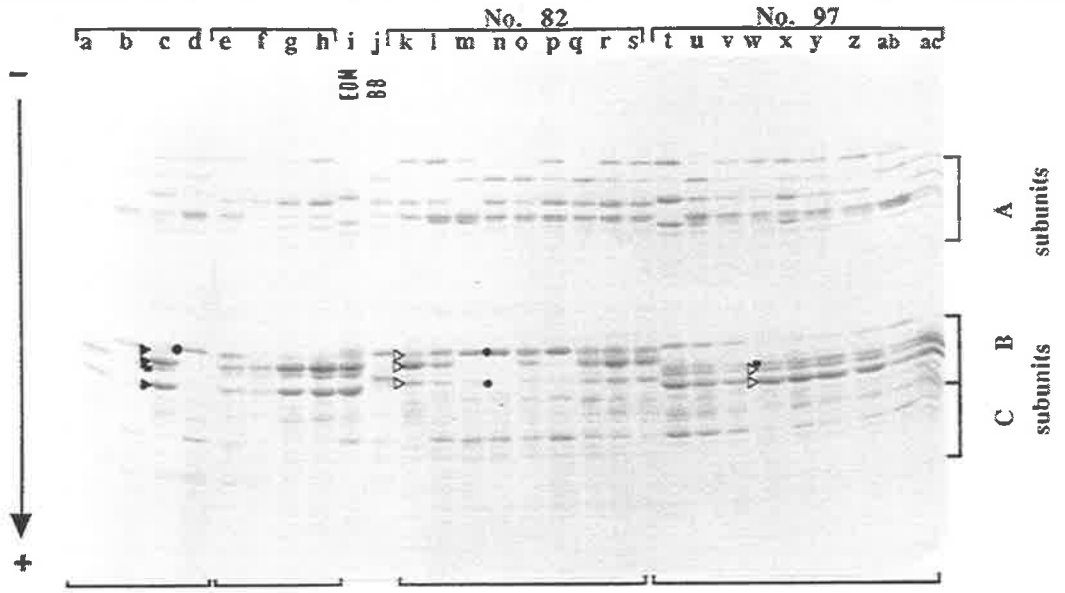
• = bands controlled by 1AS;

► = bands controlled by 1BS;

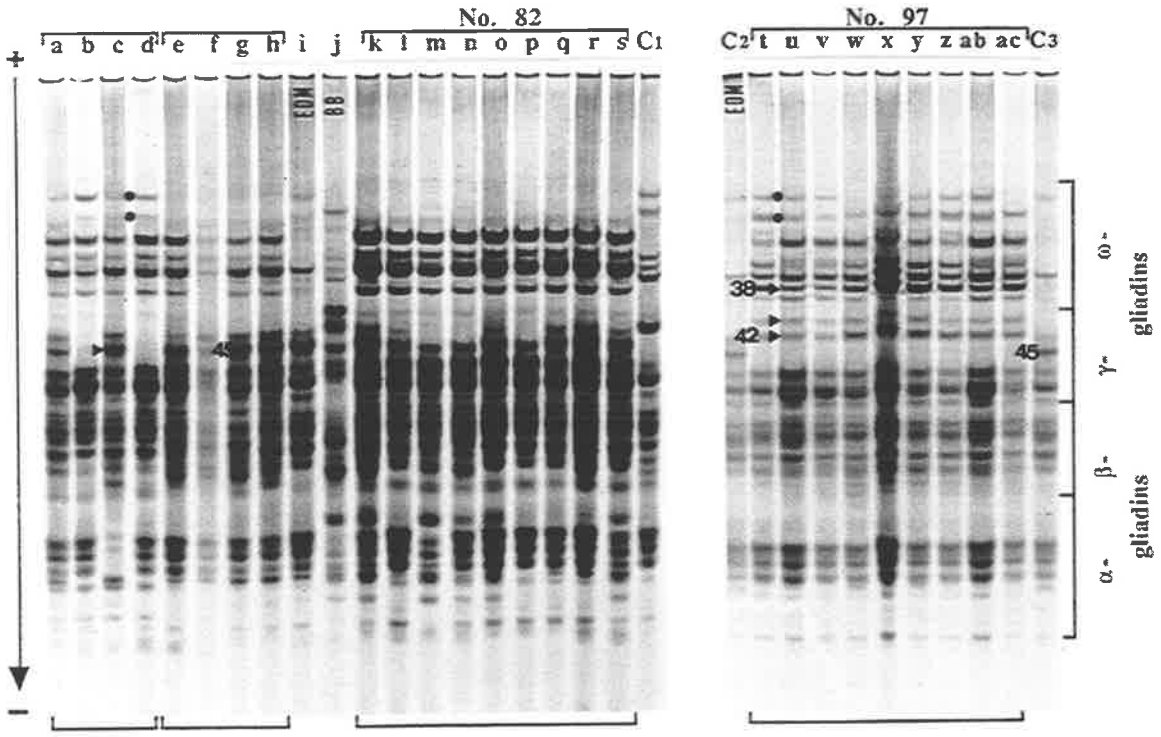
▷ = recombinant bands controlled by 1BS;

[ = secalin bands controlled by 1RS;

Numbers denoted for bands in Acid-PAGE follow the nomenclature of Bushuk and Zillman (1978).


No. 82
No. 97
a b c d e f g h i j k l m n o p q r s t u v w x y z ab ac
EOM
88
A subunits
B subunits
C subunits

A


No. 82
No. 97
a b c d e f g h i j k l m n o p q r s C1
C2 t u v w x y z ab ac C3
EOM
88
38
42
45
ω- gliadins
γ- β- gliadins
α-

B

However, progeny of the putative recombinant seed No. 82 (lanes k to s) segregated differently from the controls. They possessed a *Glu-A3$_{bb}$* gene, and a new 1BS combination of three bands, including the fastest band of Beladi Bouhi and the third and fourth fastest bands of Edmore (Fig. 5.6A, lane k, denoted as ▷), which segregated as a linkage block in the progeny. The slowest *Glu-A3$_{bb}$* band appeared to co-segregate with the fastest faint band as well (Fig. 5.6A, lane n, denoted as ●). The gliadin bands were also analysed in this progeny to find how they were associated with the new *Glu-B3* linkage block. While the *Gli-A1$_{bb}$* bands were inherited normally as expected, the *Gli-B1$_{edm}$*-coding bands were associated with the recombinant *Glu-B3* type in the progeny (Fig. 5.6B, lane c, denoted as ►), suggesting that the gene controlling the third and fourth fastest B subunit bands of Edmore was situated nearer to the ω-γ-gliadin linkage block than that controlling the fastest band. It appeared that the ω-35-γ-45 block coded by *Gli-B1$_{edm}$* has remained intact after recombination within the *Glu-B3* locus.

Progeny of another putative recombinant (seed No. 97) are included in lanes t to ac, Fig. 5.6A and Fig. 5.6B. They possessed a normal *Glu-A3$_{edm}$* band and a new 1BS-controlled block, comprising the fastest and the third fastest ■ bands of Edmore and the second fastest band of Beladi Bouhi (Fig. 5.6A, lane w, denoted as ▷).

The gliadin pattern of the segregating progeny was also analysed to determine which 1BS-controlled gliadin bands were associated with the recombinant phenotype. It was found that the recombinant possessed the 1BS-controlled gliadin *Gli-B1$_{bb}$* linkage block (Fig. 5.6B, lane u, denoted as ►), suggesting that the gene controlling the second fastest band of Beladi Bouhi was situated nearer to the ω-γ-gliadin linkage block of Beladi Bouhi than the gene controlling the fastest band (Fig. 5.6A). Again recombination within the *Glu-B3* locus, has not affected the 1BS-controlled gliadin block, since the γ-gliadin band 42 as well as band ω-38 of the triplet of bands 33-35-38 were all inherited as a block in the progeny (Fig. 5.6B, lanes t to ac). The second fastest ■ band of Edmore, which was shown earlier (section 5.3.2.2A) to be loosely linked with the *Glu-B3$_{edm}$* block, with about 17% recombination rate, segregated together with the recombinant 1BS-controlled bands because of the presence of chromosome 1RS in the TC progeny which precluded recombination along with 1BS in the progeny test.

### *5.3.2.3 [(Kharkof-5 x Langdon) $F_1$ hybrid x DTr (4x)] test-cross progeny*

The B subunit banding pattern of Kharkof-5 appeared to be similar to that of Coulter (section 5.3.2.1) but closer inspection showed that although they possessed the same *Glu-A3* bands they had different *Glu-B3* bands (Fig. 5.7A). Gene symbols *Glu-A3$_{kha}$* and *Glu-B3$_{kha}$* were assigned to the inheritance blocks of B subunits in Kharkof-5 (Fig. 5.7A, lanes h and o, denoted as ● and ►, respectively, and Fig. 5.7'A'). The ω-gliadin bands of these two cultivars were also different. With the SDS-PAGE system, Kharkof-5 had a very faint band with similar mobility to the second slowest band of Langdon (Fig. 5.7B, lane k, denoted as ► and Fig. 5.7'B'). In Acid-PAGE, γ-gliadin band 45 of Kharkof-5 was associated with ω-gliadin

**Figure 5.7** Electrophoretic banding patterns of test-cross progeny [(Kharkof-5 x Langdon) x DTr (4x)], and the controls.

**A** SDS-PAGE patterns of glutenin subunits (A, B and C subunits);

**B** SDS-PAGE patterns of gliadin bands;

(a-h, l-r), test-cross seeds; (i), DTr (4x); (j), Langdon; (k), Kharkof-5.

**C** Acid-PAGE patterns of gliadin bands.

(a-h, l-r), test-cross seeds; (i), DTr (4x); (j), Kharkof-5; (k), Langdon (γ-42 control); (s), Edmore (γ-45 control); (t), MG 2988 (γ-47 control).

**Symbols used in figures:**

Kha = Kharkof-5; Ldn = Langdon; Tr = DTr (4x);

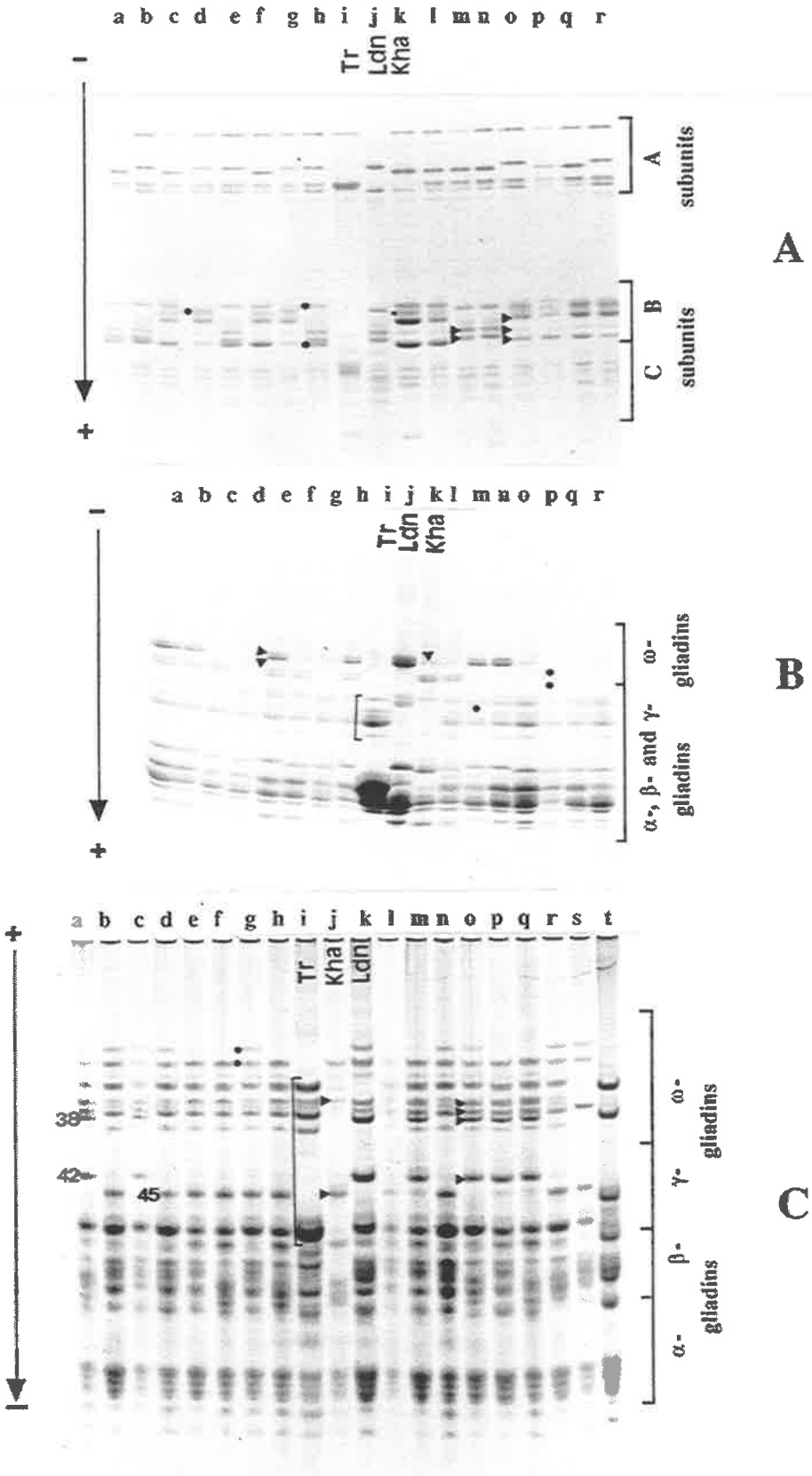
* = bands common in Kharkof-5 (and within Kharkof-5), Langdon or DTr (4x), and could not be scored in the test-cross progeny;

• = bands controlled by 1AS;

▸ = bands controlled by 1BS;

[ = secalin bands controlled by 1RS;

Numbers denoted for bands in Acid-PAGE follow the nomenclature of Bushuk and Zillman (1978).


a b c d e f g h i j k l m n o p q r
Tr
Ldn
Kha
A subunits
B subunits
C subunits
A
a b c d e f g h i j k l m n o p q r
Tr
Ldn
Kha
α-, β- and γ- gliadins
ω- gliadins
B
a b c d e f g h i j k l m n o p q r s t
Tr
Khal
Ldnl
38
42
45
α- gliadins
β- γ- gliadins
ω- gliadins
C

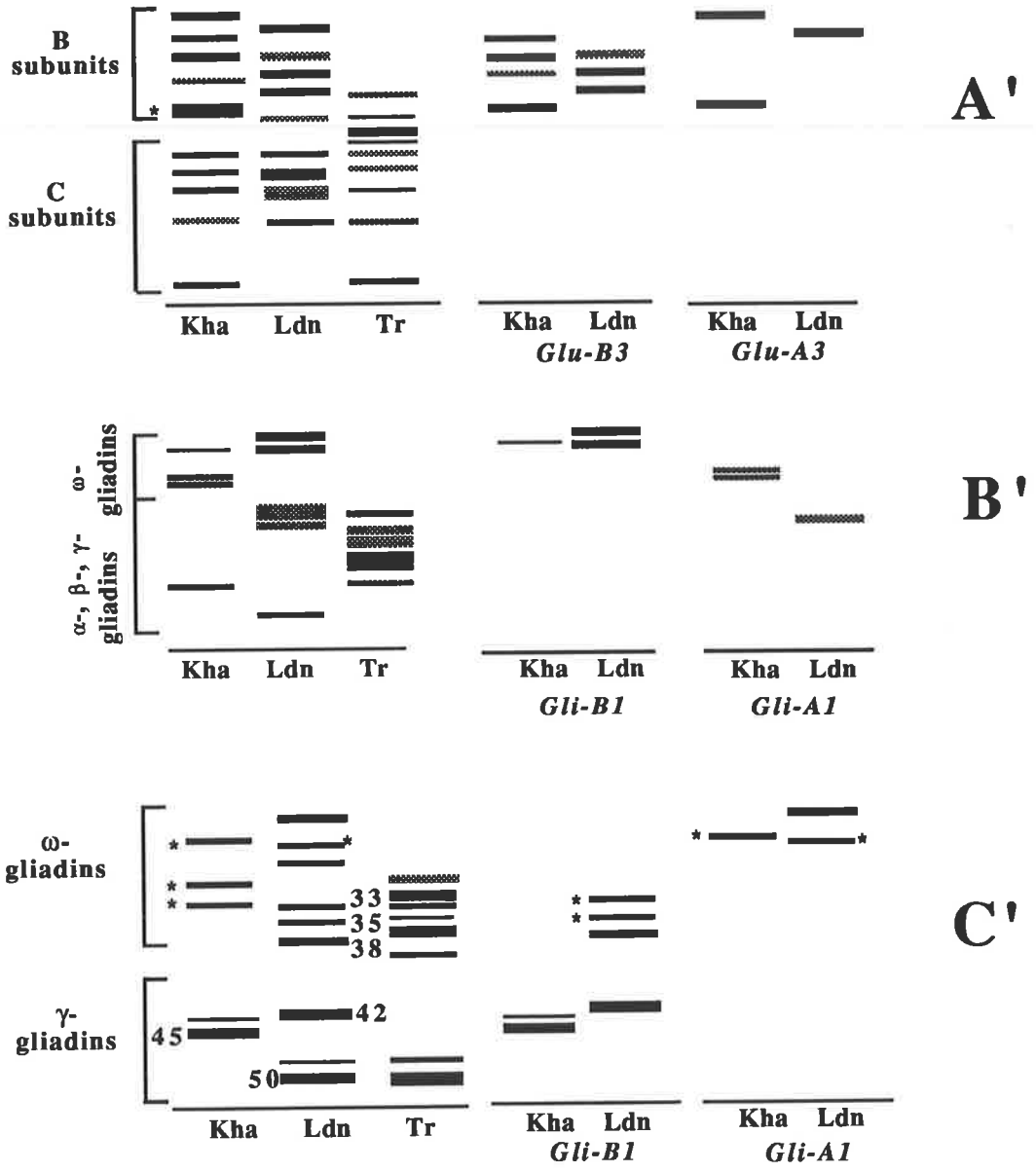



**Figure 5.7'** Diagrammatic representation of electrophoretic banding patterns of the test-cross progeny [(Kharkof-5 x Langdon) x DTr (4x)].

**A'** B subunit patterns by SDS-PAGE;

**B'** ω-gliadin patterns by SDS-PAGE;

**C'** ω- and γ-gliadin patterns by Acid-PAGE.

**Symbols used in figures:**

Same as in Fig. 5.7.

band 32 (Fig. 5.7C, lane j and 5.7'C'). The 1AS-controlled bands of Kharkof-5 were the same as for Coulter found in section 5.3.2.1 in both fractionation systems.

No recombination was detected either within the segregating blocks of bands, nor between the *Glu-3* and *Gli-1* linkage blocks (Fig. 5.7'A', 5.7'B' and 5.7'C'). The inheritance data fitted a segregation ratio of 1 : 1 for each of the *Glu-A3* and *Glu-B3* loci and a joint segregation ratio of 1 : 1 : 1 : 1 between the two loci (Tables 5.6. and 5.7). The upper limit for recombination between the *Gli-1* and *Glu-3* blocks was calculated to be 2.4% (with a 95% confidence limit).

**Table 5.6** Individual segregation of B subunit blocks among 121 [(Kharkof-5 x Langdon ) x DTr (4x)] test-cross seeds

| LMW glutenin blocks (B subunits) | Observed frequency | | $\chi^2$ ($P_1 : P_2 = 1:1$) | Probability (df = 1) |
|---|---|---|---|---|
| | Parental types ($P_1 : P_2$) | Non-parental types | | |
| kha : ldn on 1BS | 64 : 57 | 0 | 0.38 | 0.50 - 0.75 |
| kha : ldn on 1AS | 71 : 60 | 0 | 1.04 | 0.25 - 0.50 |

**Table 5.7** Joint segregation of B subunit blocks among 121 [(Kharkof-5 x Langdon ) x DTr (4x)] test-cross seeds

| | *Glu-A3$_{kha}$* | *Glu-A3$_{ldn}$* | $\chi^2$ (2 X 2) | Probability (df = 1) |
|---|---|---|---|---|
| *Glu-B3$_{kha}$* | 37 | 28 | | |
| | | | 0.00 | 1.00 |
| *Glu-B3$_{ldn}$* | 33 | 23 | | |

### *5.3.2.4 {[Edmore (and Langdon) x T. dicoccoides line 19-27] $F_1$ hybrid x DTr (4x)} test-cross progeny*

### (A) Screening of progeny

Only 55 TC progeny was available from the first parental combination and they were analysed by SDS-PAGE and Acid-PAGE. Genes coding for B subunits of 19-27 and Edmore were assigned "*td*" and "*edm*" for their respective linkage blocks. *T. dicoccoides* line 19-27 had three dark B subunit bands (Fig. 5.8A, lane o and Fig. 5.8'A'). The slowest band of 19-27 segregated as an alternative to the 1AS-band of Edmore (Fig. 5.8A, lanes l and k, denoted as ●), indicating that it was controlled by an allele or closely linked gene at the *Glu-A3* locus (Fig. 5.8'A'). The other two bands of 19-27 segregated mutually exclusively to the 1BS-bands of Edmore and were likely to be controlled by an allele or closely linked gene at *Glu-B3* (Fig. 5.8A, lanes i and g, denoted as ►).

The parents differed in both γ and ω gliadin components and their segregation was analysed in the TC progeny after Acid-PAGE separation. The gliadin pattern of 19-27 (Fig.

**Figure 5.8** Electrophoretic banding patterns of test-cross progeny [(Edmore / Langdon x 19-27) x DTr (4x)], and the controls.

**A** SDS-PAGE patterns of glutenin subunits (A, B and C subunits);

(a-l), test-cross seeds of [(Edmore x 19-27) x DTr (4x)]; (q-v), test-cross seeds of [(Langdon x 19-27) x DTr (4x)]; (m, w), Edmore; (n), DTr (4x); (o), 19-27; (p), Langdon.

**B** Acid-PAGE patterns of gliadin bands.

(a-j, m-s), test-cross seeds of [(Edmore x 19-27) x DTr (4x)]; (k), Edmore (γ-45 control); (l), 19-27; (t), DTr (4x).

**Symbols used in figures:**

Edm = Edmore; Td = 19-27; Ldn = Langdon; Tr = DTr (4x);

* = bands common in 19-27, Edmore or DTr (4x), and could not be scored in the test-cross progeny;

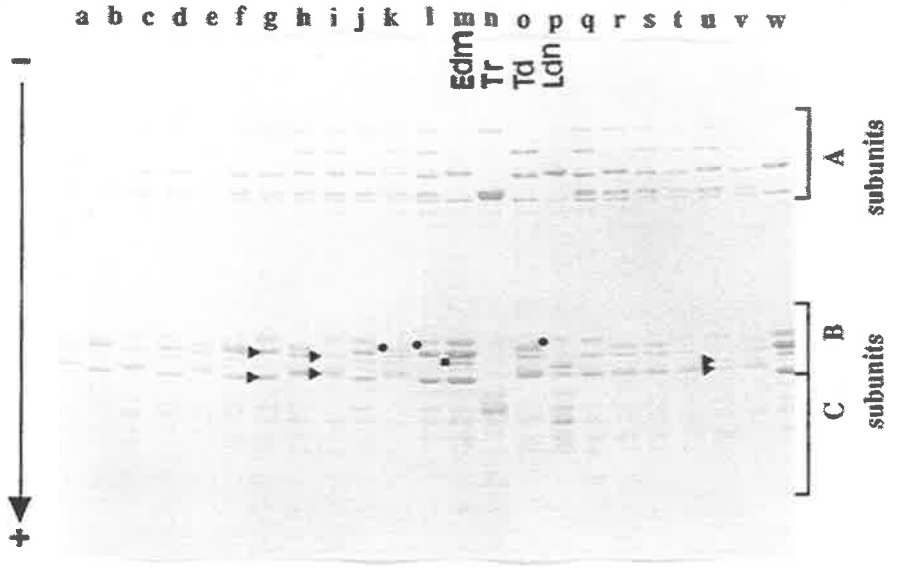
• = bands controlled by 1AS;

▸ = bands controlled by 1BS;

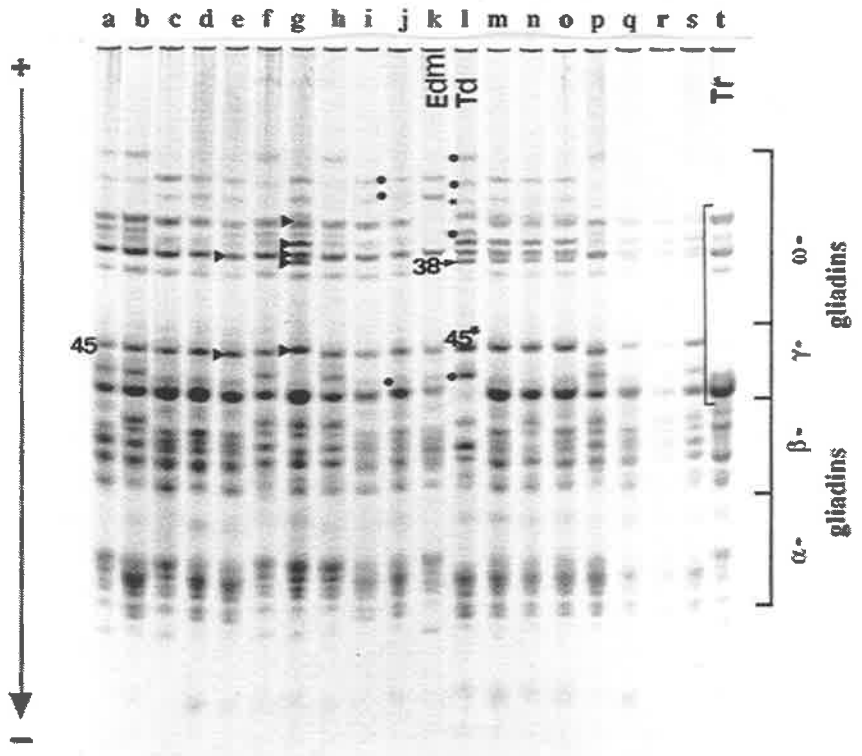
[ = secalin bands controlled by 1RS;

■ = band controlled by a gene on 1BS apparently loosely linked with that controlling other B subunits of Edmore;

Numbers denoted for bands in Acid-PAGE follow the nomenclature of Bushuk and Zillman (1978).


a b c d e f g h i j k l m n o p q r s t u v w
Edm
Tr
Td
Ldn
A subunits
B subunits
C subunits
−
+

A


a b c d e f g h i j k l m n o p q r s t
Edm
Td
Tr
38
45
45
α- gliadins
β- γ- ω- gliadins
+
−

B

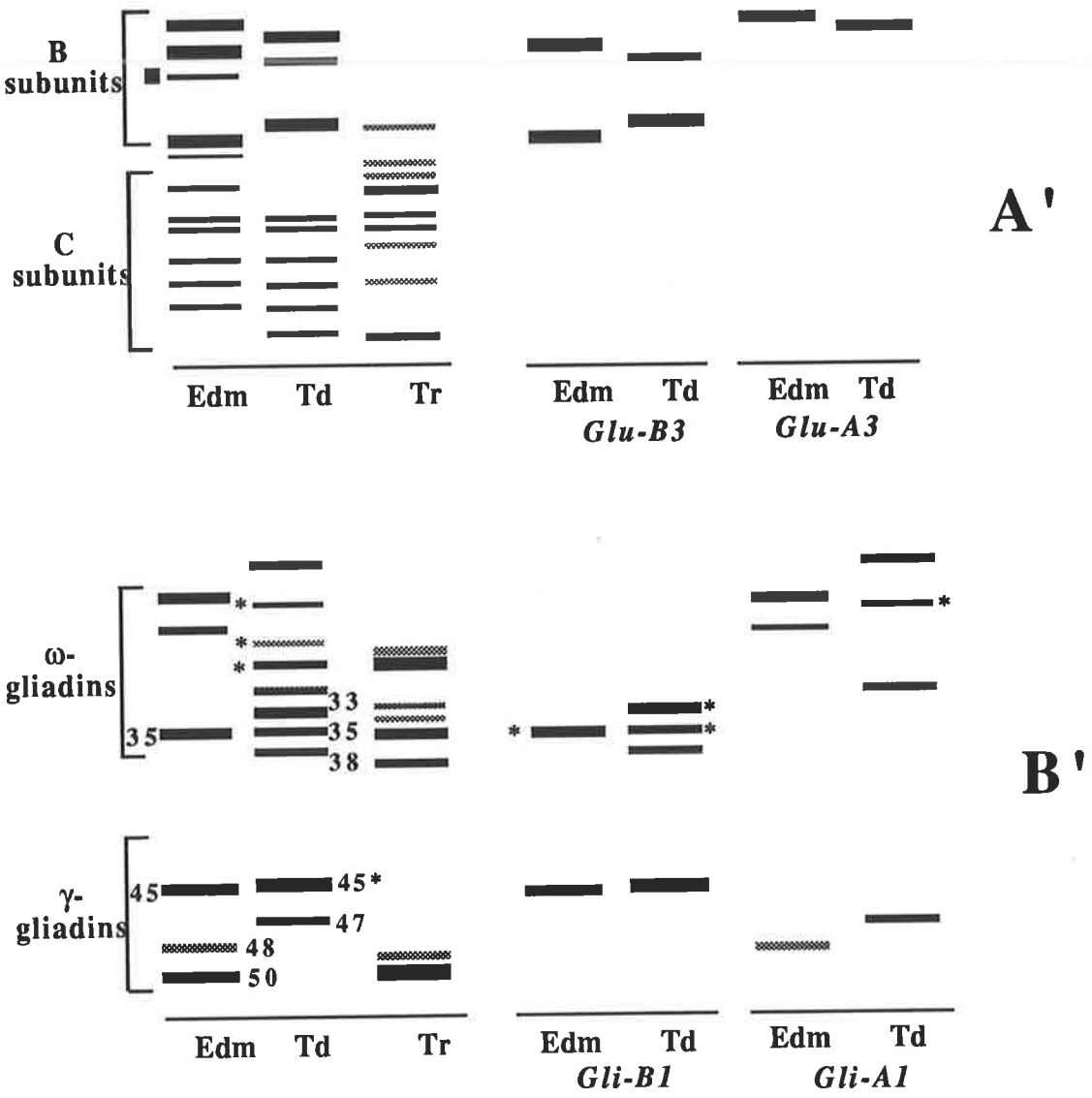



**Figure 5.8'** Diagrammatic representation of electrophoretic banding patterns of the test-cross progeny [(Edmore x 19-27) x DTr (4x)].

**A'** B subunit patterns by SDS-PAGE;

**B'** ω- and γ-gliadin patterns by Acid-PAGE.

**Symbols used in figures:**

Same as in Fig. 5.8.

5.8B) exhibited many ω-gliadin bands and a major γ-gliadin band with a mobility slightly slower than band 45 of Edmore. This band of 19-27 segregated together with the triplet (33-35-38) of ω-gliadin bands (Fig. 5.8B, lane g, denoted as ▶), normally associated with the γ-42 band of Langdon. Therefore, it was designated γ-55* to differentiate it from the γ-45 band (Fig. 5.8B and Fig. 5.8'B'). The two slowest ω-bands, a ω-31 band and a γ-47 band of 19-27 segregated as alternatives to the known 1AS-controlled bands in Edmore (Fig. 5.8B, lanes l and j, denoted as ●), thus these bands in 19-27 were thought to be controlled by a gene on 1AS as well (Fig. 5.8'B').

The individual segregation ratios of the B subunit patterns within each 1AS- and 1BS-controlled group of bands (Fig. 5.8'A' and 5.8'B'), excluding the ■ band described above (section 5.3.2.2A), segregated in a 1 : 1 ratio (Table 5.8) and when tested in a 2x2 contingency table both groups of bands segregated independently of each other (Table 5.9).

**Table 5.8** Individual segregation of B subunit blocks among 55 [(Edmore x 19-27) x DTr (4x)] test-cross seeds

| LMW glutenin blocks (B subunits) | Observed frequency | | $\chi^2$ ($P_1 : P_2 = 1:1$) | Probability (df = 1) |
|---|---|---|---|---|
| | Parental types ($P_1 : P_2$) | Non-parental types | | |
| edm : td on 1BS | 26 : 27 | 2 | 0.00 | 1.00 |
| edm : td on 1AS | 24 : 31 | 0 | 0.66 | 0.25 - 0.50 |

**Table 5.9** Joint segregation of B subunit blocks among 53 [(Edmore x 19-27) x DTr (4x)] test-cross seeds[a]

| | *Glu-A3*$_{edm}$ | *Glu-A3*$_{td}$ | $\chi^2$ (2 X 2) | Probability (df = 1) |
|---|---|---|---|---|
| *Glu-B3*$_{edm}$ | 14 | 13 | 0.02 | 0.75 - 0.90 |
| *Glu-B3*$_{td}$ | 15 | 11 | | |

[a] = Two non-parental types were excluded from the calculation.

Two possible recombinant seeds were detected involving *Glu-B3* bands (Fig. not shown). One TC seed (No. 36) had the fastest band of 19-27 and the third fastest band of Edmore, while another TC seed (No. 51) appeared to possess all of the *Glu-B3* bands from both parents representing a possible duplication or selfing product (Fig. not shown). However, the latter possibility could be eliminated because this plant carried the marker genes from the TC male parent. Another TC seed (No. 41) (Fig. not shown) possessed the *Glu-A3*$_{td}$ band as well as the *Gli-A1*$_{edm}$ block of bands and this seed was considered as a putative recombination between the *Glu-A3* and *Gli-A1* loci.

As observed in the earlier test-cross involving Edmore (section 5.3.2.2A), one of its B bands (Fig. 5.8A, lane m, denoted ■) exhibited loose linkage (20.8 ± 5.6%) with the *Glu-B3* controlled bands (Table 5.10).

**Table 5.10** Joint segregation of the second fastest B subunit of Edmore (■ in Fig. 5.8A) with the *Glu-3* blocks among 53 [(Edmore x 19-27) x DTr (4x)] test-cross seeds

| LMW glutenin blocks | ■ band[a] | Observed frequency | | Comments |
|---|---|---|---|---|
| | | 19-27 block | Edm block | |
| *Glu-B3* | + | 4 | 20 | Estimate of linkage |
| | - | 22 | 7 | = 20.8 ± 5.6% |
| *Glu-A3* | + | 14 | 12 | No linkage |
| | - | 10 | 19 | detected |

[a] + = band present; - = band absent.

There were six TC seeds resulting from the cross (Langdon x 19-27) x DTr (4x) (Fig. 5.8A, lanes q to v). Three segregating patterns were observed. These linkage blocks were consistent with those in Edmore x 19-27 TC progeny. Because there were too few seeds available in this cross to be statistically significant, it was only recorded in this section as supportive evidence for the above-mentioned B subunit blocks of 19-27.

## (B) Progeny tests of suspected recombinants

The two TC seeds Nos 36 and 51 with unique patterns and one suspected *Glu-A3/Gli-A1* recombinant (seed No. 41) detected in the TC progeny (5.3.2.4A) were germinated, grown in pots and selfed. The segregating population of the unique pattern of TC seed No. 36 is shown in Fig. 5.9A (lanes a-i) with parental lines 19-27 and Edmore in lanes j and k, four controls of TC seed patterns in lanes u to x (i. e. two parental types and two non-parental types). This plant had a normal $Glu\text{-}A3_{edm}$ coding band and a new 1BS-controlled block, comprising the fastest band of 19-27, and the third and fourth fastest bands of Edmore (Fig. 5.9A, lane c, denoted as ▷). In the present population, these two blocks segregated normally and hence confirmed the previous observation. The slowest $Glu\text{-}A3_{edm}$ band appeared to co-segregate with the fastest faint band (Fig. 5.9A, lane e, denoted as ●). A similar observation was also for the $Glu\text{-}A3_{bb}$ bands in the previous section (section 5.3.2B, Fig. 5.6). Interestingly, the gliadin 1BS-coding block $Gli\text{-}B1_{edm}$ (Fig. 5.9B, lane g, denoted as ▶) was again associated with the third and fourth fastest B subunits of Edmore (Fig. 5.9A), as observed in the previous section (section 5.3.2.2B). It was of interest to note for the purpose of future discussion (see section 5.4.2) that two seeds showed only a *Glu-B3/Gli-B1* block

**Figure 5.9** Electrophoretic banding patterns of progeny from three suspected recombinant seeds (Nos 36, 41 and 51) from the test-cross [(Edmore x 19-27) x DTr (4x)], and the controls.

**A** SDS-PAGE patterns of glutenin subunits;

**B** Acid-PAGE patterns of gliadin bands;

(a-i), progeny from test-cross seed No. 36; (j), 19-27; (k), Edmore; (l-t), progeny from test-cross seed No. 41; (u-x), four phenotypes of test-cross seed controls; ($C_1$), Edmore (LMW-B/γ-45 control); ($C_2$), Chinese Spring (hexaploid control); ($C_3$), Langdon (LMW-A/γ-42 control).

**Symbols used in figures:**

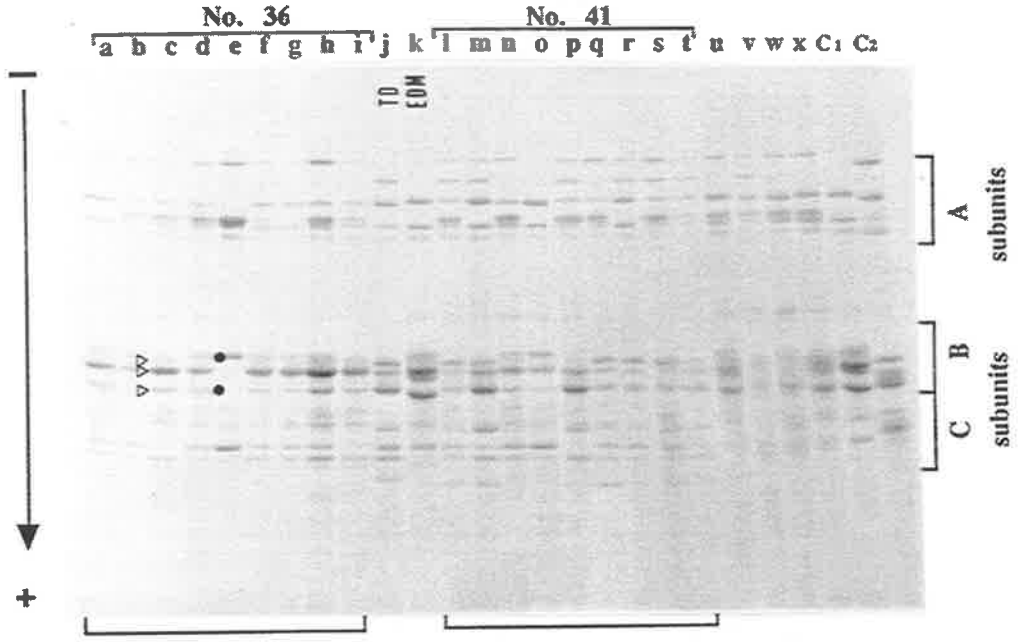
EDM = Edmore; TD = Line 19-27; $C_1$, $C_2$ and $C_3$ are cultivar controls;

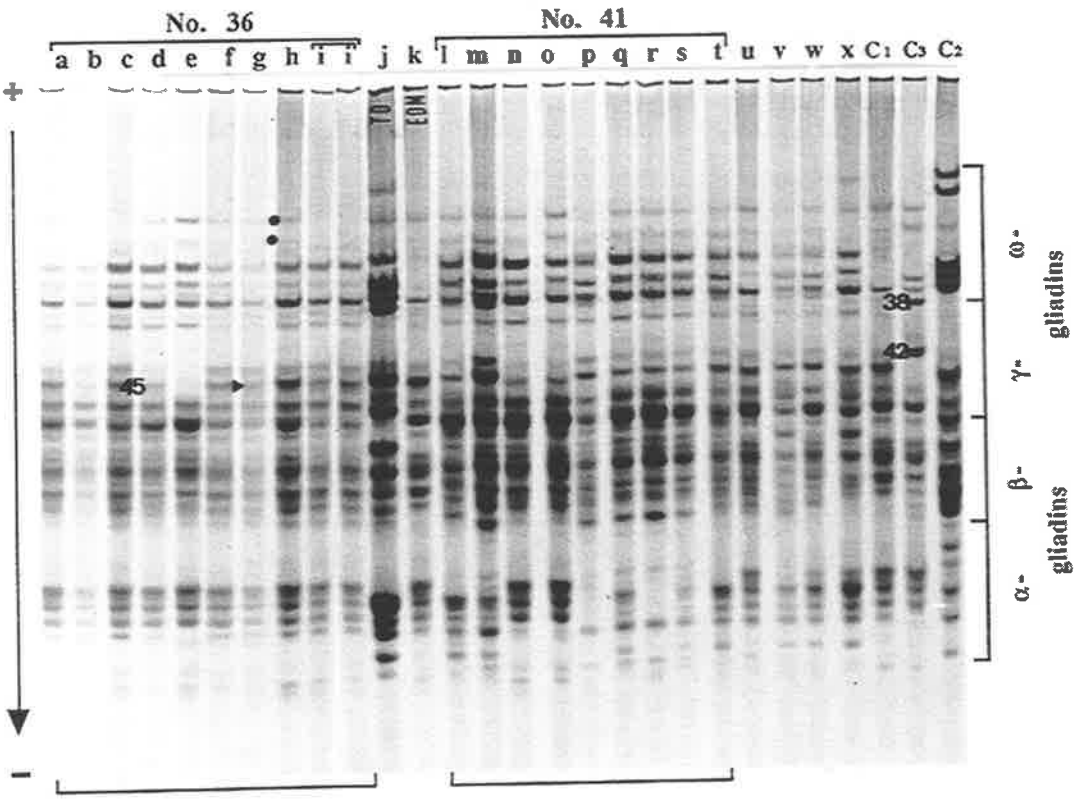
● = bands controlled by 1AS;

▸ = bands controlled by 1BS;

▷ = recombinant bands controlled by 1BS;

Numbers denoted for bands in Acid-PAGE follow the nomenclature of Bushuk and Zillman (1978).


No. 36
No. 41
a b c d e f g h i j k l m n o p q r s t u v w x C1 C2
TD
EDM
A subunits
B subunits
C
A


No. 36
No. 41
a b c d e f g h i j k l m n o p q r s t u v w x C1 C3 C2
TD
EDM
45
38
42
ω- gliadins
γ- gliadins
β- gliadins
α- gliadins
B

(lanes c and i) and one seed showed a *Glu-A3/Gli-A1* block (lane e) in the TC $F_2$ progeny, respectively (Figs 5.9A and 5.9B).

The second population tested (Fig. 5.9A, lanes l to t) was the progeny from the TC seed No. 41, tentatively identified earlier as a recombinant between the $Glu\text{-}A3_{td}$ and $Gli\text{-}A1_{edm}$ loci with a normal $Glu\text{-}B3_{td}/Gli\text{-}B1_{td}$ phenotype. Most selfed progeny seeds exhibited the expected $Glu\text{-}A3_{td}$ / $Gli\text{-}A1_{edm}$ recombination patterns (lanes l, m and p to t). However, the progeny also showed some other unexpected complicated patterns. Firstly, the *Glu-A3* linkage block segregated into three types (i. e. $Glu\text{-}A3_{edm}$, $Glu\text{-}A3_{td}$ and a null type) and the reason for this has not been elucidated. Closer inspection of the *Glu-B3* block in the progeny indicated that it comprised two different 1BS-controlled blocks as well. One was the expected $Glu\text{-}B3_{td}$ block (Fig. 5.9A, lanes l, m and p to t) and another appeared to be an intra-block recombinant, having the fastest band of 19-27 and the third fastest band of Edmore (lanes n and o). Interestingly, these two apparent recombinants (Figs 5.9A and 5.9B, lanes n and o) possessing the third fastest band of Edmore again co-inherited with the γ-45 gliadin band, as observed previously in the other progeny tests of two suspected recombinants (section 5.3.2.2B, Fig. 5.6A and this section Fig. 5.9A). Thirdly, some progeny possessed the *Gli-B1*-controlled ω-38 band of 19-27 (lanes m, p and t in Fig. 5.9B), but this was not co-inherited with either the *Glu-B3* block (Fig. 5.9A, lanes l to t), or the *Gli-B1*-controlled γ-45 (present only in lanes n, o in Fig. 5.9B) or γ-45* gliadin bands (Fig. 5.9B, lanes l, m and p to t). All the data here indicated that the original TC seed was unique and further work is required to explain these anomalies.

The progeny of the seed with the third unique pattern (seed No. 51) is shown in Fig. 5.9C. This TC seed appeared to possess both blocks of 1BS-controlled B subunits from Edmore and 19-27 (see section 5.3.2.4A). However, examination of the selfed progeny showed that it was a new 1BS-controlled block made up of the fastest and second fastest (■) bands of Edmore and the second and third fastest bands of 19-27 (Fig. 5.9C, lane j, denoted as ▷). That was this group of bands always segregated together (Fig. 5.9C, lanes a to j). It should be pointed out that the fastest band of 19-27 was separated into two bands in the progeny test (cf. Figs 5.9A and 5.9C). The 1BS-controlled gliadin bands of this progeny were shown to be those controlled by the $Gli\text{-}B1_{td}$ genes (Fig. 5.9D, lanes a to j), suggesting that the genes controlling the second and third fastest B subunits of 19-27 were situated nearer to the genes controlling 1BS-ω-γ-gliadin bands of 19-27 than the gene controlling the fastest B subunit of 19-27 (Fig. 5.9C). The band controlled by $Glu\text{-}A3_{edm}$ segregated as expected (Fig. 5.9C, lane h, denoted as o), but it was co-inherited with a gliadin band controlled by $Gli\text{-}A1_{td}$, indicating that a rare recombination must have occurred between the *Gli-A1* and *Glu-A3* loci (Fig. 5.9D, lane e, denoted as o), giving a recombination rate of 1.89 ± 1.87%.

### 5.3.2.5 *Segregation of LMW glutenin subunits and gliadin bands in Langdon $BC_1$ progeny: Langdon x [DTr (4x)]$^2$*

The reason for analysing backcross progeny of this type was to study each 1AS- and

**Figure 5.9** Electrophoretic banding patterns of progeny from three suspected recombinant seeds (Nos 36, 41 and 51) from the test-cross [(Edmore x 19-27) x DTr (4x)], and the controls.

**C** SDS-PAGE patterns of glutenin subunits;

**D** Acid-PAGE patterns of gliadin bands.

($C_1$), Langdon (LMW-A/γ-42 control); (a-j), progeny from test-cross seed No. 51; (k), 19-27; (l), Edmore; ($C_2$), Edmore (LMW-B/γ-45 control).

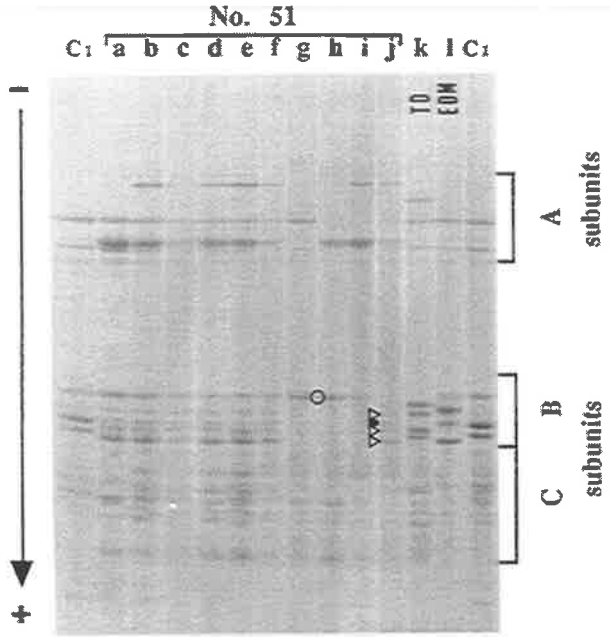
**Symbols used in figures:**

EDM = Edmore; TD = Line 19-27; $C_1$ and $C_2$ are cultivar controls;

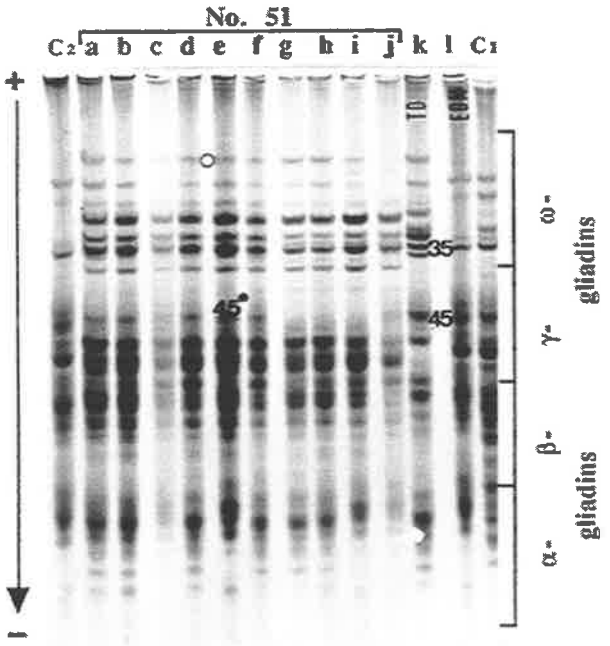
o = recombinant bands controlled by 1AS;

▷ = recombinant bands controlled by 1BS;

Numbers denoted for bands in Acid-PAGE follow the nomenclature of Bushuk and Zillman (1978).


No. 51
C1 a b c d e f g h i j k l C1
TD
EOM
A subunits
B subunits
C subunits

C


No. 51
C2 a b c d e f g h i j k l C1
TD
EOM
35
45
45
ω-gliadins
γ-gliadins
β-gliadins
α-gliadins

D

1BS-controlled block in the reference cultivar without interference of bands from another parent. The glutenin subunit patterns for the $BC_1$ progeny of Langdon x $[DTr\ (4x)]^2$ are shown in Fig.5.10A. Langdon (lanes b and k) had four B subunits with three darkly stained and one faint. It was found earlier that the slowest band appeared to be controlled by 1AS and the others controlled by 1BS (sections 5.3.2.1 and 5.3.2.3). The $BC_1$ progeny clearly showed that two distinct blocks were segregating in Langdon (Fig. 5.10A, lanes f and r, denoted as ● and ▷, respectively). An additional faint band with the same mobility as the fastest dark band of Langdon co-segregated with the slowest 1AS-band (see lanes c, d and f, as examples), implying that this fast band was also controlled by 1AS and formed a block coded by *Glu-$A1_{ldn}$*. This band could only be detected in this BC procedure because it was overlapped by the fastest 1BS-controlled band in other crosses. As expected, the 1AS- and 1BS-controlled bands segregated independently of each other.

When the gliadin bands of the progeny were separated by Acid-PAGE (Fig. 5.10B), with the same sample loading sequence as shown in Fig. 5.10A, two blocks of bands were identified. The 1AS-bands were the two slowest ω-gliadin bands (lane i, denoted as ●) while the 1BS-bands were the typical γ-42 with ω-38 gliadin bands (lane q, denoted as ▷) and these two groups segregated as alternatives (Fig. 5.10B). Only band 38 of the triplet of bands 33-35-38 in Langdon could be scored because the other bands were overlapped with the 1RS-controlled bands in the progeny. Thus, the 1AS- and 1BS-gliadin blocks of Langdon were further confirmed.

### 5.3.2.6 *Segregation of LMW glutenin and gliadin bands in Edmore $BC_1$ progeny: Edmore x $[DTr\ (4x)]^2$*

Similar $BC_1$ seeds as in section 5.3.2.5 were produced with Edmore. It was shown in earlier sections (sections 5.3.2.2A. and 5.3.2.4A) that the B subunits of Edmore appeared to be coded by three functional *Glu-3* loci and not one as suggested by Gupta (1989). In the present backcross progeny, only two different blocks were segregating. All four of the B subunits including the slowest faint band and the second fastest (■) band segregated as a 1BS-controlled block (Fig. 5.11A, lane n, denoted as ▷). However, the slowest and the fastest bands of Edmore were clearly shown to be composed of both 1AS- and 1BS-components (Fig. 5.11A, lanes k and n), except the faster moving faint 1AS-controlled band had slightly less mobility than the fastest band of the 1BS group (lane k, denoted as ●). Obviously, it was difficult to separate both of these 1AS-bands when they were co-segregating with 1BS-bands. It should be noted that sometimes the fastest 1AS-band seemed to be completely absent (lanes l, m and t) and the reason for this was not known. Unlike in the previous analyses (sections 5.3.2.2A and 5.3.2.4A), no recombination was observed in the $BC_1$ between the second fastest (■) band of the four 1BS-bands, and the other three bands. This was because the 1BS chromosome segregated as a linkage block in the $BC_1$ progeny due to the presence of 1RS in place of a normal 1BS in the $F_1$ plant, preventing any homologous pairing and recombination at meiosis.

**Figure 5.10** Electrophoretic banding patterns of backcross progeny Langdon x [DTr (4x)]$^2$, and the controls.

**A** SDS-PAGE patterns of glutenin subunits;

**B** Acid-PAGE patterns of gliadin bands.

(a), Chinese Spring; (b, k), Langdon (LMW-A/γ-42 control); (c-j, m-x), backcross progeny; (l), DTr (4x); (y), Edmore (LMW-B/γ-45 control).

**Symbols used in figures:**

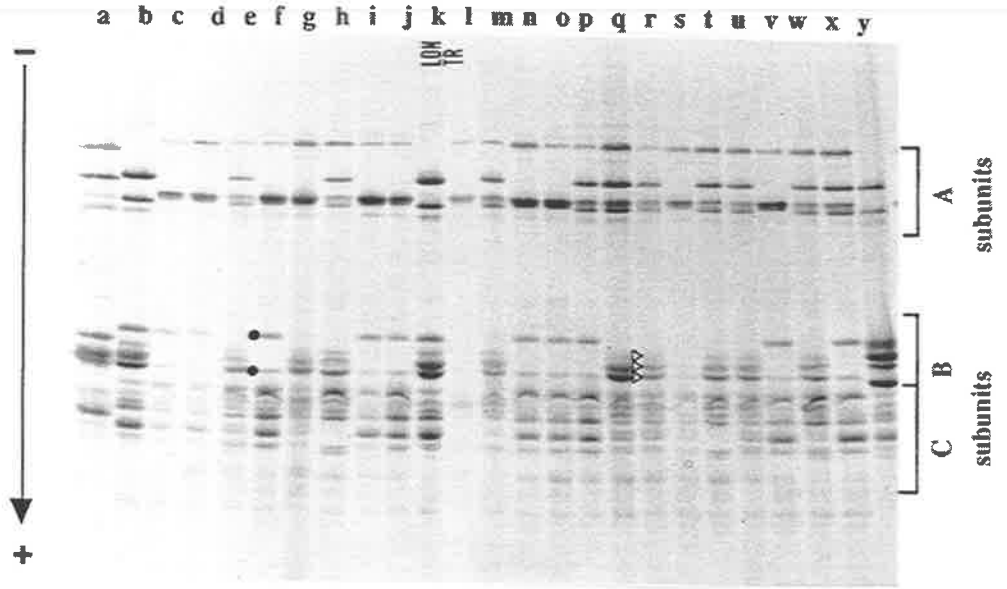
LDN = Langdon; TR = DTr (4x);

• = bands controlled by 1AS;

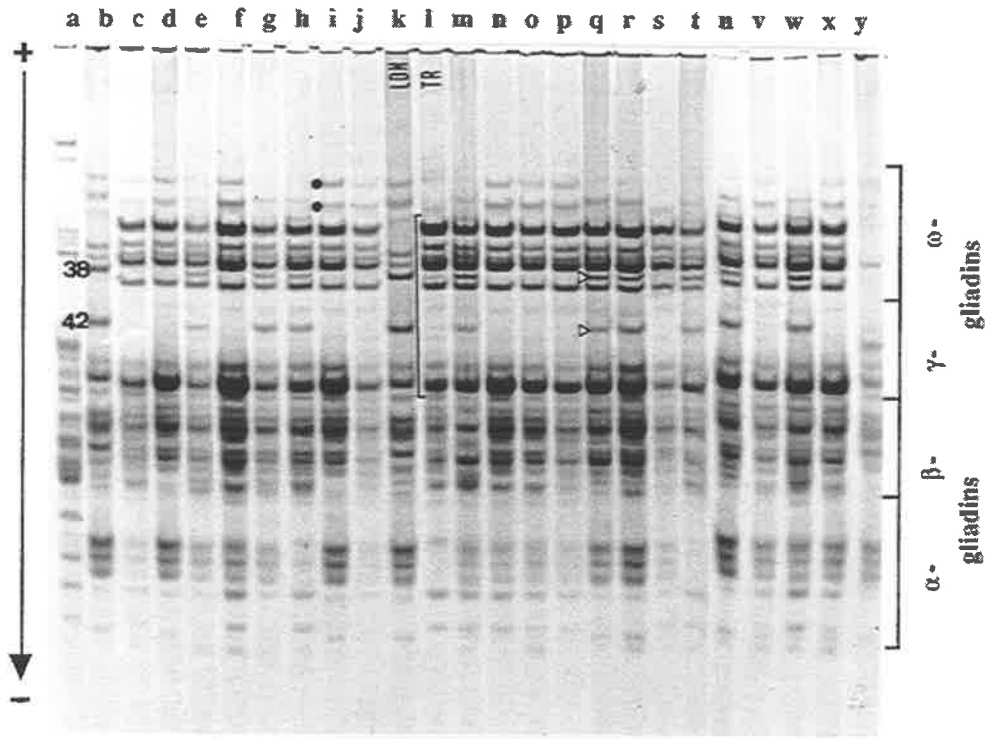
▷ = bands controlled by 1BS;

[ = secalin bands controlled by 1RS;

Numbers denoted for bands in Acid-PAGE follow the nomenclature of Bushuk and Zillman (1978).


a b c d e f g h i j k l mn o p q r s t u v w x y
LOK
TR
A subunits
B
C subunits

A


a b c d e f g h i j k l m n o p q r s t u v w x y
LOK
TR
38
42
ω-gliadins
γ-
β-
α-

B

**Figure 5.11** Electrophoretic banding patterns of backcross progeny Edmore x [DTr (4x)]$^2$, and the controls.

**A** SDS-PAGE patterns of glutenin subunits;

**B** Acid-PAGE patterns of gliadin bands.

(a, g, x), Edmore (LMW-B/γ-45 control); (b-e, h-w), backcross progeny; (f), DTr (4x); (y), Langdon (LMW-A/γ-42 control).

**Symbols used in figures:**

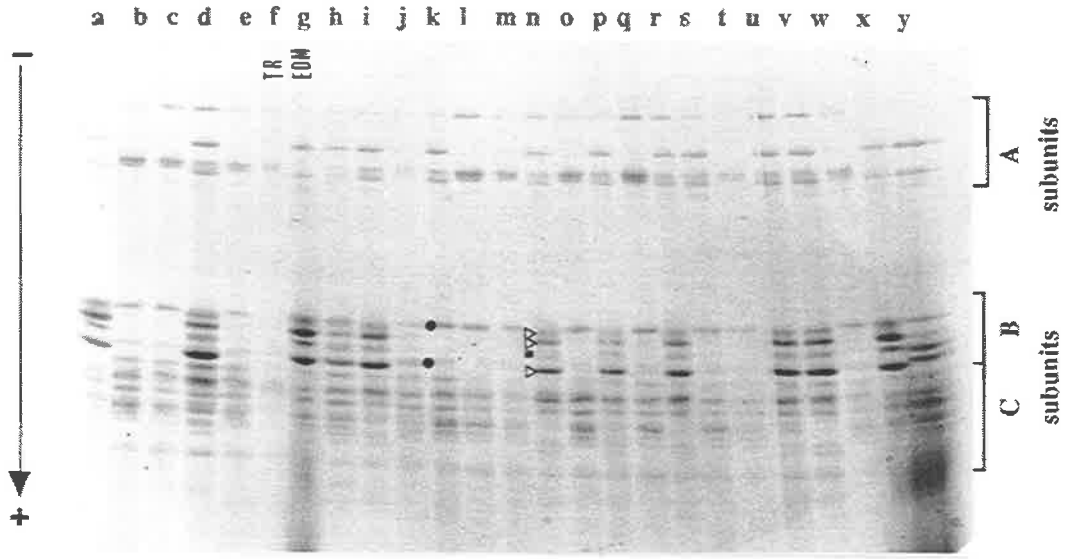
EDM = Edmore; TR = DTr (4x);

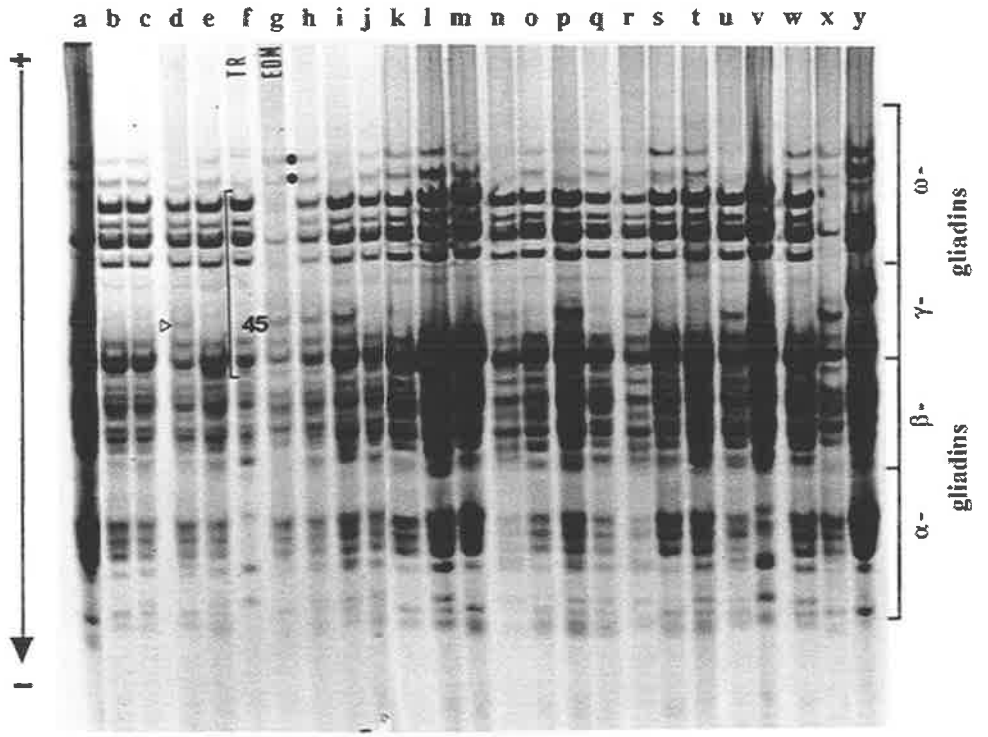
• = bands controlled by 1AS;

▷ = bands controlled by 1BS;

[ = secalin bands controlled by 1RS;

Numbers denoted for bands in Acid-PAGE follow the nomenclature of Bushuk and Zillman (1978).


a b c d e f g h i j k l m n o p q r s t u v w x y
TR
EOM
A subunits
B C subunits
A


a b c d e f g h i j k l m n o p q r s t u v w x y
TR
EOM
45
ω-gliadins
γ-gliadins
β-gliadins
α-gliadins
B

The gliadin band patterns present in Edmore $BC_1$ progeny after separation by Acid-PAGE are shown in Fig. 5.11B. The 1AS-controlled bands were the two slowest ω-gliadin bands (lane h, denoted as ●) while the 1BS-band was the gliadin band γ-45 (Fig. 5.11B, lane d, denoted as ▷). The 1BS-controlled ω-35 band could not be detected because of overlap with the secalin bands in this region. The *Gli-A1* linkage block in Edmore seemed to be identical to that of Langdon (cf. Figs 5.10B and 5.11B), contrary to the different gene designations given by Gupta (1989). Also they had the same 1AS-controlled B subunit bands in SDS-PAGE (cf. Figs 5.10A and 5.11A), supporting this argument.

## 5.4 DISCUSSION

The inheritance of B subunits of glutenin and γ- and ω-gliadin bands of durum and *T. dicoccoides* was clearly demonstrated in the present experiment. The production of the DTr (4x) was based on the previous study of Gupta and Shepherd (1993), who showed that the triple wheat rye translocation stock in hexaploid wheat which did not contain any LMWGS could be used for studying the inheritance of LMWGS. As hybrid seeds from crosses between hexaploid and tetraploid wheats were usually shrivelled and had a low level of germination, a new program was initiated to select a double-rye translocation in durum wheats. Since this stock produced only one faint B subunit of glutenin and therefore contributed virtually no overlapping bands, all the LMWGS from the TC progenies were clearly resolved by SDS-PAGE and identification of each band was unambiguous.

### 5.4.1 Use of test-cross technique

The varieties intercrossed were selected from a survey of many durum wheat cultivars (see Chapter 4) to give $F_1$ hybrids with contrasting LMW glutenin subunit banding patterns. In order to determine the allelic relationships of the genes controlling the LMWGS, two control cultivars i. e. Langdon and Edmore with known LMWGS (Gupta, 1989; Chapter 4), were chosen as one of the parents in each of the inheritance studies.

#### *5.4.1.1 Segregation of the Glu-3 loci*

The B subunit patterns encoded by *Glu-3*, with a few exceptions (see below), were inherited as units or blocks in all four TC experiments, similar to the results observed in hexaploid wheat (Gupta and Shepherd, 1990a, 1990b). The test-cross procedure using the DTr (4x) has allowed the minor differences in mobility of bands controlled by $Glu\text{-}A3_{edm}$ and $Glu\text{-}A3_{bb}$ in section 5.3.2.2A, for example, to be detected. Also the TC approach allowed the similar banding patterns of Coulter and Kharkof-5 to be differentiated by revealing that the fastest band of $Glu\text{-}B3_{clt}$ was slightly slower and could be separated into two bands when the $Glu\text{-}A3_{clt}$ bands were present but not in the case of Kharkof-5 (cf. sections 5.3.2.1 and 5.3.2.3).

The B subunits of durum and bread wheat have been shown to be encoded by a series of closely linked structural genes (Gupta and Shepherd, 1990a and 1990b; Sozinov and Poperelya, 1982, 1988). It appeared that the *Glu-B3* genes generally coded for at least two polypeptides, as there was no case of *Glu-B3* coding for a single band. Similarly, the *Glu-A3* locus, previously thought to code for nil (null), or one or two bands, (Gupta and Shepherd, 1988; Liu and Shepherd, 1991) has been confirmed in the present analysis to code for one or two bands. The exact band blocks could only be detected when they were not overlapping each other in the parents and when there was no overlap of 1AS- and 1BS-coding bands.

With this expected close linkage, it was surprising to detect at least five different recombinants within the B subunit blocks in two of the inheritance studies involving Edmore as a common parent. Rare intra-locus recombinants have been reported earlier for the HMWGS of hexaploid wheats eg. a 5+12 band pair (Pogna *et al*, 1987), but the current experiments gave the first report of such recombination within a locus of LMWGS in tetraploid wheats. All of these recombinants were confirmed by progeny tests and these new combinations of bands segregated normally as new linkage blocks in the subsequent progenies (sections 5.3.2.2B and 5.3.2.4B).

Pooled data from two experiments (sections 5.3.2.2 and 5.3.2.4) gave a calculated value of 3.07 ± 1.35% recombination between the genes controlling the fastest B subunit band and the other major 1BS-controlled bands (Fig. 5.12), suggesting that the fastest B subunit bands studied appeared to be coded by a separate locus away from the normal *Glu-B3* locus. Furthermore, the major *Glu-B3* locus [coding for the slower band(s)] did not recombine with the ω- and γ-gliadin blocks whereas Pogna *et al* (1988, 1990) reported a rare recombination between genes controlling the ω-(33-35-38 vs. 35) and genes controlling the γ-(42 vs. 45) gliadin bands with 2.0 ± 0.8% recombination rate. Hence it was possible that the fastest B subunits of the three parents studied were controlled by genes at another locus, tentatively designated the *Glu-B4* locus (Fig. 5.12).

It was originally thought that there were just two groups of homoeoloci, namely the *Gli-1* and *Glu-3* loci, controlling ω- and γ-gliadins and the major (B and C) LMW glutenin subunits, respectively, on the distal part of the short arms of homoeologous group 1, and the *Gli-2* genes, controlling α- and β-gliadins, on the distal part of the short arms of group 6 chromosomes (Payne *et al*, 1982a, 1984c). The distance between *Glu-1* and *Gli-1* was calculated to be 66 cM (Payne *et al*, 1982a) (Fig. 5.12). However, it now appeared that the number and structure of these loci were more complex. A new locus, designated as *Gli-3* (Payne *et al*, 1988b), has been located approximately midway between the *Glu-1* and *Gli-1* loci on chromosomes 1A and 1B of hexaploid wheats (Galili and Feldman, 1984a; Jackson *et al*, 1985; Payne *et al*, 1986; Sobko, 1984). The *Gli-B3* locus (Payne *et al*, 1988b) [synonymous with the earlier *Gld-B6* (Galili and Feldman, 1984a) and *Glu-B2* (Jackson *et al*, 1985) loci on chromosome 1B, and equivalent to a *Gld-2-1A* locus on chromosome 1A (Sobko, 1984)] was mapped with a genetic distance of 22-28 cM from the *Gli-B1* locus (Galili and Feldman, 1984a; Jackson *et al*, 1985; Metakovsky *et al*, 1986; Dachkevitch *et al*, 1992). Recently, several

other gliadin loci have been detected on chromosome arm 1BS. The *Gli-B5* locus was mapped 1.8 ± 0.4 cM away from the *Gli-B1* locus, located distally in hexaploid wheat (Pogna *et al*, 1993). It was possible that a comparable locus i. e. *Gli-B4* on chromosome 1BS exists, since a *Gli-A4* locus was already mapped 10 cM from the *Gli-A1* locus, located between the *Gli-A1* and *Gli-A3* loci (Redaelli *et al*, 1992). These newly discovered loci were comparable to a former report by Metakovsky *et al* (1986) who detected three loci on chromosome 1AS with 13 ± 3%, 5 ± 1% and 1% recombination among the ω-gliadin bands of hexaploid wheat.

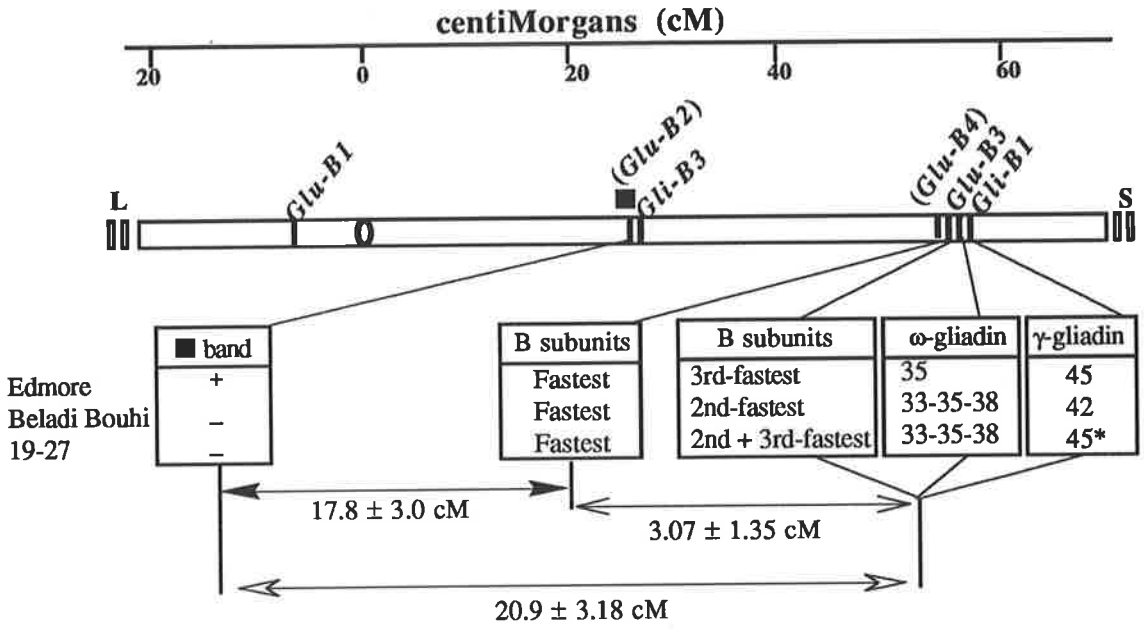



**Figure 5.12** Location of the genes controlling storage protein on chromosome 1B of wheat (Lawrence and Shepherd, 1981; Payne *et al*, 1982a; Singh and Shepherd, 1988a; Pogna *et al*, 1990). Because recombination frequency was affected by environmental factors and by the genetic background of the heterozygous parents, the values given should not be regarded as absolute. However, the relative distances between loci were considered to be reliable.

**Symbols used in figure:** Glu = glutenin, Gli = gliadin, S = Short arm, L = long arm.

A unique feature observed in the current inheritance studies here was that the B subunits of Edmore segregated into three groups rather than the expected two. One of the interesting findings was that from the recombination behaviour of the B subunit in Edmore, the genes controlling the second fastest (■) band of Edmore were apparently located in similar region as the *Gli-B3* locus, since it recombined with the major 1BS-controlled bands [i. e. the slower B subunit(s) and ω- and γ-gliadin blocks] with about 21% recombination rate, suggesting the presence of a previously unidentified locus on chromosome 1BS (see Fig. 5.12 and sections 5.3.2.2A and 5.3.2.4A). Estimates of the distance between the genes coding for the ■ band and the 1BS-coded faster-moving B subunits was 17.8 ± 3.0 cM, and the 1BS-coded slower-

moving B subunits was 20.9 ± 3.2 cM, in crosses between Edmore and Beladi Bouhi and 19-27. Clearly further investigations are required, since formerly the *Gli-B3* locus was reported to code for ω-gliadins (eg. Galili and Feldman, 1984a), or D-subunits of glutenin (eg. Jackson *et al*, 1985), whereas in the present study, it seemed to code for a B subunit of glutenin. Because this band appeared to have aggregating ability and biochemically similar to glutenin, its locus was tentatively given the previous *Glu-B2* designation (Jackson *et al*, 1985).

In the light of these inheritance studies, the extensive variation detected in Chapter 4 could be interpreted more easily, if the B subunits were in fact controlled by at least three different loci i. e. *Glu-3*, *Glu-2* and *Glu-4*. For example, the γ-45 and γ-47 types were associated with more than 20 different '*Glu-B3* linkage blocks' deduced previously (Figs 4.1A to 4.1C), which consisted of the third and fourth fastest bands of Edmore, presumed to be controlled by the *Glu-B3* locus. The many differences observed in the former *Glu-B3* linkage blocks were either due to changes to the fastest band(s), which might be controlled by different alleles at the *Glu-B4* locus; or differences in the middle bands, which might be controlled by different alleles at the *Glu-B2* locus. This also applied to the γ-40- and γ-42-associated LMWGS patterns, which also had a 'common' *Glu-B3* block (i. e. *Glu-B3a'*). The reason that γ-40 vs. γ-42, and γ-45 vs. γ-47 possessed similar respective *Glu-B3* blocks might be due to their similarity in ω-γ-gliadin-LMWGS block structures. Nevertheless, this was just an indication of the need to perform more work, to investigate this problem. This can be done with detailed inheritance studies on the linkage blocks in several variants recorded in Chapter 4. More work is required on the biochemical properties and molecular relationship of the bands controlled by these different loci in this region of chromosome 1BS.

### *5.4.1.2 Segregation of the Gli-1 loci*

The purpose of studying the segregation of the gliadin bands was to monitor the co-inheritance of these bands with the B subunit of glutenin and to look for rare recombination between the *Glu-3* and *Gli-1* loci. It was found that all the ω- and γ-gliadin patterns were inherited as blocks or clusters as found in previous inheritance studies in both hexaploid and durum wheats (Singh and Shepherd, 1988a; Sozinov and Poperelya, 1982, 1988; Pogna *et al*, 1990). For example the γ-gliadin band 42 was known to form a block with ω-33-35-38 bands and γ-45 with ω-35 (Pogna *et al*, 1988, 1990), controlled by *Gli-B1* genes on chromosome 1BS. There were also some new blocks identified such as the 1BS-coding bands of the *T. dicoccoides* line 19-27. The first rare recombination event was reported by Pogna *et al* (1988) when it was found that gliadin band γ-42 must have recombined with ω-35 giving rise to the γ-42-ω-35-LMW-2 phenotype in the Italian durum wheat cv. Berillo. They constructed a genetic map showing the locus sequence (LMW-ω-gliadin)-γ-gliadin with a map distance 2.0 ± 0.8 cM between the genes controlling the ω-35-gliadin-B-glutenin subunit and genes controlling the γ-gliadin bands (Pogna *et al*, 1990). However, all the *Gli-B1* blocks studied had remained intact in the present study. That was, the rare recombination observed between different B subunits did not change these gliadin blocks, further suggesting that these

structural genes were more tightly linked and more stable than the B subunit blocks, which now appeared to be coded by at least three separate loci i. e. *Glu-B3*, *Glu-B2* and *Glu-B4*. It was difficult to detect recombination in the 1AS-block, because of the large number of secalin bands in the TC plant which overlapped the segregating bands. Variation at the *Gli-2* loci on homoeologous group 6 chromosomes was not investigated.

#### *5.4.1.3 Recombination between the Glu-3 and Gli-1 loci*

The co-inheritance of the ω- and γ-gliadin patterns with the major B subunits was consistent with the findings of previous work (Payne *et al*, 1984c; Singh and Shepherd 1988a; Gupta and Shepherd 1990a, 1990b) indicating that genes encoded by the *Glu-3* and *Gli-1* loci were closely linked. Most segregating populations failed to yield any recombination between these two loci.

A rare recombination event was reported in hexaploid wheat between *Gli-B1* and *Glu-B3* loci with a recombination rate of 1.7% (Singh and Shepherd, 1988a) whereas another recombination recorded in durum cv. Berillo by Pogna *et al* (1988) had in fact occurred between the ω- and γ-gliadin components. In the present experiment, the TC progeny populations were small, ranging from 55 to 121 seeds and two such rare recombinants were observed between *Glu-A3* and *Gli-A1* loci at a recombination rate of 3.78%, higher than the recombination rate reported above for chromosome 1BS in bread wheat.

### 5.4.2 Value of the backcross procedure

DTr (4x) was used as the recurrent parent in backcrosses to study the inheritance of band blocks within a cultivar. Because of the virtual absence of major B subunit bands in DTr (4x) stock, the structure of the actual band blocks could be determined more clearly since segregation in the $BC_1$ progeny would give 1/4 of the progeny with either a 1AS- or 1BS-block alone and the other 1/2 would have both blocks. In such circumstances, the major bands in a block would segregate without any band overlap from the other block (i. e. 1AS- or 1BS-bands). Thus, some new bands, especially those having the same or similar mobility with other major bands could be detected in this system (sections 5.3.2.5 and 5.3.2.6). This information, provided a more precise interpretation of the banding patterns in progeny where segregation for several loci was occurring simultaneously.

With Langdon the results obtained were similar to that obtained in previous work (Gupta, 1989) except an extra band coded by 1AS was detected (see section 5.3.2.5). With Edmore, this approach revealed some important new information. It had been reported earlier from comparing the patterns of two pairs of disomic substitution lines, which could not detect the overlapping bands, that all the B subunits of Edmore were controlled by 1BS and that the *Glu-A3* allele was a null type not coding for any B subunit (Gupta, 1989). However, the backcross analysis showed that the band patterns in Edmore could be grouped into 1A and 1B blocks. The slowest band was clearly shown to be composed of overlapping 1AS- and 1BS-

components (section 5.3.2.6). All of the B subunits including the slowest band with reduced intensity segregated as a block. Also, a new 1AS-controlled component with slightly less mobility than the fastest B subunit band was detected with the backcross procedure. These components were also observed in two separate progeny tests earlier (Figs 5.6A and 5.9A).

It should be noted that the selfing of test-cross seeds obtained from using the DTr (4x) stock was also functionally similar to the backcross procedure, since the 1RS chromosome was present in these progenies in heterozygous state. One fourth of the $F_2$ progeny would either have the 1AS- or 1BS-block alone. Examples of this were found with the two normal TC progenies in Fig. 5.6A (lanes a to d, e to h, respectively) in section 5.3.2.2. The drawback of this $BC_1$ procedure for inheritance studies was that rare recombination between closely linked genes would never occur because the short arms of group 1 chromosomes in wheat could not pair with the 1RS chromosome arms in the $F_1$ hybrid. Therefore, all genes located on the short arm would segregate as one unit, as showing the different inheritance behaviour of the ■ band of Edmore in the TC and BC procedures (sections 5.3.2.2A, 5.3.2.4A, 5.3.2.5 and 5.3.2.6).

In general, the current results were consistent with previous reports on recombination between genes controlling γ- and ω-gliadins and LMW glutenin subunits in durum (Pogna *et al* 1988, 1990) as well as in bread wheat (Sozinov and Poperelya 1980; Payne *et al* 1984c; Singh and Shepherd 1988a; Gupta and Shepherd 1990a, 1990b). This study has demonstrated clearly that all B subunits in tetraploid wheats were controlled by genes on chromosomes 1AS and 1BS, and the tight linkage between the *Gli-1* and *Glu-3* was further confirmed. In addition, some new information was provided on rare recombination within the *Glu-3* blocks. The tight linkage between gliadins and LMW glutenin subunit genes on group 1 chromosomes supported the hypothesis that they have evolved from the same ancestral gene through duplication and divergence (Kreis *et al*, 1985 for a review; Bartels *et al*, 1986; Colot *et al*, 1989). In addition, they were known to share very high homology at the amino acid and nucleotide sequence levels as well. However, the ancestral genes have shown much divergence at genomic level. For example, the genes on chromosome 1BS always synthesised the predominant protein amounts (either gliadin, or LMW glutenin subunits) whereas the genes on chromosome 1AS produced relatively less as found in the present study. This also applied to other prolamins in hexaploid as well as tetraploid wheats (Lawrence and Shepherd, 1980; Payne *et al*, 1980, 1984d; Payne, 1987c; Singh and Shepherd, 1988a, 1988b; Gupta and Shepherd, 1990a, 1990b; Sozinov and Poperelya, 1982, 1988), suggesting that the A and B genomes have been subjected to quite different evolutionary environments and selective processes.

Although many differences were observed in the C subunits of glutenin, they were not analysed in the present study mainly due to the unavailibility of a proper tester parent and the seemingly complicated genetic control and expression of these bands. Genes at the *Glu-3* locus have been partly implicated in their formation and genes on group 6 chromosomes also seemed to contribute to these patterns (Payne, 1987c; Gupta and Shepherd, 1993). Much

further work is required on these proteins but a prerequisite would be the development of new methods of extraction and separation which give reproducible patterns.

The value of knowledge on the genetic control of LMWGS related to the allelic variation in both gliadin and LMW glutenin subunits and association with differences in flour quality (Sozinov and Poperelya, 1980; Gupta *et al*, 1989, 1990; Metakovsky *et al*, 1990; Pogna *et al*, 1990). The LMW subunits appeared to have a cause-effect relationship with flour quality (Pogna *et al*, 1990) and the association of gliadin bands with quality could be ascribed by the tight linkage between the *Gli-1* and *Glu-3* loci. However, the *Gli-1* genes could still be used as genetic markers selecting for appropriate *Glu-3* genes in breeding programs, since these markers were much easier to screen for in a breeding program than the LMW genes.

# CHAPTER 6. COMPARATIVE GRAIN YIELD AND QUALITY CHARACTERISTICS OF DURUM AND BREAD WHEATS

## 6.1 INTRODUCTION

Genetic studies using electrophoretic analysis of aneuploid stocks have now demonstrated that chromosomes 1A, 1B, and 1D, carry genes coding the majority of the glutenin subunits (Lawrence and Shepherd, 1980) and other inheritance studies have shown that these subunits have a large impact on the bread-making quality of bread wheats (Payne *et al*, 1980, 1984b). The association between HMW glutenin subunits and quality characteristics of durums was not as strong as that shown in the hexaploids, although some reports indicated that certain HMW alleles influenced the viscoelastic properties of durum doughs (du Cros, 1987a; Boggini and Pogna, 1989; Pogna *et al*, 1990). The association between γ-gliadin bands and dough strength in durums (Damidaux *et al*, 1978; Pogna *et al*, 1987; du Cros *et al*, 1982) has been shown to be due to their linkage to different LMW glutenin alleles, which were directly responsible for variation in gluten strength (Payne *et al*, 1984c; Pogna *et al*, 1988; Boggini and Pogna, 1989).

Most research has been concentrated on the pasta-making aspects of durum wheat and investigations on the baking quality of durums have been rare (Boggini and Pogna, 1989). Boggini and Pogna (1989) pointed out that a durum possessing both satisfactory bread-making characteristics and acceptable pasta-making quality would be a desirable goal for breeders. Hence the first part of this chapter compares the dough strengths and yields of a large set of durums from a world collection, including some Australian advanced lines, with bread wheats. The association of the gliadin and glutenin banding patterns of these durums with the quality characters has been studied as well.

Traditionally, durum wheat has been regarded as being unsuitable for bread-making due to its poor gluten strength, attributed either to the absence of the D-genome chromosomes (Morris *et al*, 1966; Konzak, 1977; Payne, *et al*, 1988c) or to the almost complete absence of *Glu-A1* alleles (du Cros, 1987a; Boggini and Pogna, 1989). Joppa and Williams (1988) produced a complete set of D-genome disomic-substitution lines by crossing the Chinese Spring (CS) nullisomic-tetrasomic stocks with the durum cv. Langdon. Two intervarietal substitution lines Langdon (Edmore-1B) and Langdon (Kharkof-5-1B) were developed from cultivars Edmore and Kharkof-5 by using the Ldn 1D(1B) line (Joppa *et al*, 1983a) and these two lines were found to have greater gluten strength than the recurrent parent but similar in strength to the 1B donor parents (Joppa *et al*, 1983b). However, the technological qualities of this whole set of individual D-genome substitutions for their A and B homoeologous chromosomes in tetraploids have not been studied in detail. Hence, in the latter part of this chapter, the set of D-genome disomic substitution lines in Langdon from CS, and the two

durum intervarietal substitution lines mentioned above, were used to evaluate the effect of these chromosomes on quality under South Australian growing conditions.

## 6.2 MATERIALS AND METHODS

### 6.2.1 Plant materials and field experiment 1

Eighty durum lines chosen from different countries (including eight advanced lines from the Australian durum breeding program), believed to have diverse quality range, were kindly provided by Dr A. J. Rathjen of the Plant Science Department, Waite Institute. All of these lines had been analysed electrophoretically for their gliadin and glutenin compositions previously (see Chapter 4). Eight hexaploid $F_5$ lines (*Triticum aestivum*) having different HMW and LMW glutenin subunits from a cross between {[(Mexico 120 x Koda) x Raven]/111/8 x Kite} (Gupta *et al*, 1989) and two parents (Kite and Line 111/8) were grown as controls.

These lines were sown in two replicated randomised-block designs at normal sowing rate and plot size (four rows, 4.3 x 0.75 $m^2$/plot) in two locations (Roseworthy Agricultural College - RAC and Saddleworth, South Australia). The agronomic practices including seeding times and rates, fertilizer and herbicide applications were typical of those normally adopted by wheat growers in the areas. The trials were harvested and the grain yield measured after the samples had been re-cleaned to remove chaff and rachis debris.

### 6.2.2 Plant materials and field experiment 2

Joppa and Williams (1988) produced a complete set of D-genome disomic-substitution lines by crossing the CS nullisomic-tetrasomic stocks with the durum Langdon. Each of the $F_1$s with different D-genome chromosomes was backcrossed to Langdon at least five times to ensure the homogeneity for the other 13 chromosomes. Thus Langdon 1D(1A) had the pair of 1D chromosomes from CS replacing the 1A pair while other 13 pairs of chromosomes were from Langdon. [Note: The designation of chromosomes 4A and 4B was changed in 1989 to 4B and 4A, respectively (7th Int. Wheat Genetics Symp., Miller, T. E. and Koebner, R. M. D. eds) and the new system is used in the present study] Two intervarietal substitution lines Langdon (Edmore-1B) and Langdon (Kharkof-5-1B) were also developed by using the Ldn 1D(1B) line to substitute pairs of 1B chromosomes from cultivars Edmore and Kharkof-5 into Langdon (Joppa *et al*, 1983b).

All these durum lines i. e. Langdon (Ldn), Edmore, Langdon intervarietal substitution lines - Ldn (Edmore-1B) and Ldn (Kharkof-5-1B), and the whole set of Langdon D-genome disomic-substitution lines [i. e. Ldn 1D(1A), Ldn 1D(1B) to Ldn 7D(7B)] were kindly provided by Dr L. R. Joppa, North Dakota State University, Fargo, USA. All of these lines were multiplied under quarantine at the Waite Institute. The control cultivars with varying gluten strengths, Stewart, Vic, Coulter and Yallaroi were supplied by Mr M. Mackay, AWCC,

Tamworth, New South Wales. The prolamin compositions of these lines were presented in Table 6.7.

These lines were grown in the field at the Waite Institute along with the parents and control cultivars. The materials were sown in a randomised-block design with two replicates at normal sowing rate in double-rowed plots (4.3 m x 0.5 m).

## 6.2.3 Analytical methods

### *6.2.3.1 Electrophoresis*

Half seed or 20 mg flour samples from each line used in the experiment were analysed for gliadin and glutenin subunits by electrophoretic procedures (Acid-PAGE, SDS-PAGE) as described previously (see Chapter 3).

### *6.2.3.2 Milling*

#### (A) Experiment 1

The grain moisture content ($m_o$%) was measured on grain ground in a coffee grinder (ESM DIE KRONE) from representative samples by using a Moisture Meter (TF933A, Marconi Instruments Ltd, England). Cleaned sub-samples of each plot (200-300 g) were tempered to 16.5% in a sealed plastic bag overnight before being milled on a Brabender Quadrumat Junior Mill equipped with a 1.0 mm screen. The flour extraction rate averaged 62% with a range of 41.6 to 71.5%.

The water (Ha in ml) required to bring the sample $M_o$ (in g) to 16.5% moisture content ($m_s$ = 16.5%) was calculated by the following formula:

$$Ha = M_o * \frac{m_s - m_o}{100}$$

#### (B) Experiment 2

Wholemeal samples (20-30 g) were prepared by grinding the grain in a hammer mill (Falling Number 3100) equipped with a 0.8 mm screen. No tempering was involved.

### *6.2.3.3 Quality tests*

#### (A) For field experiment 1

The total protein concentration (%) of the whole grain samples was measured by the micro-Kjeldahl method. Twenty seed samples including a control and a blank were accurately weighed (0.9900 to 1.0100g) and digested with concentrated $H_2SO_4$ for 45 mins on a pre-programmed heat-block. After digestion, 50 mls of water were added and the sample measured for nitrogen concentration on a Kjeltec Auto 1030 Analyzer (Tecator A B, Sweden).

The average seed moisture content for every batch of samples was calibrated by testing another four of the samples (0.9900 to 1.0100 g) in each batch, measured by calculating differences in weight before and after the oven drying.

$$\text{Moisture content \%} = \frac{\text{Pre-drying wt. - After drying wt.}}{\text{Pre-drying wt.}} * 100$$

The seed protein concentration (in %) was expressed on a 16.5% moisture basis by the following formula:

$$\text{Protein concentration \%} = \frac{\text{Kjeltec Analyzer reading}}{\text{Sample wt.} * \text{Moisture content}} * \frac{100\text{-}16.5}{100} * 100$$

The SDS-sedimentation test was performed to measure the gluten strength following the procedure of Axford *et al* (1979) with a control sample in every batch of ten. Five grams flour samples were mixed with 50 mls of water in a 100 mls measuring cylinder. The material was dispersed by rapid shaking for 15 seconds at 0 min, 2 mins and 4 mins after mixing. Immediately after the last shaking, 50 mls of the SDS-lactic acid reagent {20g SDS/l, (lauryl sulfate, Sigma) and 20 mls/l lactic acid solution [88% AnalaR grade lactic acid : water = 1 : 8 (v/v)]} were added and mixed by inverting the cylinder four times. The material was inverted four times again at 2, 4 and 6 mins.

## (B) For field experiment 2

Total protein concentration (%) and grain hardness index (HI) of the samples were determined by Near-infrared Reflectance Spectroscopy - NIRS (Technicon Infra-Alyzer 450) calibrated specifically for durum wheat at the Cereals Laboratory at the South Australian Research and Development Institute. The wholemeal colour was measured by a Minolta Chromameter CR-200 and expressed in absolute values of whiteness and yellowness of the wholemeal samples.

The rheological properties were determined with a two-gram mixograph (Fig. 6.1) using the method of Gras and O'Brien (1992) with modifications described below. The actual weight of sample used ($M_2$ in g) was calculated according to the following formula:

$$M_2\ (g) = \frac{M_1 * (100\text{-}m_1)}{(100\text{-}m_2)}$$

Where $m_1$ and $M_1$ were the standard moisture content (14%) and sample amount (2.0 g), respectively. $m_2$ refers to the measured moisture content of the sample.

The amount of water (W in ml) added in the mixograph was determined by the moisture content and flour protein concentration of the sample as well as seed kernel hardness.

$$W\ (ml) = W_1 + W_2$$

$W_1$ was the amount of water (ml) added to adjust the sample moisture content to the set point, as determined by the following function,

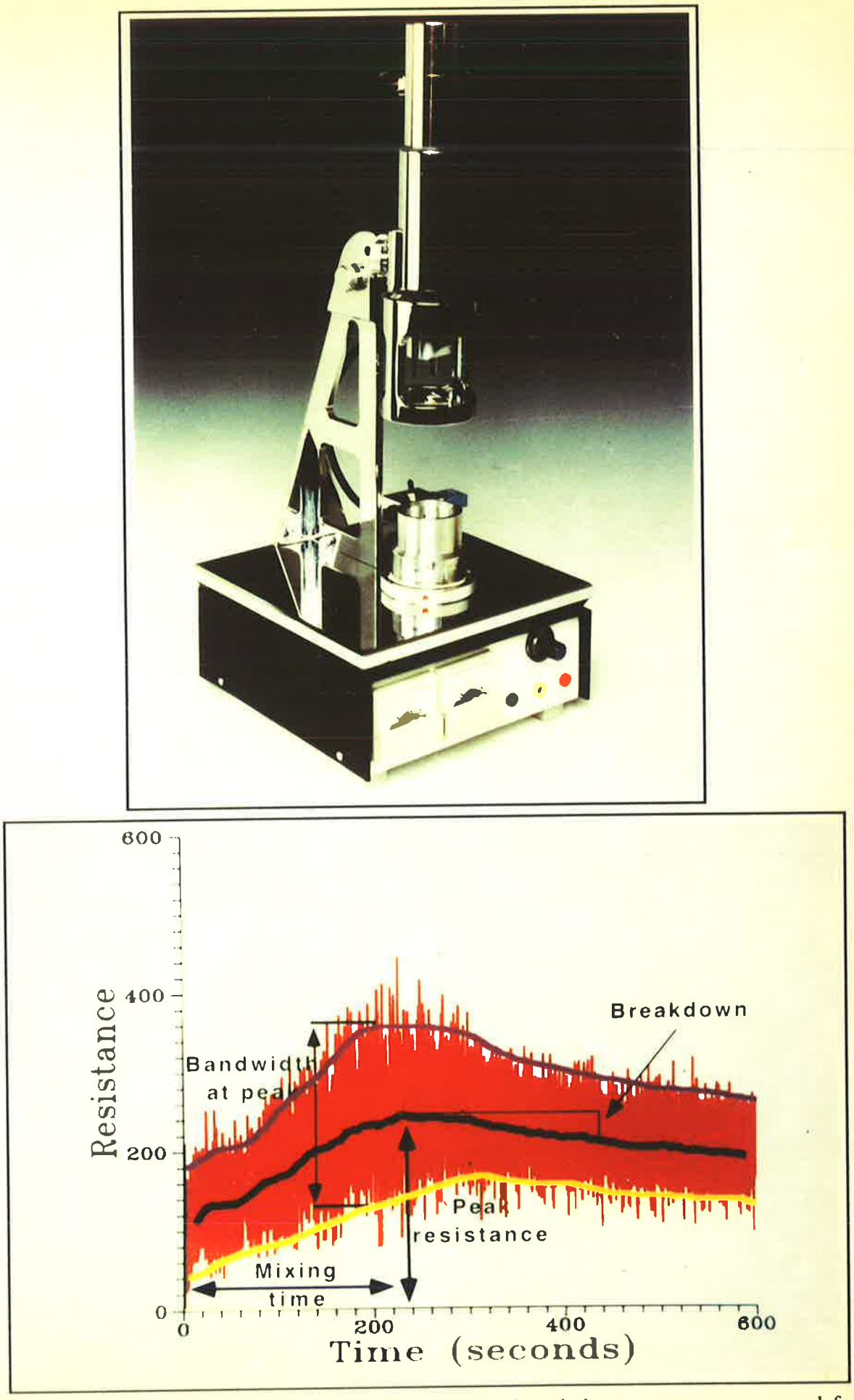



**Figure 6.1** Diagram showing the apparatus (top) and the parameters measured from a mixogram trace (bottom) of two-gram mixograph.

$$W_1 \text{ (ml)} = M_1 - M_2$$

$W_2$ was the amount of water (ml) added dependent on the sample protein concentration and grain hardness, as determined by a modified AACC method (54-40A):

$$W_2 \text{ (ml)} = 2 * \frac{(A \times PC + B)}{100}$$

Where A depends on grain hardness, for durums, A = 2.025;

PC was the protein concentration and B accounts for differences in experimental conditions (41.15 for the two-gram mixer).

Parameters (Fig. 6.1) measured from the mixograph curve were:

i. time (secs) to reach the maximum height (mix time - MT),
ii. height at peak resistance (PR) in mixograph Brabender units,
iii. differences of height at PR and 3 minutes later - resistance breakdown (RBD),
iv. differences of bandwidth at PR and 3 minutes later - bandwidth breakdown (BBD),
v. time (secs) to reach peak bandwidth (TPBW), and
vi. peak bandwidth (PBW) in mixograph Brabender units.

The SE-HPLC (Waters WISP 710B liquid chromatography) was used to measure quantitatively the molecular weight distribution of the total endosperm proteins following the procedure of Singh *et al* (1990a) modified by Batey *et al* (1991). To extract the total endosperm proteins, ten mg meals were extracted with an SDS phosphorous buffer (50 mM $Na_2HPO_4$ and 50 mM $NaH_2PO_4$ in 2% SDS solution, pH 6.8) with sonication. The extracted protein supernatants were filtered (0.45 µm, Millex-$HV_{13}$, Millipore) and placed in a WISP 712 automatic sampler. Samples (20 µls) were injected automatically by the automatic sampler and the columns were eluted isocratically by 0.1 % trifluoroacetic acid in 50% acetonitrile (pH 6.9) at the flow rate of 0.5 mls/min at 26°C. Eluent was delivered by a model 510 pump and separation was achieved with a Waters Protein-Pak™ 200 SW size-exclusion analytical column (7.5 x 300 mm), effective in separating total endosperm proteins over a large range of molecular weight (from 10,000 to one million Dalton).

The major peaks, referred to as $P_1$, $P_2$ and $P_3$, were eluted between 9 and 19 min as detected by a Lambda-Max Model 481 Spectrophotometer at a wavelength of 214 nm (Fig. 6.2). Peak one ($P_1$) mainly consisted of glutenins, peak two ($P_2$) of gliadins and peak three ($P_3$) of non-prolamins (Singh *et al*, 1990a). The peak area was measured by a Waters software package in Digital Professional 350 computer. It was observed that $P_1$ could be divided into two adjacent peaks (Fig. 6.2), corresponding to the highly-aggregated and intermediate-aggregated glutenin fractions. The terms $P_{1\text{-}1}$ and $P_{1\text{-}2}$ were used to represent these.

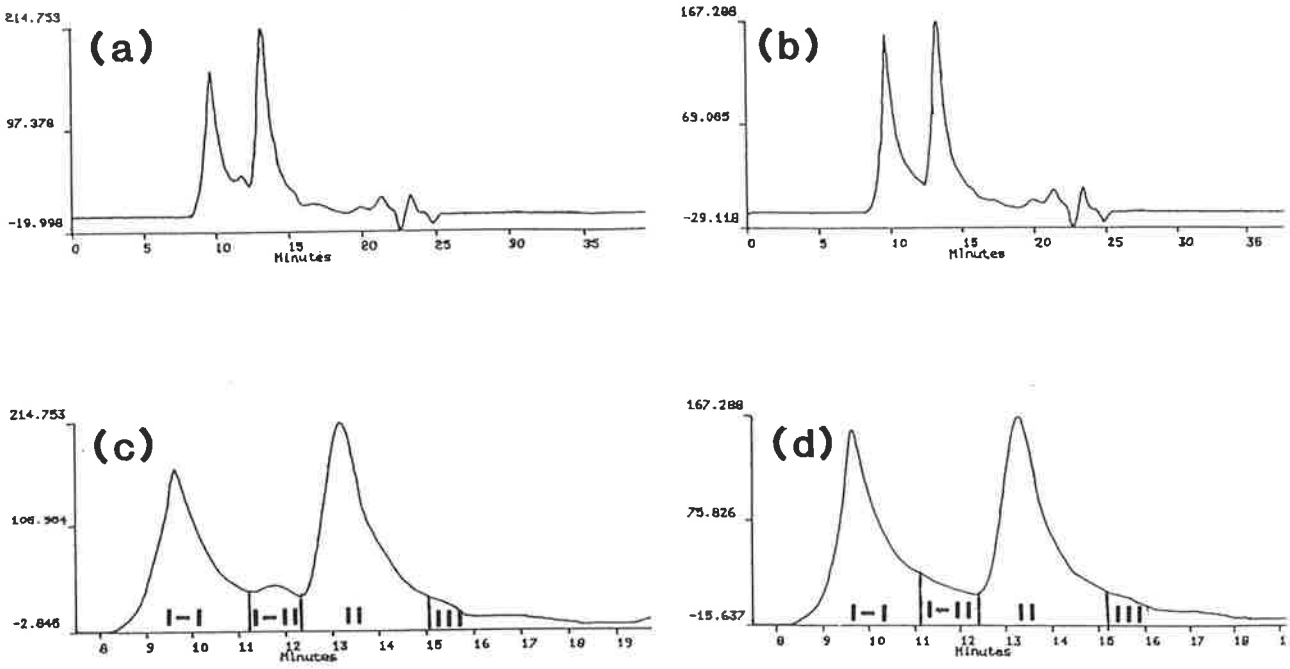



**Figure 6.2** The size-exclusion high performance liquid chromatography (SE-HPLC) traces of a poor (a) and a better (b) quality durum wheat and their respective expanded traces (c and d).

It is clear that the first glutenin peak is separated into two peaks (i. e. I-I and I-II) in poor quality eluents (a and c) but it resolved into a shoulder in the better quality durum wheat samples.

When wholemeal samples were used in the SDS-sedimentation test, six grams of samples were taken instead of five grams and analysed as described before (section 6.2.3.3A). Fig. 6.3 shows the SDS-sedimentation values of different Langdon D-genome disomic substitution lines and controls.

#### *6.2.3.4 Statistical analysis*

The Genstat 5 software on DEC station 5000/240 was used for Analysis of Variance and Principal Components Analysis (PCA), and the Macintosh Statview (Version 4.02) and Super ANOVA (2v1.11) programs were also employed to analyse the experimental data.

## 6.3 RESULTS

### 6.3.1 Varietal survey

#### *6.3.1.1 Yield and quality range of genotypes and influence of growing locations*

Analysis of variance showed that the grain yield per plot, protein concentration, SDS-sedimentation (SDSS) values and protein yield per plot (= grain yield x protein concentration) were all highly significantly different between the lines studied (Table 6.1). However, SDSS and protein yield values were not significant in different sites, suggesting that these quality parameters were mainly genotype dependent. There were also highly significant differences for lines*sites interactions, indicating that these agronomic and quality factors were different in different growing locations. Hence, further analyses was conducted separately for each site.

**Table 6.1** Analysis of variance of the yield and quality data of the 90 wheat lines from two sites (RAC and Saddleworth)[a]

| Source of variance | df | Yield | Protein concentration | SDSS values | Protein yield |
|---|---|---|---|---|---|
| Site | 1 | 18.8* | 517.5* | 3863.5 n.s. | 29808.3 n.s |
| Lines | 89 | 0.2*** | 6.4*** | 713.5*** | 426.3*** |
| Lines x Sites | 89 | 0.1*** | 0.94** | 83.3*** | 194.5** |
| Residues | 178 | 0.03 | 0.6 | 28.5 | 119.2 |

[a] = Values given were mean squares. *, **, *** and **** were significant at a 5%, 1%, 0.1% and 0.01% probability level.

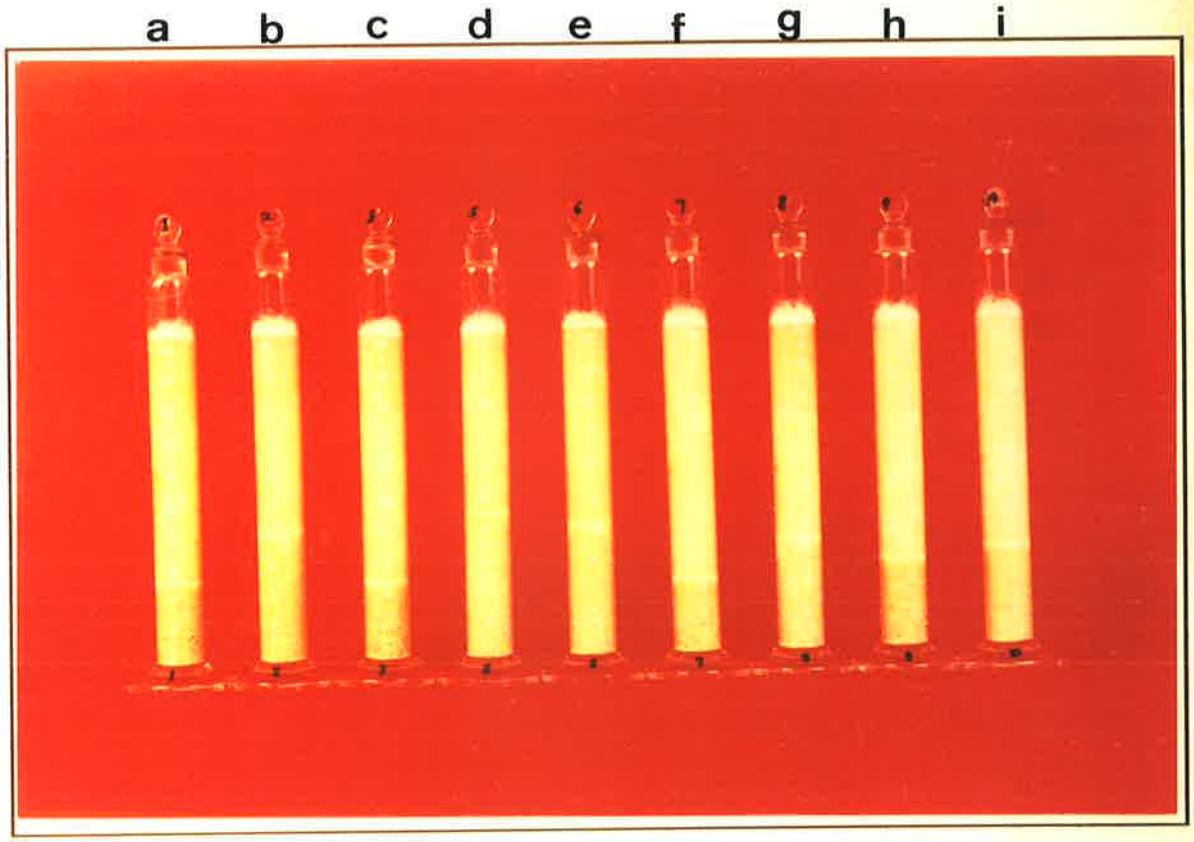



**Figure 6.3** SDS sedimentation test of different durum Langdon D-genome disomic substitution lines and controls.

(a), Langdon 2D(2A); (b), Yallaroi; (c), Langdon; (d), Langdon 1D(1A); (e), Langdon 1D(1B); (f), Langdon; (g), Langdon 5D(5A); (h), Langdon 6D(6B); (i), Langdon 7D(7A).

### 6.3.1.2 *Distribution of flour protein concentration, plot yield, SDS-sedimentation (SDSS) volumes and protein yield between durum and bread wheats, and correlation among these parameters*

There was a wide range of protein concentration variation among the 79 durum and 11 bread wheat lines (see next paragraph) (Table 6.2 and Fig. 6.4). The protein concentration of the durum wheats ranged from 9.7% to 18.1% with an average of 14.0%, while the protein percentage of bread wheats ranged from 9.5 to 14.8% with an average of 11.9%.

**Table 6.2** Ranges and means of the grain yield (g), protein concentration, SDSS values and protein yield of the 90 wheat lines grown at RAC and Saddleworth (SDW) in two replications

| Traits | Type | No. of samples | Site | Mean | Range | SD[a] | SE[a] |
|---|---|---|---|---|---|---|---|
| Grain protein concentration (%) | Durum | 158 | RAC | 15.2 | 10.5 - 18.0 | 1.40 | 0.11 |
| | | 158 | SDW | 12.7 | 9.7 - 18.1 | 1.61 | 0.13 |
| | Hexaploid | 22 | RAC | 12.8 | 11.2 - 14.8 | 0.71 | 0.15 |
| | | 22 | SDW | 10.9 | 9.5 - 12.3 | 0.69 | 0.15 |
| Grain yield (g) | Durum | 158 | RAC | 373 | 139 - 615 | 104 | 8.2 |
| | | 158 | SDW | 597 | 263 - 943 | 142 | 11.3 |
| | Hexaploid | 22 | RAC | 547 | 436 - 706 | 68.3 | 14.6 |
| | | 22 | SDW | 670 | 531 - 863 | 91.0 | 19.4 |
| SDSS values (mls) | Durum | 158 | RAC | 48.4 | 25.0 - 80.0 | 12.05 | 0.96 |
| | | 158 | SDW | 43.2 | 15.0 - 67.0 | 9.07 | 0.72 |
| | Hexaploid | 22 | RAC | 84.4 | 64.5 - 94.0 | 8.61 | 1.84 |
| | | 22 | SDW | 68.0 | 45.0 - 90.0 | 11.27 | 2.40 |
| Protein yield (g) | Durum | 158 | RAC | 55.8 | 24.6 - 87.9 | 13.49 | 1.07 |
| | | 158 | SDW | 76.1 | 31.8 - 123.5 | 16.83 | 1.34 |
| | Hexaploid | 22 | RAC | 70.0 | 55.3 - 89.6 | 8.16 | 1.74 |
| | | 22 | SDW | 73.2 | 54.4 - 94.3 | 10.58 | 2.26 |

[a] = SD (standard deviation), SE (standard error).

The overall average protein concentration was significantly different between the two locations. At Roseworthy, the average protein concentration for durum wheat was 15.2% but only 12.7% at Saddleworth, with bread wheats giving 12.8% at RAC but only 10.9% at Saddleworth (Table 6.2). Generally, durum wheat appeared to possess higher protein concentration than bread wheat under the same growing conditions (Fig. 6.4A and Fig. 6.4B).

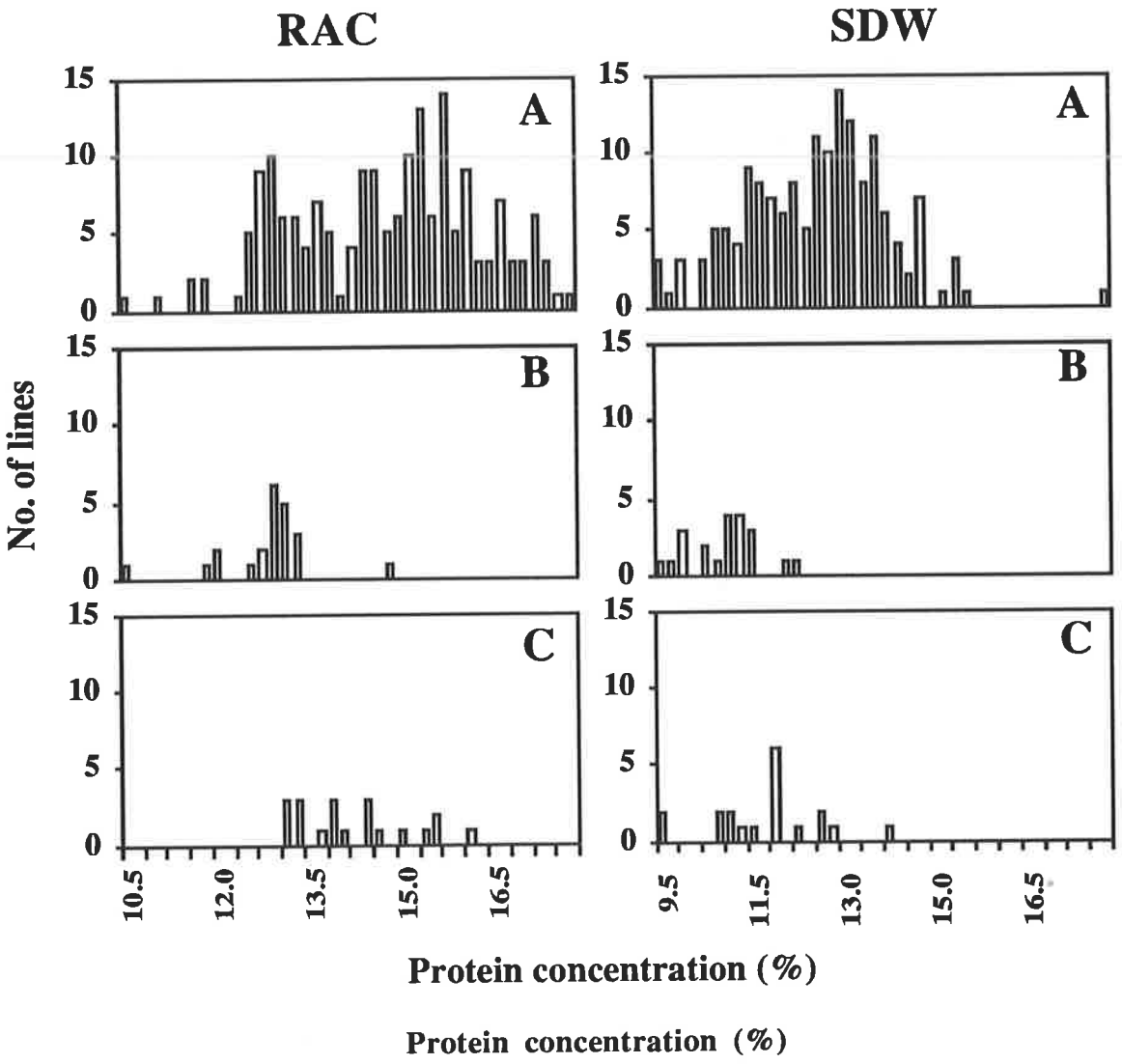



**Figure 6.4** The protein concentration distributions of (A) total samples, (B) bread wheat and (C) Australian advanced durum lines at Roseworthy (RAC) and Saddleworth (SDW) in 1991.

The mean grain yield of durums was significantly lower than bread wheat over the two sites (486.7 vs. 608.1), and there was a significant difference in grain yield between two sites (RAC 393.9 vs. Saddleworth 609.3). However, at Saddleworth the mean yield of the durums was much closer to that of bread wheats (597 vs. 670) than at RAC (373 vs. 547). Again, the general trend was demonstrated in Fig. 6.5A and 6.5B.

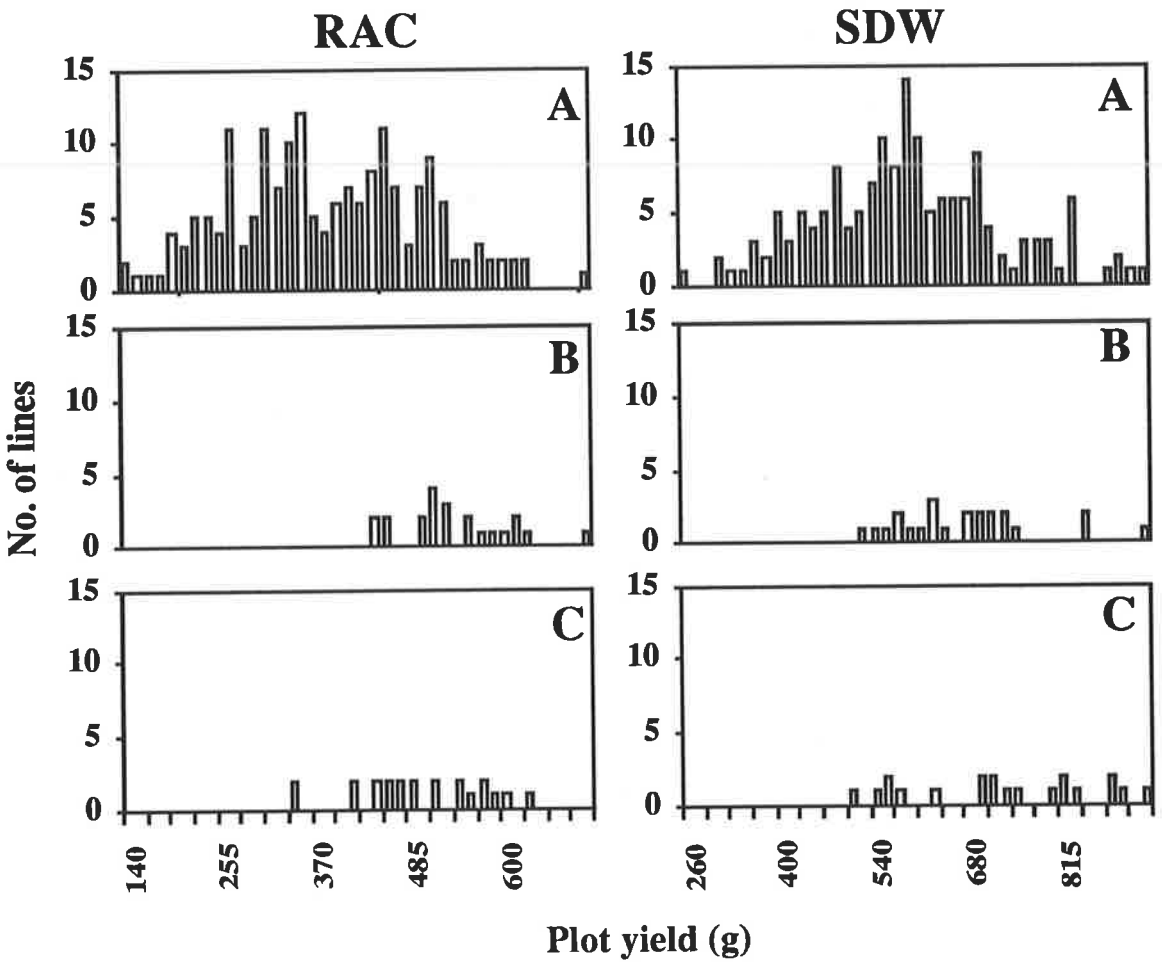



**Figure 6.5** The grain yield per plot distributions of (A) total samples, (B) bread wheat and (C) Australian advanced durum lines at Roseworthy (RAC) and Saddleworth (SDW) in 1991.

The sedimentation values of durum wheats ranged from 15.0 to 80.0 mls, mostly clustered around 46 mls. One wheat labelled Italian durum had a very high sedimentation volume (75 and 91 mls) when compared to all other durum wheat lines, suggesting it might be incorrectly identified. It was confirmed by electrophoresis that it was, in fact, a bread wheat. The misclassified cultivar was therefore grouped under hexaploid wheats. The eight bread wheat advanced lines, two parents and the Italian cultivar had a mean sedimentation value of 76.2 mls, ranging from 45.0 to 94.0 mls, implying a substantial variation of technological properties, which agreed with a previous report on quality parameters measured by extensograph (see Gupta *et al*, 1989). Fig. 6.6A and 6.6B show the clear difference of the two groups for their SDSS values at two locations.

Protein yield was a measure of actual protein production at each plot. In general, durum wheat synthesized less protein quantity than bread wheat (66.5 vs. 71.6). However, at the higher protein concentration level (RAC), durum wheat produced less protein than bread wheat, but it produced more at the lower protein concentration level (Saddleworth) (Table 6.2 and Fig. 6.7).

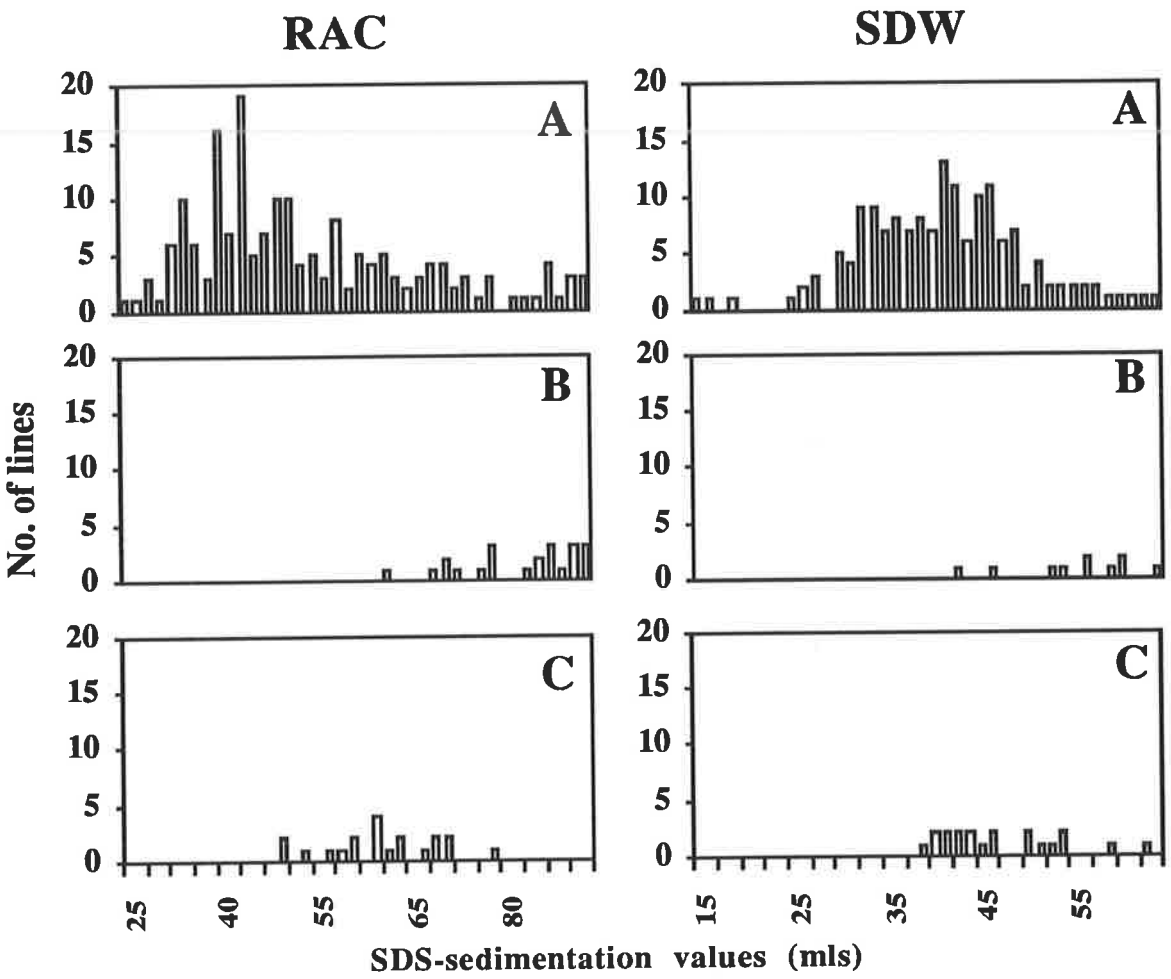



**Figure 6.6** The SDS-sedimentation values of (A) total samples, (B) bread wheat and (C) Australian advanced durum lines at Roseworthy (RAC) and Saddleworth (SDW) in 1991

The eight Australian durums (one line was repeated three times) can be compared with the rest of the durum and bread wheats for yield and quality (Figs 6.1C to 6.4C). On average, these lines had protein concentrations and SDSS values between those of hexaploid and the other durums. These lines had yields and protein yields either outyielding or similar to the bread wheats at Saddleworth but a lower grain yield at RAC, indicating a substantial commercial potential at some locations for these varieties.

Among the durum wheat lines, significant or highly significant correlations were observed between protein concentration, SDSS and protein yield at each location, suggesting that these quality parameters were inter-related. On the other hand, there were no significant correlations among the bread wheats at both growing sites, for these three quality parameters.

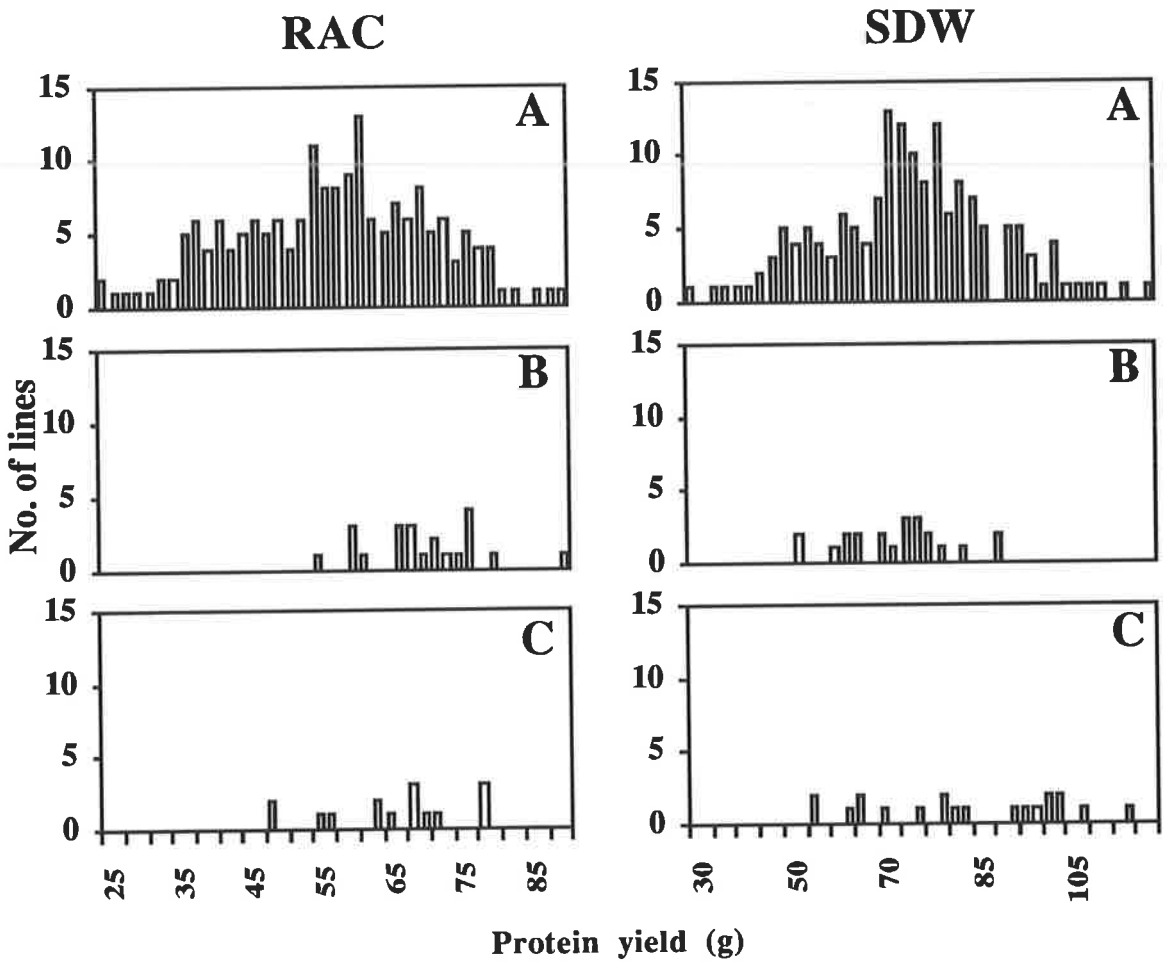



**Figure 6.7** The protein yield distribution of (A) total samples, (B) bread wheat and (C) Australian advanced durum lines at Roseworthy (RAC) and Saddleworth (SDW) in 1991

### *6.3.1.3 Associations between prolamin alleles and quality properties among the durum wheat lines*

The 79 lines of durum wheat had been analysed previously for their protein composition (see Chapter 4) and the banding patterns for LMW and HMW glutenin subunits and γ-gliadin are summarised in Fig. 6.8. Only those types occurring more than once were subjected to further analysis in this chapter. Due to the un-balanced groups of banding patterns involved in this study, Scheffe's tests (at the significance level of 5%) were chosen to perform multiple comparisons. Therefore, to be significant the differences between groups should be large.

#### (A) Gliadin bands

Table 6.3 shows the association between the major γ-gliadin bands, controlled by *Gli-B1* alleles and the average SDSS values per unit protein concentration at two growing sites. The γ-45 type was associated with a significantly higher SDSS value than γ-42 at both sites. Thus the current results were consistent with previous observations (Damidaux *et al*, 1978, 1990b;

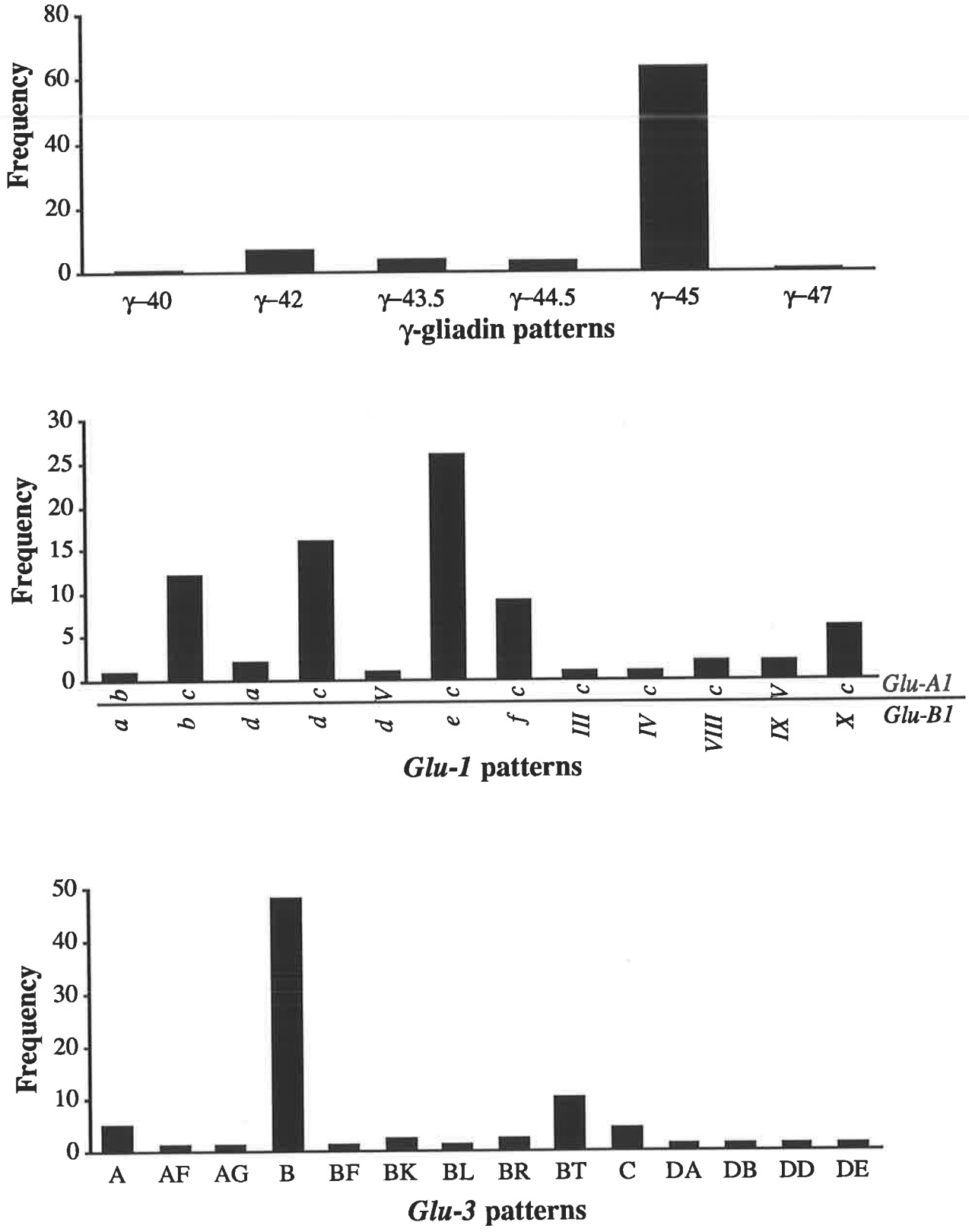



**Figure 6.8** Frequency of occurrence of (a) γ-gliadin, (b) HMW glutenin subunit patterns (*Glu-1*) and (c) LMW glutenin subunit patterns (*Glu-3*) patterns within the durum wheats.

**Table 6.3** Average SDSS values per unit protein concentration (SDSS/PC) for different *Gli-B1* and *Glu-3* alleles at two sites (RAC, upper line and Saddleworth, lower line)[a]

| *Gli-B1* | | | *Glu-B3* | | | *Glu-A3* | | |
|---|---|---|---|---|---|---|---|---|
| Allele | Freq | SDSS/PC (Mean ± SE) | Allele | Freq | SDSS/PC (Mean ± SE) | Allele | Freq | SDSS/PC (Mean ± SE) |
| γ-42 | 8 | 2.44 ± 0.17 | *a'* | 6 | 2.50 ± 0.19 | *a'* | 68 | 3.38 ± 0.09 |
| | | 2.46 ± 0.19 | | | 2.54 ± 0.21 | | | 3.55 ± 0.07 |
| γ-43.5 | 4 | 3.25 ± 0.37 | *b'* | 49 | 3.16 ± 0.09 | *c'* | 4 | 2.37 ± 0.18 |
| | | 3.04 ± 0.32 | | | 3.47 ± 0.08 | | | 3.26 ± 0.32 |
| γ-44.5 | 2 | 2.52 ± 0.27 | *d'* | 4 | 3.49 ± 0.45 | *f* | 4 | 2.79 ± 0.30 |
| | | 2.73 ± 0.09 | | | 3.29 ± 0.30 | | | 2.87 ± 0.28 |
| γ-45 | 63 | 3.38 ± 0.09 | *i'* | 3 | 2.40 ± 0.22 | *g'* | 2 | 2.30 ± 0.17 |
| | | 3.62 ± 0.07 | | | 3.22 ± 0.45 | | | 2.31 ± 0.33 |
| | | | *k'* | 11 | 4.25 ± 0.20 | | | |
| | | | | | 4.21 ± 0.14 | | | |
| Significant level[b] | | ** | | | **** | | | ** |
| | | **** | | | **** | | | ** |
| Scheffe's comparison[b] | | * | | | * | | | * |
| | | * | | | * | | | * |

[a] = For *Gli-B1* alleles, the nomenclature of Bushuk and Zillman (1978) was used. The allele systems followed that of Chapter 4 for durum wheat;

[b] = *, **, *** and **** were significant at a 5%, 1%, 0.1% and 0.01% probability level for analysis of variance, respectively; n. s. = non-significant. Multiple comparisons were performed by Scheffe's tests at a level of significance of 5% due to the un-balanced groups.

Kosmolak *et al*, 1980; du Cros *et al*, 1982). The γ-43.5 and γ-44.5 types were associated with higher quality values than γ-42, but lower than the γ-45 types, and these differences were not significant. However, *Gli-A1* alleles were not considered.

## (B) LMW glutenin alleles

Table 6.3 shows the relationship of LMW glutenin subunits controlled by *Glu-B3* and *Glu-A3* alleles to SDSS volume. At *Glu-B3* locus, the allele *Glu-B3b'* had significantly higher SDSS/PC values than the *Glu-A3a'* allele at Saddleworth but not at RAC. The alleles *Glu-B3b'* and *Glu-B3a'* corresponded to the previously reported LMW-2 and LMW-1 types, respectively (Payne *et al*, 1984c). Hence this result was consistent with the previous reports that LMW-2 was stronger than LMW-1 in dough strength (Pogna *et al*, 1988, 1990).

Other alleles also exhibited some significant differences at *Glu-B3* locus at both sites. Allele *Glu-B3k'* were significantly higher in SDSS than *Glu-B3b'* and *Glu-B3a'* at both sites and *Glu-B3k'* was also significantly different from *Glu-B3i'* at RAC. Moreover, the *Glu-B3k'* allele appeared to be associated with the highest values in SDSS values and all the Australian advanced lines had this allele. Alleles affected quality in the order of $k' > d' > b' >> a' > i'$ at RAC and $k' > b' > d' > i' >> a'$ at Saddleworth (Table 6.3).

At *Glu-A3* loci, allele *Glu-A3a'* had significantly higher SDSS/PC values than alleles *Glu-A3c'* at RAC and *Glu-A3g'* at Saddleworth (Table 6.3). These new *Glu-A3* alleles have not been tested before and the present result suggested that the *Glu-A3a'* was superior to other alleles in the decreasing order of $a' >> f' > c' > g'$ at RAC and $a' > c' > f' > g'$ at Saddleworth.

Furthermore, when the *Glu-A3* and *Glu-B3* patterns are combined to give the LMWGS patterns, LMW-BT types (i. e. *Glu-A3a'* + *Glu-B3k'*) were associated with significantly higher SDSS/PC values than LMW-BR (i. e. $f' + b'$), LMW-B ($a' + b'$) and LMW-A ($a' + a'$) at both sites (extracted from Table 6.3). LMW-B types had higher SDSS values than LMW-A at Saddleworth and LMW-BT higher than LMW-BK (i. e. $c' + l'$) at RAC.

## (C) HMW glutenin alleles

Four *Glu-A1* alleles (*Glu-A1a*, *Glu-A1b*, *Glu-A1c* and *Glu-A1v*) occurred (Fig. 6.8) in the durum wheats but only three were examined (Table 6.4). Analysis of variance showed that there were no significant differences from SDSS/PC values among these types at Saddleworth. *Glu-A1v* types were associated with significantly higher average SDSS values than the null allele at RAC. In contrast, the general conclusion that alleles $a > c$ at *Glu-A1* locus for dough quality in hexaploid wheats (Payne, 1987c) did not occur in the durum wheats grown at RAC.

For the *Glu-B1*-coding alleles, *Glu-B1f* and a new allele *Glu-B1IX* at RAC and *Glu-B1f* at Saddleworth gave higher SDSS values than *Glu-B1e* (Table 6.4). Furthermore, when the *Glu-B1* and *Glu-A1* patterns are combined to give the *Glu-1* patterns, the *Glu-1* combination *Glu-B1f* plus *Glu-A1c* vs. *Glu-B1e* plus *Glu-A1c* at RAC exhibited significant differences for their SDSS/PC values (Table 6.4). In brief, the *Glu-1* patterns were correlated with the quality parameter, consistent with previous results in durum wheats (du Cros, 1987a; Boggini and Pogna, 1989) and broadly in line with those in hexaploid wheats (Payne *et al*, 1988a, 1988b).

## (D) Combined alleles

Fig. 6.9A shows all the combinations of LMW and HMW glutenin alleles. At RAC, it was apparent that lines having *Glu-B1b*, *Glu-A1c* with LMW-BT gave significantly higher SDSS/PC values than lines having *Glu-B1e*, *Glu-A1c* with LMW-B;

or lines having *Glu-B1VIII* and *Glu-A1c* with LMW-B;
or lines having *Glu-B1X* and *Glu-A1c* with LMW-BR;
or lines having *Glu-B1b* and *Glu-A1c* with LMW-A.

**Table 6.4** Average SDSS values per unit protein concentration (SDSS/PC) for different *Glu-1* alleles and combinations at two sites (RAC, upper line and Saddleworth, lower line)[a]

| *Glu-A1* | | | *Glu-B1* | | | *Glu-1* | | |
|---|---|---|---|---|---|---|---|---|
| Allele | Freq | SDSS/PC (Mean ± SE) | Allele | Freq | SDSS/PC (Mean ± SE) | Allele (*Glu-B1+Glu-A1*) | Freq | SDSS/PC (Mean ± SE) |
| *a* (1) | 2 | 2.98 ± 0.07 | *b* (7+8) | 12 | 3.53 ± 0.24 | *b* + *c* | 12 | 3.59 ± 0.26 |
| | | 3.58 ± 0.57 | | | 3.68 ± 0.18 | | | 3.68 ± 0.18 |
| *V* | 4 | 4.21 ± 0.34 | *d* (6+8) | 19 | 3.30 ± 0.17 | *d* + *a* | 2 | 2.98 ± 0.07 |
| | | 4.06 ± 0.18 | | | 3.33 ± 0.12 | | | 3.58 ± 0.57 |
| *c* (null) | 72 | 3.23 ± 0.08 | *e* (20) | 26 | 2.80 ± 0.10 | *d* + *c* | 16 | 3.32 ± 0.19 |
| | | 3.44 ± 0.08 | | | 3.26 ± 0.12 | | | 3.25 ± 0.12 |
| | | | *f* (13+16) | 9 | 4.16 ± 0.17 | *e* + *c* | 26 | 2.88 ± 0.12 |
| | | | | | 4.11 ± 0.18 | | | 3.32 ± 0.13 |
| | | | *VIII* | 2 | 2.62 ± 0.16 | *f* + *c* | 9 | 4.16 ± 0.17 |
| | | | | | 2.56 ± 0.07 | | | 4.11 ± 0.14 |
| | | | *IX* | 3 | 4.64 ± 0.31 | *VIII* + *c* | 2 | 2.62 ± 0.16 |
| | | | | | 4.04 ± 0.14 | | | 2.56 ± 0.07 |
| | | | *X* | 5 | 3.04 ± 0.22 | *IX* + *V* | 2 | 4.33 ± 0.29 |
| | | | | | 3.67 ± 0.38 | | | 3.74 ± 0.18 |
| | | | | | | *X* + *c* | 6 | 3.06 ± 0.18 |
| | | | | | | | | 3.57 ± 0.32 |
| Significant level[b] | | * | | | **** | | | **** |
| | | n. s. | | | *** | | | ** |
| Scheffe's comparison[b] | | * | | | * | | | * |
| | | n. s. | | | * | | | n. s. |

[a] = Vallega's (1986, 1988b) nomenclature for new HMW glutenin subunit alleles of durums has been adopted in this table; and the common nomenclature for the *Glu-A1* and *Glu-B1* alleles is bracketed;

[b] = same as in Table 6.3.

Lines having *Glu-B1f* and *Glu-A1c* with LMW-BT also gave higher SDSS/PC values than lines having *Glu-B1e* and *Glu-A1c* with LMW-B. However, no significant differences were associated with these patterns which materials grown at Saddleworth, where the lines had a low protein level.

When these lines were compared with respect to the combination of γ-gliadin bands and *Glu-1* patterns, numerous significant differences were also exhibited (Fig. 6.9B). At RAC, lines having *Glu-B1b* or *Glu-B1f* and *Glu-A1c* with γ-45 were significantly higher than lines

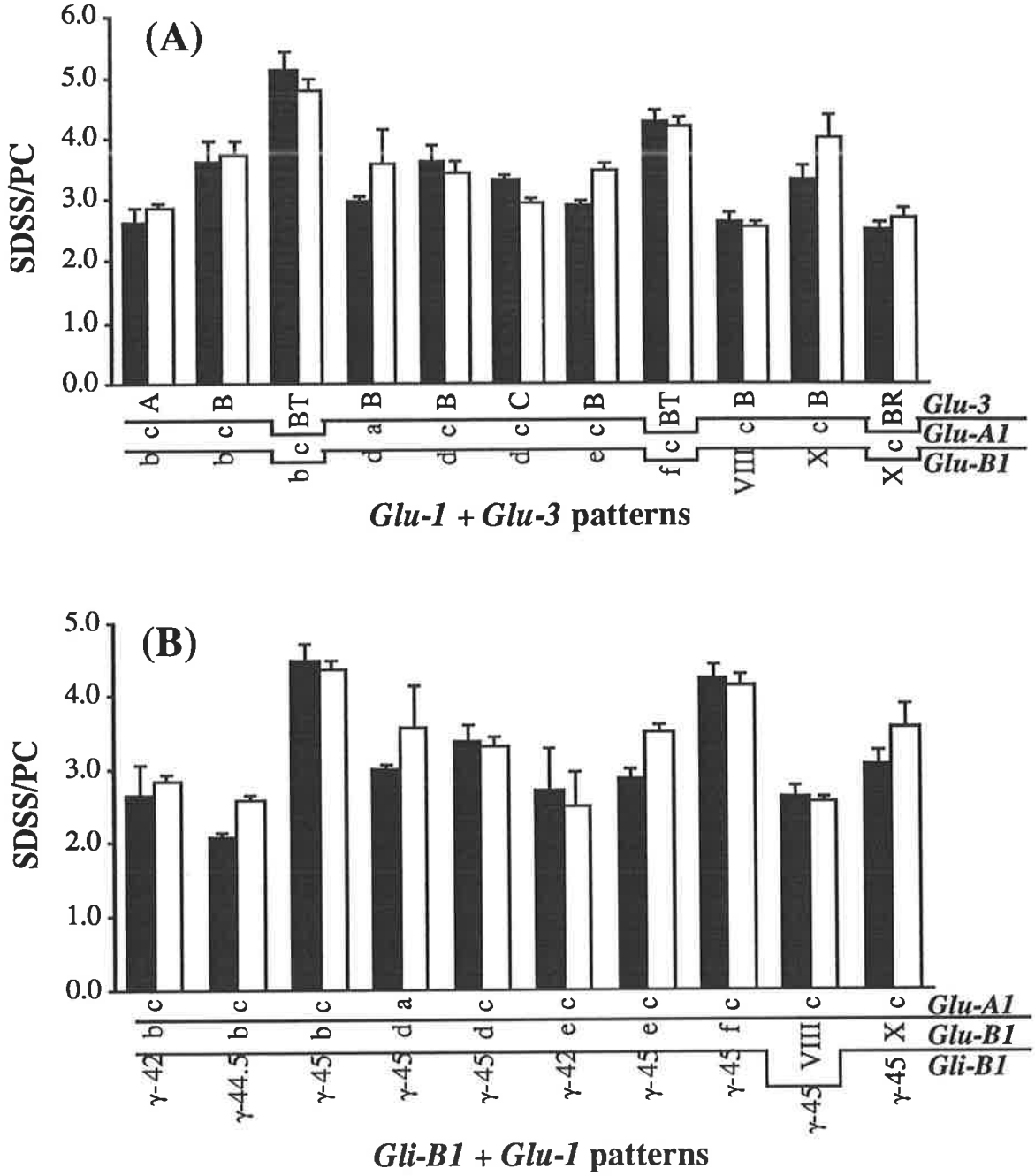



**Figure 6.9** Association of (A) allelic combinations of *Glu-1* and *Glu-3*, (B) allelic combinations of *Gli-B1* and *Glu-1* patterns with the averaged SDSS values per protein concentration (SDSS/PC) from RAC (solid column) and Saddleworth (blank column) of durum wheat studied. The error bars indicate the standard errors of means.

having *Glu-B1b* and *Glu-A1c* with γ-44.5 or lines having *Glu-B1e* and *Glu-A1c* with γ-42 or with γ-45.

Lines which both possessed *Glu-A1c* with γ-45 but differed at the *Glu-B1* locus (*b* vs. *x*) were significant different with SDSS values.

Similarly at Saddleworth, lines significantly different in SDSS values were those both
having *Glu-A1c*, γ-45 but differed *Glu-B1b* vs. *d*; or
having *Glu-A1c* and *Glu-B1b* but differed γ-45 vs. γ-44.5; or
having *Glu-A1c* but differed γ-45 vs. γ-44.5 and *Glu-B1f* vs. *b*.

In summary, it appeared that the combinations of *Glu-A1c*, *Glu-B1b* with LMW-BT/γ-45 (Fig. 6.9) at both growing locations gave the highest quality scores.

### (E) Areas of origin

Since the SDSS values within some of the patterns varied drastically, the question was raised whether the breeding program might play an important role. On average, Australian and CIMMYT (Mexico) lines showed significantly higher quality values (SDSS/PC) when tested at two sites than lines from most of the other regions tested (Table 6.5). This was probably due to the lines from these two regions having relatively lower protein concentrations and only the LMW-B or LMW-BT types present. All lines having *Glu-B1f* were from Australia. When lines within different regions were subdivided into their LMW-1 or LMW-2 related (i. e. corresponding to γ-42 or 45) types and other LMW types (mostly γ-43.5), significant differences in the quality parameter were observed from the Moroccan and Tunisian but not from Syrian and UAR (i. e. Egypt) materials. This could explain why there was no significant difference between the LMW-A and LMW-B related types in respect to quality when the whole data set was analysed at RAC.

In general, the *Glu-1* and *Glu-3* alleles were well correlated with the SDSS values as expected, except for the poor relationship between *Glu-A1* alleles and quality parameters, which was probably due to less variability encountered. Hence, the present results were not only consistent with those of hexaploid (Payne and Lawrence, 1983) and durum wheats (Pogna *et al*, 1988), but expanded our knowledge of the effect of numerous alleles in the durums substantially.

## 6.3.2 Langdon D-genome disomic substitution lines

Since the first replication of the experiment suffered from bird damage when the plants were nearly ripe, only grain yield data from the second replication data is considered. However, the grain quality of the first replication was not likely to be affected by the bird damage and both replications were used for quality evaluations.

The Langdon D-genome disomic substitution lines were generally lower in grain yield and vigour (some were sterile) than the parental and control cultivars. Ldn 1D(1A) and 2D(2B) were the highest yielding among the substitution lines but were still an average 10% lower in yield than the recurrent parent Langdon (Fig. 6.10A). Ldn 3D(3B) was almost sterile when multiplied in the glasshouse and was not included in the field experiment. Ldn 2D(2A) and Ldn 4D(4A) were also partially sterile in the glasshouse and even more sterile under field

**Table 6.5** Average SDSS values per unit protein concentration (SDSS/PC) the from different major growing regions at two sites (RAC, upper line and Saddleworth, lower line)[a]

| | Total patterns | | LMW-A related | | LMW-B related | | Other patterns | |
|---|---|---|---|---|---|---|---|---|
| Country | No. | SDSS/PC (Mean ± SE) | No. | SDSS/PC (Mean ± SE) | No. | SDSS/PC (Mean ± SE) | No. | SDSS/PC (Mean ± SE) |
| **Algeria** | 10 | 2.74 ± 0.08 | | | 10 | 2.74 ± 0.08 | | |
| | | 3.53 ± 0.17 | | | | 3.53 ± 0.17 | | |
| **Australia** | 10 | 4.45 ± 0.16 | | | 10 | 4.45 ± 0.16 | | |
| | | 4.30 ± 0.14 | | | | 4.30 ± 0.14 | | |
| **Italy** | 7 | 2.88 ± 0.25 | | | 6 | 3.02 ± 0.27 | 1 | 2.08 |
| | | 3.19 ± 0.26 | | | | 3.40 ± 0.25 | | 1.91 |
| **CIMMYT** | 12 | 4.34 ± 0.15 | | | 12 | 4.34 ± 0.15 | | |
| | | 4.17 ± 0.13 | | | | 4.17 ± 0.13 | | |
| **Morocco** | 13 | 2.54 ± 0.10 | 1 | 1.51 | 10 | 2.56 ± 0.87 | 2 | 2.94 ± 0.25 |
| | | 2.74 ± 0.12 | | 1.26 | | 2.87 ± 0.11 | | 2.85 ± 0.19 |
| **Syria** | 4 | 2.50 ± 0.16 | 1 | 2.33 | 3 | 2.55 ± 0.21 | | |
| | | 3.26 ± 0.29 | | 2.98 | | 3.36 ± 0.39 | | |
| **Tunisia** | 12 | 2.54 ± 0.10 | 1 | 2.04 ± 0.09 | 9 | 2.70 ± 0.11 | 1 | 2.09 |
| | | 3.04 ± 0.14 | | 2.00 ± 0.03 | | 3.31 ± 0.12 | | 2.66 |
| **UAR** | 10 | 3.69 ± 0.20 | 3 | 3.79 ± 0.50 | 4 | 3.46 ± 0.33 | 3 | 3.89 ± 0.21 |
| | | 3.41 ± 0.16 | | 3.52 ± 0.37 | | 3.27 ± 0.27 | | 3.47 ± 0.21 |
| Significant level[b] | | **** | | | | | | |
| | | **** | | | | | | |
| Scheffe's comparison[b] | | * | | | | | | |
| | | * | | | | | | |

[a] = The allele and pattern systems followed that of Chapter 4 for durums;
[b] = same as in Table 6.3.

conditions. On the other hand, the intervarietal substitution line Ldn (Kharkof-5-1B), did not show any sign of yield reduction (Fig. 6.10A).

Protein concentration was negatively correlated with grain yield ($r = -0.772$, $P < 0.01\%$) but with the exception of Ldn 2D(2A), Ldn 6D(6B) and Ldn 7D(7B), the grain from most lines had a similar protein concentration (Fig. 6.10B). Protein concentration was also highly significantly correlated with other quality characters such as SDSS, MT values, and the protein components $P_{1-1}\%$, $P_1\%$ and $P_2\%$ (Table 6.6). $P_{1-1}\%$, $P_1\%$ and $P_1/P_2$ were all correlated positively with the mixograph parameters MT, PR, BWAP, TPBW and PBW

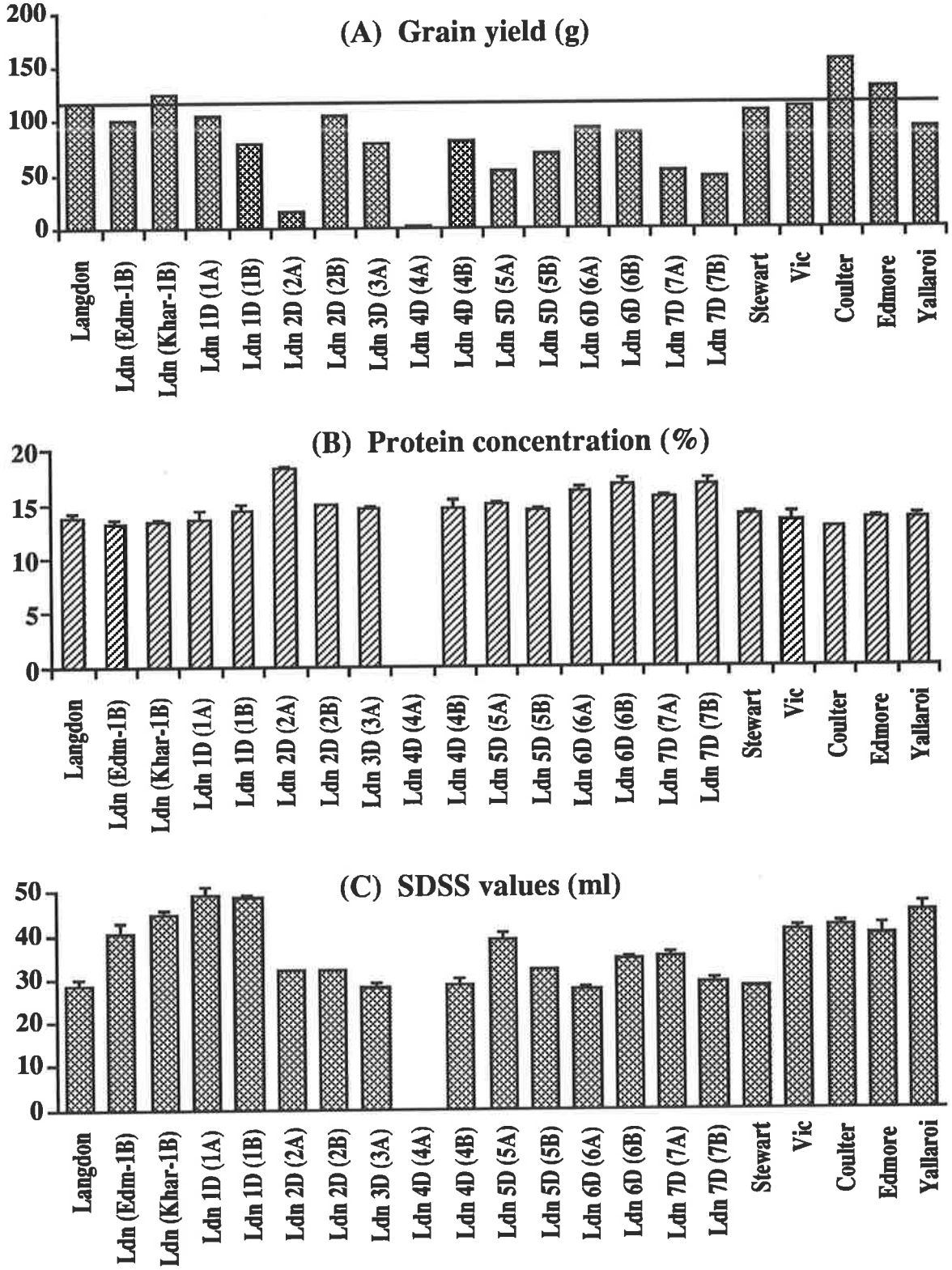



**Figure 6.10** Comparisons of (A) grain yield (second replication only), (B) protein concentration and (C) SDSS values of the intervarietal substitution and D-genome disomic substitution lines of Langdon and other durum check varieties. Ldn 4D(4A) was not measured for protein concentration because an insufficient sample was available. The error bar of each column represents the standard error of mean.

**Table 6.6** Simple correlation among individual yield and quality parameters of the D-genome disomic substitution lines of Langdon and the intervarietal substitution lines and other durum wheat controls[a]

| Name | PC | HI | Yellow-ness | White-ness | SDSS | $P_1/P_2$ | $P_1$% | $P_{1-1}$% | $P_{1-1}/P_2$ | $P_2$% | MT | PR | BWAP | RBD | BBD | TPBW |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| HI | -0.14 | | | | | | | | | | | | | | | |
| Yellowness | -0.51 | 0.77 | | | | | | | | | | | | | | |
| Whiteness | 0.28 | -0.91 | -0.94 | | | | | | | | | | | | | |
| SDSS | -0.51 | 0.07 | 0.50 | -0.31 | | | | | | | | | | | | |
| $P_1/P_2$ | -0.60 | 0.32 | 0.63 | -0.49 | 0.72 | | | | | | | | | | | |
| $P_1$% | -0.63 | 0.37 | 0.69 | -0.54 | 0.72 | 0.99 | | | | | | | | | | |
| $P_{1-1}$% | -0.68 | 0.37 | 0.74 | -0.57 | 0.79 | 0.96 | 0.98 | | | | | | | | | |
| $P_{1-1}/P_2$ | -0.64 | 0.32 | 0.67 | -0.51 | 0.76 | 0.99 | 0.98 | 0.98 | | | | | | | | |
| $P_2$% | 0.62 | -0.36 | -0.66 | 0.52 | -0.70 | -0.99 | -0.99 | -0.97 | -0.98 | | | | | | | |
| MT | -0.53 | 0.37 | 0.61 | -0.50 | 0.79 | 0.61 | 0.63 | 0.71 | 0.66 | -0.61 | | | | | | |
| PR | -0.23 | -0.06 | 0.28 | -0.13 | 0.66 | 0.34 | 0.38 | 0.45 | 0.38 | -0.35 | 0.42 | | | | | |
| BWAP | -0.51 | 0.09 | 0.49 | -0.32 | 0.75 | 0.67 | 0.68 | 0.74 | 0.71 | -0.67 | 0.65 | 0.69 | | | | |
| RBD | 0.61 | -0.37 | -0.71 | 0.55 | -0.78 | -0.66 | -0.68 | -0.77 | -0.71 | 0.65 | -0.86 | -0.46 | -0.66 | | | |
| BBD | 0.09 | -0.39 | -0.39 | 0.42 | -0.28 | -0.25 | -0.24 | -0.27 | -0.26 | 0.24 | -0.54 | 0.18 | -0.06 | 0.46 | | |
| TPBW | -0.52 | 0.37 | 0.61 | -0.50 | 0.79 | 0.66 | 0.66 | 0.74 | 0.70 | -0.65 | 0.97 | 0.37 | 0.71 | -0.84 | -0.56 | |
| PBW | -0.51 | 0.02 | 0.44 | -0.23 | 0.82 | 0.58 | 0.62 | 0.69 | 0.63 | -0.56 | 0.64 | 0.87 | 0.80 | -0.64 | 0.09 | 0.59 |

[a] = Symbols used in this table are bracketed. Protein concentration (PC) (in %); Hardness Index (HI) (in %); SDS sedimentation volume (SDSS) (in ml); Percentage of highly aggregating glutenin, total glutenin and gliadin ($P_{1-1}$%, $P_1$% and $P_2$%); Mix time (MT) (in sec); Peak resistance (PR), Band Width at Peak resistance (BWAP), Resistance breakdown (RBD), Bandwidth breakdown (BBD) (in mixograph Brabender units); Time to peak bandwidth (TPBW) (in sec); Peak bandwidth (PBW) (in mixograph Brabender units). Data ≤ 0.281, n.s., ≥ 0 314, 0.288, 0.497 and 0.529, were significant at 5%, 1%, 0.1% and 0.01% level, respectively.

**Table 6.7** Prolamin compositions and mean values of major quality parameters of the D-genome disomic substitution lines of Langdon and the intervarietal substitution lines and other durum wheat controls[a]

| | Prolamin compositions[c] | | | | | Quality parameters | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Genotypes | γ-gliadin type | *Glu-B3* | *Glu-A3* | *Glu-B1* | *Glu-A1* | HI | Yellow-ness | White-ness | $P_1$% | $P_{1-1}$% | $P_2$% | $P_1/P_2$ | MT | PR | BWAP | RBD | TPBW | PBW |
| **Langdon** | **42** | ***a'*** | ***a'*** | ***d*** | ***c*** | **101** | **18.3** | **61.2** | **44.7** | **30.7** | **51.9** | **0.9** | **218** | **172** | **118** | **14.5** | **154** | **142** |
| Ldn (Edm-1B) | 45 | *b'* | *a'* | *d* | *c* | 101 | 19.3 | 60.2 | 52.7 | 39.4 | 44.3 | 1.2 | 284 | 224 | 145 | 8.8 | 197 | 187 |
| Ldn (Khar-5-1B) | 45 | *i'* | *a'* | *b* | *c* | 101 | 18.8 | 60.4 | 46.2 | 34.8 | 50.1 | 0.9 | 597 | 185 | 168 | 0.8 | 516 | 186 |
| Ldn 1D (1A) | 42 | *a'* | - | *d* | - | 92.0 | 18.1 | 62.0 | 57.3 | 41.7 | 40.2 | 1.4 | 430 | 311 | 227 | 6.8 | 356 | 252 |
| Ldn 1D (1B) | - | - | *a'* | - | *c* | 92.0 | 20.2 | 58.7 | 46.8 | 34.4 | 50.2 | 0.9 | 322 | 263 | 159 | 7.8 | 224 | 208 |
| Ldn 2D (2A) | 42 | *a'* | *a'* | *d* | *c* | 92.0 | 16.3 | 62.7 | 40.6 | 25.8 | 55.0 | 0.7 | 205 | 202 | 124 | 20.8 | 124 | 149 |
| Ldn 2D (2B) | 42 | *a'* | *a'* | *d* | *c* | 97.5 | 18.0 | 60.9 | 43.4 | 29.3 | 52.7 | 0.8 | 193 | 197 | 123 | 19.0 | 131 | 166 |
| Ldn 3D (3A) | 42 | *a'* | *a'* | *d* | *c* | 98.5 | 16.9 | 61.4 | 42.4 | 27.9 | 53.3 | 0.8 | 205 | 149 | 112 | 18.0 | 129 | 132 |
| Ldn 4D (4A)[b] | 42 | *a'* | *a'* | *d* | *c* | | | | 45.9 | 32.7 | 50.2 | 0.9 | | | | | | |
| Ldn 4D (4B) | 42 | *a'* | *a'* | *d* | *c* | 93.0 | 17.8 | 61.3 | 45.5 | 30.3 | 50.4 | 0.9 | 130 | 146 | 141 | 23.8 | 97.8 | 143 |
| Ldn 5D (5A) | 42 | *a'* | *a'* | *d* | *c* | 45.0 | 13.2 | 71.5 | 42.6 | 28.3 | 53.3 | 0.8 | 158 | 233 | 132 | 20.0 | 81.0 | 179 |
| Ldn 5D (5B) | 42 | *a'* | *a'* | *d* | *c* | 45.5 | 13.4 | 71.3 | 41.0 | 27.4 | 54.2 | 0.8 | 144 | 181 | 142 | 21.4 | 69.2 | 164 |
| Ldn 6D (6A) | 42 | *a'* | *a'* | *d* | *c* | 98.0 | 16.9 | 61.9 | 43.7 | 28.5 | 50.7 | 0.9 | 129 | 164 | 123 | 24.5 | 79.5 | 141 |
| Ldn 6D (6B) | 42 | *a'* | *a'* | *d* | *c* | 97.0 | 18.0 | 60.4 | 48.1 | 32.7 | 48.8 | 1.0 | 213 | 189 | 116 | 21.3 | 143 | 150 |
| Ldn 7D (7A) | 42 | *a'* | *a'* | *d* | *c* | 94.0 | 16.3 | 63.6 | 47.8 | 32.2 | 48.3 | 1.0 | 245 | 175 | 116 | 13.7 | 165 | 148 |
| Ldn 7D (7B) | 42 | *a'* | *a'* | *d* | *c* | 96.0 | 17.0 | 61.6 | 46.0 | 30.6 | 50.3 | 0.9 | 185 | 173 | 123 | 14.0 | 118 | 141 |
| Coulter | 45 | *l'* | *e'* | *d* | *c* | 98.5 | 19.4 | 60.1 | 55.1 | 41.1 | 42.2 | 1.3 | 319 | 244 | 153 | 10.0 | 219 | 219 |
| Edmore | 45 | *b'* | *a'* | *d* | *c* | 103 | 21.4 | 57.5 | 53.2 | 39.1 | 44.1 | 1.2 | 361 | 210 | 157 | 5.0 | 214 | 200 |
| Stewart | 42 | *a'* | *a'* | *b* | *c* | 96.5 | 18.6 | 60.7 | 46.8 | 31.6 | 49.9 | 0.9 | 211 | 173 | 115 | 12.8 | 130 | 142 |
| Vic | 45 | *b'* | *a'* | *d* | *c* | 96.5 | 20.5 | 58.7 | 52.9 | 38.5 | 44.3 | 1.2 | 283 | 239 | 199 | 5.6 | 242 | 203 |
| Yallaroi | 45 | *k'* | *a'* | *h?* | *c* | 98.5 | 20.2 | 58.5 | 57.3 | 42.6 | 40.4 | 1.4 | 382 | 233 | 205 | 6.5 | 371 | 196 |
| Mean | | | | | | 92 | 18.0 | 61.6 | 48.0 | 33.7 | 48.5 | 1.0 | 259.1 | 201.0 | 143.4 | 13.1 | 188.4 | 169.5 |
| Fisher's Protected LSD[d] | | | | | | 4.3 | 0.69 | 1.17 | 3.45 | 2.50 | 3.03 | 0.13 | 80.96 | 74.63 | 26.25 | 6.31 | 48.81 | 19.26 |

[a] = Symbols as in table 6.6;
[b] = HI, colour and mixograph statistics were not measured due to a limited amount of sample;
[c] = The γ-gliadin types 42 and 45, coded by *Gli-B1* alleles, are tightly linked to the LMW glutenin subunit alleles coded by *Glu-B3* (for LMW-1 and LMW-2 types, respectively). The *Glu-3* and *Glu-1* alleles were referred to the results in Chapter 4 and presented in Appendix A;
[d] = Results were significant at $P < 0.1\%$.

**Table 6.8** Mean effects of substitution of the individual D-genome chromosomes of Chinese Spring into Langdon on the yield and major quality characters of durum wheat[a]

| | Plot yield[b] | PC | HI | Yellow -ness | SDSS | $P_1$% | $P_{1-1}$% | $P_2$% | $P_1/P_2$ | MT | PR | BWAP | RBD | TPBW | PBW |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1D | -25.5 | 0.10 | -8.50 | 0.81 | 20.4 | 7.33 | 7.40 | -6.71 | 0.32 | 157 | 115 | 75.0 | -7.25 | 136 | 87.90 |
| 2D | -56.5 | 2.80 | -5.75 | -1.16 | 3.50 | -2.73 | -3.10 | 1.92 | -0.08 | -19.2 | 27.4 | 5.10 | 5.38 | 27.0 | 15.00 |
| 3D[c] | -37.0 | 0.65 | -2.00 | -1.46 | -0.75 | -2.27 | -2.76 | 1.39 | -0.07 | -13.5 | -23.5 | -6.5 | 3.50 | -24.7 | -10.50 |
| 4D[c] | -16.5 | 0.75 | -7.50 | -0.51 | 0.00 | 0.97 | 0.82 | -1.64 | 0.05 | -43.9 | 21.5 | 21.6 | 4.38 | -27.1 | 18.75 |
| 5D | -56.0 | 0.85 | -55.25 | -5.04 | 6.88 | -2.89 | -2.79 | 1.86 | -0.08 | -67.3 | 35.1 | 18.5 | 6.20 | -79.9 | 29.30 |
| 6D | -26.0 | 2.58 | -3.00 | -0.85 | 2.50 | 1.21 | -0.05 | -2.17 | 0.07 | -47.4 | 4.15 | 1.00 | 8.38 | -43.0 | 3.40 |
| 7D | 67.0 | 2.30 | -5.50 | -1.69 | 3.50 | 2.23 | 0.74 | -2.61 | 0.09 | -3.25 | 1.70 | 0.95 | -0.68 | -12.5 | 2.60 |
| Mean | -21.50 | 1.43 | -12.50 | -1.41 | 5.14 | 0.55 | 0.04 | -1.14 | 0.04 | -5.26 | 25.9 | 16.5 | 2.84 | -3.44 | 20.92 |
| P[d] | / | n. s. | **** | **** | **** | ** | *** | * | ** | *** | *** | ** | *** | **** | *** |
| Fisher's Protected LSD[d] | / | 6.53 | 3.64 | 0.62 | 3.41 | 3.89 | 2.11 | 3.79 | 0.14 | 50.3 | 29.1 | 22.52 | 3.58 | 38.44 | 22.70 |

[a] = Symbols as in table 6.6. Data were from figure 6.6, and Table 6.5 and expressed as the differences of the average D-genome substitution lines - cv. Langdon;

[b] = second replicate data only;

[c] = Since 3D(3A) and 4D(4A) were sterile, only 3D(3B) and 4D(4B) were used to calculate the effect of 3D and 4D, respectively.

[d] = The averaged values of each D genome substitution lines [i. e. 1D(1A) and 1D(1B)] within each replication were used as an observation for analysis of variance and the Fisher's Protected LSD was employed to compare the average differences of individual chromosome substitutions.

(hence positive characters) and negatively with $P_2$ % and RBD (Table 6.6). $P_1$% was strongly negatively correlated with $P_2$ % (r = -0.99). However, analysis of variance showed no significant difference between the average values of grain protein concentration of each individual chromosome substitution line and the other durum varieties (Table 6.8).

Chromosome 5D had a major effect on the reduction of grain hardness (50%) ($P < 1\%$), with lesser effects associated with substitution of chromosomes 1D, 4D, 2D and 7D (Tables 6.7 and 6.8). Chromosome 5D also had a larger influence on flour colour while the other chromosomes had a lesser but significant effect. These two substitution lines [Ldn 5D(5A) and Ldn 5D(5B)] were paler yellow and brighter ($P < 5\%$) in colour. Edmore had the most intense yellow colour and the lowest whiteness scores (Table 6.7), since whiteness and yellowness were highly negatively correlated (Table 6.6).

Chromosome 1D showed the largest effect on SDSS values (Fig. 6.10C) and on the prolamin fractions $P_1$%, $P_1/P_2$ (Tables 6.7 and 6.8). For the mixograph parameters, Ldn 1D(1A) had the highest MT, PR, BWAP, TPBW and PBW and lowest RBD and BBD among the substitution lines, suggesting dough properties of 1D substitution lines were stronger than the euploid Langdon (Table 6.8). Chromosomes 5D, 2D and 7D also gave higher SDS-sedimentation values (Fig. 6.10C) but none of the D-genome chromosomes except 1D were associated with a significant increase in the proportion of glutenin ($P_1$%) measured by SE-HPLC (Table 6.7). Chromosome 5D in contrast to chromosome 1D had a significant detrimental effect on MT (Tables 6.7 and 6.8), which was considered as an important criterion of dough strength (MacRitchie, 1987). Chromosome 5D had higher PR values when compared to Langdon whereas chromosomes 2D, 4D, 5D and 6D had significantly higher RBD values than Langdon, further indicating the negative influence of these chromosomes on dough mixing tolerance.

Many of the quality characters measured were highly inter-correlated, a result not unexpected as some were derivatives of the other characters (eg. $P_1/P_2$). The eleven quality parameters [protein concentration, HI, Yellowness, $P_1$%, MT, PR, BWAP, RBD, TPBW and PBW, SDSS (y)] were analysed by PCA to determine the component contributing most to dough quality. The data were first standardised - character x was transformed to [x-mean(x)]/st dev(x) - and then subjected to PCA. The average of the two replicates for each line was used as an observation. As interest lay in seeing the relationship of SDSS to the quality characters, SDSS was excluded from the PCA. The first principal component explained 63.1% of the total variation in the original variates, with positive loading coefficients for protein concentration and RBD, and negative coefficients for the remaining eight characters. The second principal component was dominated by the positive contribution of HI and the negative response of PR. The first two principal components accounted jointly for approximately 80% of the variation in the original set of ten variables.

A plot of Principal Component 1 versus Principal Component 2 was used to distinguish between the lines (Fig. 6.11). In the vertical direction (i. e. Principal Component 1) two groups were clear, with the groupings based on γ-gliadin compositions (γ-42/-45 types), coded

by genes at *Gli-B1* locus (Table 6.7). All varieties belonging to the γ-42 types were clearly separated from γ-45 types except the 1D substitution lines (γ-42 type or null), which were grouped with the γ-45 due to these substitutions (with HMWGS band 2+12) possessing large influence on quality parameters. When Principal Component 2 was also considered, four groups were evident due to the effects of HI and mixing properties. These corresponded to chromosome 5D, Ldn 1D(1A), the γ-42 types and the γ-45 types [including Ldn 1D(1B)], as shown in Figure 6.11. A multiple linear regression of SDSS on Principal Components 1 and 2 showed both components was significant and gave $r^2 = 84\%$.

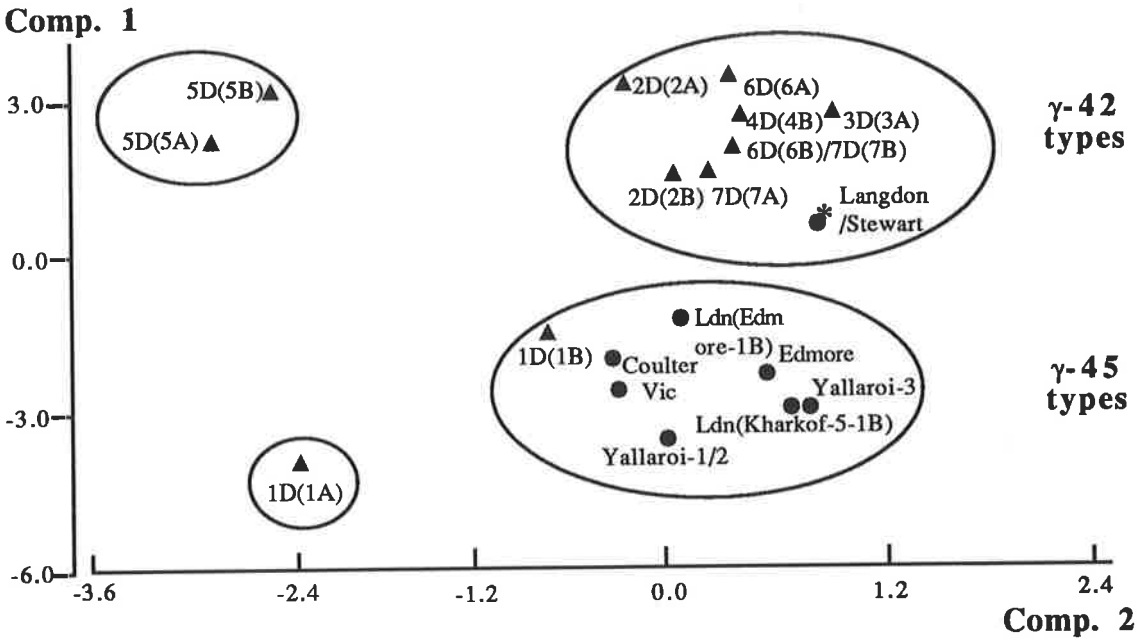



**Figure 6.11** Distribution of individual lines, according to the principal components 1 and 2 from the Principal Component Analysis of Langdon and its disomic D-genome substitution lines as well as durum controls. Ten quality parameters were used in this analysis. ● = Control cultivars, * = Langdon and ▲ = Langdon substitution line.

When the HI and colour were omitted from the analysis, the effect of chromosome 5D was diminished and the 5D substitution lines were clustered within the γ-42 group (Fig. 6.12). Ldn (Kharkof-5-1B) and Yallaroi-3 (Yallaroi repeated three lines in the experiment as controls) were separated from the γ-45 group, which was mainly due to the longer MT and larger PBW values associated with these two lines.

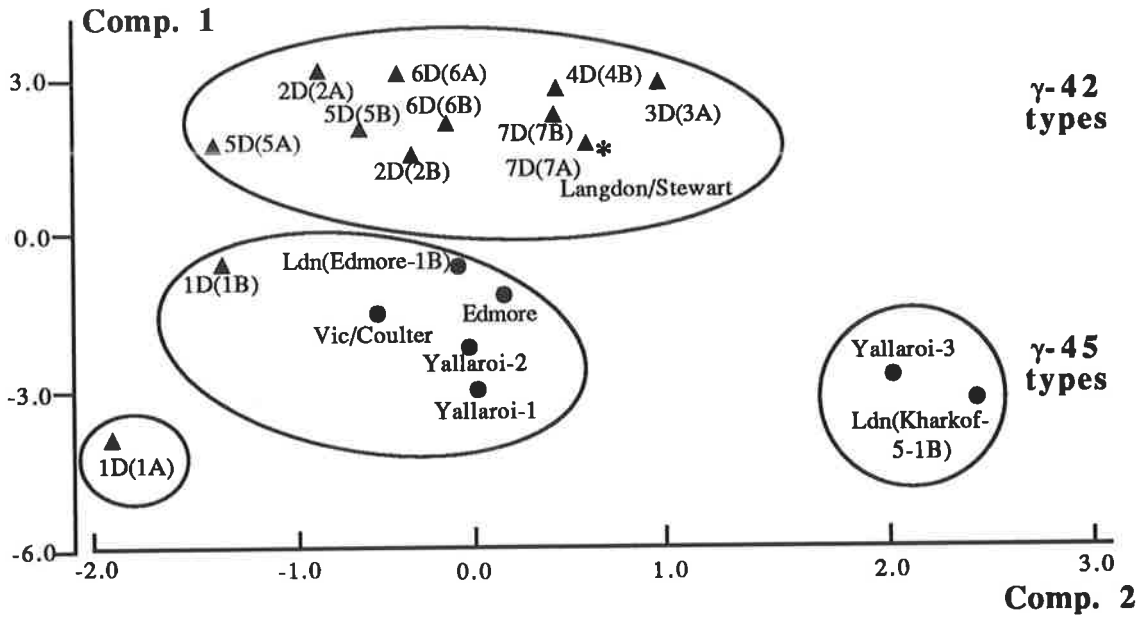



**Figure 6.12** Distribution of individual lines, according to the principal components 1 and 2 from the Principal Component Analysis of Langdon and its disomic D-genome substitution lines as well as durum controls. Analysis was the same as in Fig. 6.11 except two parameters (hardness index and colour) were excluded. Symbols as in Fig. 6.11.

## 6.4 DISCUSSION

### 6.4.1 Yield and quality differences between the durum and hexaploid wheats from the varietal survey

It was clearly demonstrated in the present study that most durums possessed higher protein concentration and had a lower yield than bread wheat when compared in the same growing conditions. In contrast, the new Australian durums from Dr R. Hare's breeding program at Tamworth, N. S. W., were comparable to commercial bread wheats in grain yield while retaining about 1~1.5% higher protein concentration. Most durums coming from countries outside Australia had relatively lower yields which might simply be due to their poor adaptation to South Australian conditions, many being late maturing, tall and susceptible to soil factors including pests and micro-nutrient deficiencies (Rathjen and Brooks, pers. comm.).

The SDSS test has been widely used for evaluating the gluten strength in both bread and durum wheats (Axford *et al*, 1979; Dexter *et al*, 1980; Preston *et al*, 1982). Good pasta quality and good bread-making quality were associated with a large sedimentation volume, although the latter was affected by environmental factors, mainly fluctuations in protein concentrations (Taha and Sagi, 1987; Preston *et al*, 1982). All durums studied had, on average, lower SDSS values than the bread wheat controls, consistent with the previous

conclusion by Dexter *et al* (1981) and Finney *et al* (1987), although some lines possessed relatively higher values including the advanced lines from Dr R. Hare's program.

### 6.4.2 Quality associations with particular gliadin and glutenin alleles from the varietal survey

Chromosome 1B of durum wheat has been shown to have a special influence on gluten strength (Josephides *et al*, 1987) and this has been associated with the presence of certain γ-gliadin bands (Damidaux *et al*, 1978). It was found that cultivars having gliadin band 45 showed strong gluten in contrast to cultivars with band 42, which had weak gluten (Damidaux *et al*, 1978) and this association was confirmed by many other research groups (eg. Kosmolak *et al*, 1980, du Cros *et al*, 1982). The general conclusion was again confirmed with a diverse sources of durum lines grown in South Australian conditions. The γ-gliadin bands affecting quality were in the decreasing order of γ-45 > γ-43.5 >> γ-44.5 > γ-42. The present study suggest that use of gliadin marker for selection of durums with good cooking quality is still applicable.

The relationship between the presence of particular HMWGS and the pasta-making quality of durums was either poor or nil from previous investigations (eg. Vallega, 1986; du Cros, 1987a), presumably due to narrow genetic variability they studied. At *Glu-A1* locus, this appeared true as no particular relationship was observed, hence the previous results that 1 = 2* > 0 in hexaploids were not confirmed here. However a novel allele *Glu-A1v* was clearly shown to be superior to the null allele (Table 6.4). Furthermore, the present study clearly showed that HMWGS at the *Glu-B1* locus (i. e. alleles coding for bands 13+16 and 7+8) correlated with dough strength, in the order 13+16 > 7+8 > 6+8 > 20 (Table 6.4). Boggini and Pogna (1989) and Autran and Feillet (1985) had similar results suggesting that 7+8 >> 20 > 6+8 in bread-making of durums. In brief, the results of effect of HMWGS on dough quality in tetraploids were similarly to the results in hexaploids for the bread-making properties (Payne *et al*, 1987a, 1987b), hence those results obtained from HMWGS in hexaploids could be directly used as guidance in durum wheat quality improvement.

LMW glutenin subunits also exhibited influence on quality parameters, both at the *Glu-A3* and *Glu-B3* loci (Table 6.3). As a close linked locus to *Gli-B1* coding for γ-gliadin components, *Glu-B3* alleles showed similar trend in quality characters in the order of *Glu-B3b'* > *a'*, confirming recent conclusion that LMWGS were the causal factors influencing dough viscoelasticity and firmness properties (Feillet *et al*, 1989; Pogna *et al*, 1988, 1990). In addition, the allele *Glu-B3k'* appeared to be the best one studied, this allele being in all the higher quality Australian advanced lines. Interestingly, the *Glu-A3* alleles also exhibited significant influence on quality. These alleles have not been studied before and more attention should be direct to them in the future.

In summary, the present study for the first time investigated in detail the effect of both HMW and LMW glutenin subunits (as well as γ-gliadins) on the quality aspect of durum

wheat. It could be concluded from the present study that both HMW and LMW glutenin subunits (or gliadins) were important in influencing dough quality except *Glu-A1* locus where there was little variation encountered in the present study. It would be expected the *Glu-A1* alleles would also play equivalent role in durums as they do in the bread wheats when more variation at *Glu-A1* locus has been incorporated into durum wheat in the future.

### 6.4.3 Effect of Langdon D-genome disomic substitution lines on yield and quality

As expected, the substitution of individual chromosome pairs from the D-genome into the tetraploid Langdon only partially compensated for the loss of their respective homoeologous counterparts (Joppa and Williams, 1988). All of them showed less vegetative vigour which resulted in lower grain yields when compared to the euploid parent. However, some, such as the 1D substitution lines were better, and others, including the 3D and 4D substitution lines producing shrivelled seeds or being sterile, were poorer. These substitution lines, in general, cannot be grown commercially because of the yield inferiority. The yield and agronomic characters of the D-genome lines were consistent with the previous results obtained by Joppa and Williams (1988).

Differences in grain protein concentration were well known to affect rheological properties of durum and bread wheat (Dexter and Matsuo, 1977b). In the present experiment all the Langdon substitution lines, except Ldn 2D(2A), 6D(6B) and 7D(7B), had similar grain protein concentrations to Langdon. Therefore, most of the observed differences in quality among these lines were most likely due to variation in protein quality rather than protein quantity (Table 6.8).

Changes in dough quality appear to depend predominantly on the glutenin fraction, because glutenin alone can endow dough with viscoelastic properties (Wall, 1979). Gluten strength has been shown to be important in the production of pasta products (Matsuo and Irvine 1970, Dexter and Matsuo 1977). Strong gluten cultivars produce pasta with greater firmness after cooking and increased tolerance to overcooking. The substitution of chromosome 1D had a large effect on durum wheat rheological properties, indicating that genes on this chromosome could be used to improve the tetraploid wheats for bread-making (Table 6.8). The large positive effect of chromosome 1D was associated with increases in the amount of glutenin and decreases in gliadin i. e. a large increase in $P_1/P_2$ (Tables 6.7 and 6.8) and this was probably due to the major storage protein genes (i. e. *Glu-D1* and *Glu-D3*) located on each arm of that chromosome (Payne, 1987c; MacRitchie *et al*, 1990). The substitution of chromosome 1D for 1A compared to 1D for 1B had a much larger effect on rheological quality, suggesting that chromosome 1B had a larger influence on quality in tetraploids than 1A (Fig. 6.10 and Table 6.7). This was confirmed by the two intervarietal chromosome 1B substitution lines i. e. Ldn (Edmore-1B) and Ldn (Kharkof-5-1B), which carried *Glu-B3b'* and *Glu-B1d*, *Glu-B3i'* and *Glu-B1b*, respectively, giving relatively higher values for quality characters than the parental line (Fig. 6.10C and Table 6.7), and also consistent with other results reported earlier in durum

and hexaploid wheat (Josephides *et al*, 1987; Rogers *et al*, 1988, 1990). In particular, Ldn (Kharkof-5-1B) line that had very high dough strength among the normal durums might be due to the cumulative effect of *Glu-B1b*, *Glu-B3i'* and *Glu-A3a'* (see Tables 6.3, 6.4 and 6.7 and Fig. 6.12). In general, the current results were consistent with a previous study where the observed negative correlation between major dough quality characters and protein concentration was found to be due mainly to changes in the glu/gli ratio i. e. $P_1/P_2$ (see Liu *et al*, 1992 and Table 6.6). An increase in the glutenin proportion ($P_1$%) was accompanied by a decrease in the gliadin proportion ($P_2$%), and an increase in SDSS, MT and PR values (Liu *et al*, 1992). The high inter-correlation among all the quality parameters indicated a tight intrinsic relationship among these characters.

Chromosome 5D was known to carry major genes controlling grain softness, protein quantity and glycolipids, all of which had some impact on flour quality (see review of Law and Krattiger, 1987). In the present study, the 5D substitution lines were the only two with soft grain, resembling that of hexaploid parent CS. This was in agreement with the hexaploid wheat Bezostaya I-Cappelle-Desprez substitution lines, of which all were soft except the 5D substitution lines (Law and Krattiger, 1987). As there were no significant changes in protein concentration or protein components for these two substitution lines compared to the parent Langdon, it was not consistent with one of Law and Krattiger's (1987) conclusion above. The increase in SDSS with the 5D substitution locus when compared to Langdon might be both due to either the grain structural changes or other chemical changes.

Chromosome 5D also had a major gene determining flour colour, which has not been reported previously. Durum semolina or flours generally had a yellow to bright yellow colour. The 5D substitution lines were pale-white rather than yellow (Table 6.7) and was presumed to be caused by the presence of a major inhibitor gene for the pigment synthesis in bread wheats.

Group 6 chromosomes had loci controlling the synthesis of α-, β- and a few γ-gliadins (Wrigley and Shepherd, 1973; Sozinov and Poperelya, 1980; Payne *et al*, 1984b). Substitution of chromosome 6D for 6A [i. e. Ldn 6D(6A)] resulted in a non-significant increase in SDSS and a significant decrease in MT compared to Langdon, whereas when 6B was replaced [i. e. Ldn 6D(6B)], a significant decrease occurred in SDSS and a non-significant increase in MT (Table 6.7). The results were in contrast to a previous result in hexaploid wheat where group 6 chromosomes had no beneficial or negative effects on quality (Mansur *et al*, 1990). The influence in quality of chromosome 6D could be either due to the increases in gliadin proportion or other chemical components in the endosperm.

Chromosomes 2D and 7D, for which no recognized storage protein loci have been reported, gave an increase in SDSS values when compared to Langdon (Table 6.8). In a previous report, chromosome 2D of CS had a regulatory effect on protein content and exhibited the lowest protein production while 2A had the highest effect on protein content when compared to a series of 'alien' chromosome substitutions in CS (Law *et al*, 1978). Although the present study did not show any significant changes in the protein concentration of the

substitution lines studied, it was possible that the results here were consistent with the previous result. Penner *et al* (1988) reported that chromosome 7D of hexaploid Canthatch also affected rheological and milling qualities although it did not affect protein level, consistent with the present result.

Modification of quality has also been reported earlier in hexaploid wheat from Cheyenne chromosomes 3A, 3B, 4D and 7B (Morris *et al*, 1966; Law and Krattiger, 1987; Mansur *et al*, 1990) or Bezostaya I chromosomes 4D, 5A, 5D and 6B (Krattiger *et al*, 1987), suggesting that there might be some unidentified genetic background influences. The hexaploid wheat substitution lines with storage genes were reported to account for only about 75% of the difference in loaf volume (Krattiger *et al*, 1987). Hence chromosomes such as 5D, 2D and 7D which contributed minor but significant quality effects here may have similar background effects to those reported for bread wheats (Morris *et al*, 1966; Law and Krattiger, 1987; Krattiger *et al*, 1987; Mansur *et al*, 1990). In both tetraploids and hexaploids, the major effects were associated with the group 1 chromosomes which carry genes coding for the HMW and LMW glutenin subunits.

### 6.4.4 Future breeding objectives

Results in this chapter demonstrated clearly that major dough quality of durum wheats was mainly influenced by group 1 chromosomes, which carried major seed endosperm protein genes. Alleles from both HMWGS and LMWGS affected quality. Although the Australian advanced lines had significantly higher dough strength than lines from other countries except CIMMYT, the dough strength of the best lines was still lower than that of bread wheats, as measured by SDSS values. Accumulation of the functional active quality genes from different loci would be a feasible way to further improve the dough quality of the present durum cultivars. For example, Yallaroi (the current commercial variety in South Australia) could be further manipulated by substituting better quality alleles as listed in the following table (Table 6.9). Lines have active alleles at *Glu-A1* locus (i. e. allele *a* from Kharkof-5 or *b* from Duramba A) could be considered at the first instance, since these alleles have been ignored and are rarely seen in the modern cultivars but have been shown to possess significant influence on dough quality in bread wheat (Payne *et al*, 1984b, 1988a, 1988c). Alleles with a better quality score [*Glu-1* score, Payne *et al* (1987a)] at *Glu-B1* locus should also be considered, such as *Glu-B1b* (coding for bands 7+8) or *Glu-B1f* (bands 13+16), which have also been consistently shown to associate with significantly higher SDSS values than other alleles both in the present study and elsewhere (Payne *et al*, 1988a; Pogna *et al*, 1990). It has been hypothesised that glutenin alleles coding for higher number of bands or darker-stained band(s) are better in quality than those coding for faint or null bands (Lawrence *et al*, 1988; Singh *et al*, 1990b) and could be used as selection criteria for better quality in relation to quality improvement of durum cultivars in the future breeding work. Therefore, the newly identified allele on *Glu-A3* locus (i. e. *e'* from Kharkof-5 and Coulter, or *a'* from Edmore and Yallaroi) and *Glu-B3* locus (i. e. *k'*

from Yallaroi or *l'* from Coulter), some of which have not been tested for quality effect, could also be incorporated in the breeding program to test their influence on dough strength.

**Table 6.9** Glutenin subunit and gliadin compositions of important lines for further dough strength improvement of durum cultivars[a]

| | *Glu-A1* | *Glu-B1* | *Glu-A3* | *Glu-B3* | *Gli-B1* |
|---|---|---|---|---|---|
| Yallaroi | *c* (0) | *h* (14+15)?[b] | ***a'*** | ***k'*** | *b'* (γ-45) |
| Kharkof-5 | ***a*** (1) | ***b*** (7+8) | ***e'*** | ***i'*** | *b'* (γ-45) |
| Coulter | *c* (0) | *d* (6+8) | ***e'*** | ***l'*** | *b'* (γ-45) |
| Duramba A | ***b*** (2*) | ***f*** (13+16) | ***a'*** | ***i'*** | *b'* (γ-45) |
| Cappelli | *c* (0) | *e* | ***a'*** | ***b'*** | *b'* (γ-45) |
| Australian lines | *c* (0) | ***b/f*** | ***a'*** | ***b'*** | *b'* (γ-45) |
| Italian lines | ***c/b/a*** | ***b/e/x/a/l/v/d/II*** | ***a'/c'/j'*** | ***b'/k'/i'/c'/e'/d'/a'*** | ***a'/b'*** (γ-42/45) |
| CIMMYT's lines | *c* (0) | *d/b/e/x* | ***a'/c'*** | ***b'/k'*** | *b'* (γ-45) |

[a] = Common designation of bands in brackets. The proposed favourable alleles in bold types;

[b] = The *Glu-B1* allele of Yallaroi was not checked with the standard control but clearly different from *Glu-B1f* bands.

# CHAPTER 7. GRAIN YIELD AND QUALITY CHARACTERISTICS OF $F_2$-DERIVED 1D/1B SUBSTITUTION LINES

## 7.1 INTRODUCTION

The development of a disomic-substitution line 1D(1B) in tetraploid wheat (*Triticum turgidum* var. *durum*) cultivar Langdon (Ldn) has facilitated the transfer of chromosome 1B from one durum variety to another (Joppa *et al*, 1983a, 1983b). For example, two substitution lines of Langdon were produced containing chromosome 1B from two strong gluten strength durum wheat cultivars Edmore (Edm) and Kharkof-5 (Joppa *et al*, 1983b). Subsequently, the dough quality characteristics (SDS-sedimentation values and mixing scores) of these two 1B substitution lines exhibited more similar to the chromosome 1B donor parents, although they were similar in plant morphology to the recurrent parent Langdon (Joppa *et al*, 1983b). This demonstrated clearly that substitution of chromosome 1B from a durum wheat cultivar with strong gluten into one with weak gluten was sufficient to change the dough quality characteristics of the recipient cultivar.

Durum wheat is characterised by its unique spaghetti-making dough properties (Kerber and Tipples, 1969). It has long been argued that the low-molecular weight (LMW) subunits of glutenin rather than the high-molecular weight (HMW) subunits of glutenin in durum wheat had a large effect on dough properties, which was contrary to the findings in hexaploid wheats (Payne, 1987c). Whether this was due to the lack of the whole D genome (Konzak, 1977; Welsh and Hehn, 1964) or other factors has not been explained clearly. When a pair of 1D chromosomes of Chinese Spring carrying genes for both HMW and LMW glutenin alleles (*Glu-D1a* , *Glu-D3a*) (Payne *et al*, 1979; Gupta and Shepherd, 1988) was substituted for chromosome 1B of Langdon, an increase in gluten strength was also achieved (Joppa *et al*, 1983b). It was hypothesized that the quality characteristics of durum wheat cultivar Langdon might be significantly improved further if chromosome 1B of Edmore was substituted with 1D as a double substitution for chromosomes 1B and 1A of Langdon, respectively.

In the present study, a 1D (1A) substitution line has been produced carrying the 1B prolamin alleles (*Glu-B3/Gli-1*) of Edmore to test the hypothesis. Several quality tests were performed including the SDS-sedimentation test (SDSS), size-exclusion high-performance liquid chromatography (SE-HPLC) and direct-drive two-gram mixograph tests to characterise the rheological and technological properties of wholemeal flour from the $F_2$-derived progeny lines. In a separate part of the study, comparisons were made between the 1D substitution lines and ordinary durum and bread wheat cultivars as controls with respect to their grain yield and quality properties.

## 7.2 MATERIALS AND METHODS

### 7.2.1 Parental materials

Durum wheat cultivars Langdon and Edmore and Ldn 1D(1A), Ldn 1D(1B) and Ldn (Edmore-1B) disomic-substitution lines were originally obtained from Dr L. R. Joppa, North Dakota State University, Fargo, USA and then maintained at the Waite Institute. Hexaploid wheat cultivars Chinese Spring(CS), Halberd, Warigal and Gabo (ranging from weak to medium strong dough quality) were from the Waite Institute collection. Durum wheat cv. Vic was obtained from the AWCC, NSW. Yallaroi was supplied by Dr R. Hare, NSW Department of Agriculture, Tamworth via Dr A. J. Rathjen, Department of Plant Science, Waite Institute.

### 7.2.2 Production of 1D (1A) substitution line with Edmore 1B prolamin alleles

Durum wheat Langdon disomic 1D substitution line [Ldn 1D(1A)] and Langdon 1B substitution line [Ldn (Edmore-1B)] were hybridized. The major prolamin band differences of these parents are shown in Table 7.1. $F_2$-derived lines were selected from progeny of this $F_1$ hybrid as shown in the diagram (Fig. 7.1). The prolamin phenotypes of progeny were analysed electrophoretically in the $F_2$ and $F_3$ generations to select out the desired four genotypes having chromosome 1D or 1A with allelic changes on chromosome 1B.

### 7.2.3 Field experiments

The four selected $F_2$-derived progeny types (type-I, -II, -III and -IV) (see Fig. 7.1) were grown in $F_3$ generation in a bird-proof enclosure in the field at the Waite Institute along with the three parental types i. e. Langdon, Ldn (Edmore-1B) and Ldn 1D(1A). The plant materials were sown in a randomised-block design with each entry being a single row (three metres long). Plants were spaced 10 cm within rows and 30 cm between rows. The four $F_2$-derived selections and their parental types were replicated six times.

In addition, the above mentioned four hexaploid cultivars and four durum wheat lines Ldn 1D(1B), Edmore, Vic and Yallaroi were included as controls in another two replicates comprising the $F_2$-derived progeny lines and their parents. They were grown at the same location but in a separate experiment to the one described above.

### 7.2.4 Methods

#### *7.2.4.1 Electrophoresis*

Acid-PAGE, unreduced SDS-PAGE and modified gradient 1-D SDS-PAGE were employed to identify the gliadin or glutenin bands (see Chapter 3). To test for homogeneity, seven to nine $F_3$ seeds of each $F_2$-derived family were analysed separately for their gliadin and glutenin composition.

**Table 7.1** Prolamin alleles on homoeologous group 1 chromosomes present in the parents Langdon, Ldn 1D(1A) and Ldn (Edmore-1B)[a]

| | | Alleles present in | | |
|---|---|---|---|---|
| Prolamin type | Locus | Langdon | Ldn 1D(1A) | Ldn (Edmore-1B) |
| HMW | *Glu-D1* | - | *a* (2+12) | - |
| glutenin | *Glu-B1* | *d* (6+8) | *d* (6+8) | *d* (6+8) |
| subunits | *Glu-A1* | *c* (0) | - | *c* (0) |
| LMW | *Glu-D3*[b] | - | *a* | - |
| glutenin | *Glu-B3*[c] | *a'* (LMW-1) | *a'* (LMW-1) | *b'* (LMW-2) |
| subunits | *Glu-A3*[c] | *a'* | - | *a'* |
| | *Gli-D1* | - | *a'* | - |
| Gliadins[d] | *Gli-B1* | *a'* (γ-42) | *a'* (γ-42) | *b'* (γ-45) |
| | *Gli-A1* | *a'* | - | *a'* |

Note: [a] = Common band designation (Payne and Lawrence, 1983) included in parentheses;
[b] = *Glu-D3* allele symbols from Singh and Shepherd (1988a);
[c] = *Glu-A3* and *Glu-B3* allele symbols from Chapter 4. The prime distinguished the symbols from those used by Gupta and Shepherd (1988);
[d] = *Gli-1* allele symbols were arbitrary.

### 7.2.4.2 *Cytological analysis*

The standard Feulgen procedure was used to produce mitotic chromosome preparations from root tips (see section 5.2.6, Chapter 5). For each $F_3$ protein phenotype, four to six seedlings were checked for their chromosome constitution. Only the plants having 28 chromosomes were retained for further study.

### 7.2.4.3 *Wholemeal preparation*

Wholemeal flour samples (20-30 g) were ground in a hammer mill (Falling Number 3100) equipped with an 0.8 mm screen.

### 7.2.4.4 *Quality tests*

All the procedures were the same as described in Chapter 6, section 6.2.3.3B.

### 7.2.4.5 *Statistical analyses*

The same as described in Chapter 6, section 6.2.3.4.

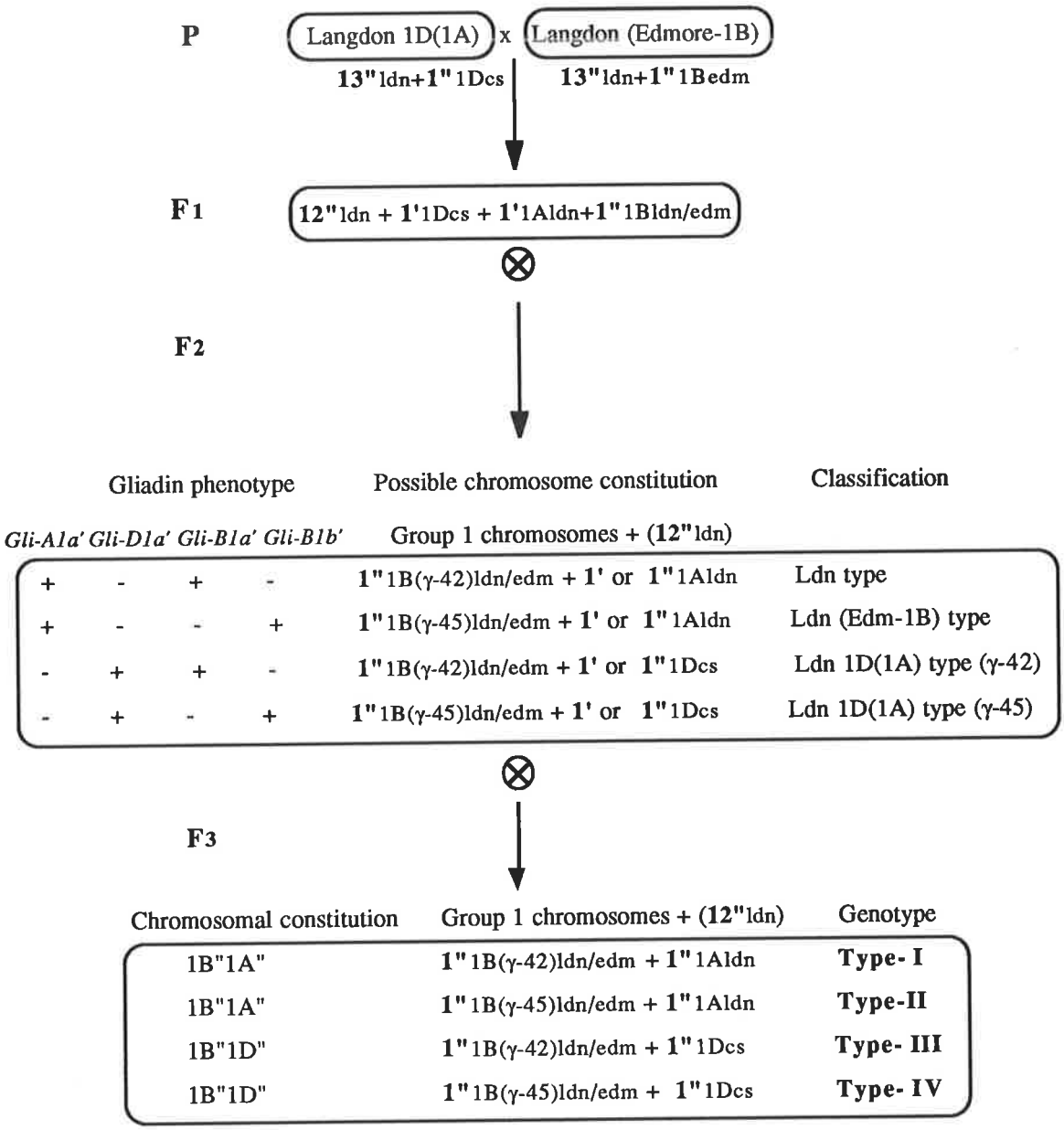



**Figure 7.1** Selection of $F_2$-derived genotypes from a cross between Langdon 1D(1A) and Langdon (Edmore-1B).

Abbreviations used in the figure are given in brackets. Langdon (ldn), Edmore (edm), Chinese Spring (cs). 1" and 1' denote the bivalent and univalent status of a particular chromosome. $1D_{cs}$, $1B_{edm}$ and $1A_{ldn}$ and $1B_{ldn/edm}$, represent chromosomes 1D of CS, 1B of Edmore, 1A of Langdon and the heterozygous constitution of chromosome 1B, respectively.

## 7.3 RESULTS

### 7.3.1 Selection and isolation of the $F_2$-derived lines

Since the parental genotypes are inter-specific and inter-varietal chromosome substitution lines of Langdon, the $F_2$-derived progeny from a cross between them were expected to segregate for the presence of chromosome 1D and 1A intact and for any allelic differences on chromosomes 1B of Langdon and Edmore but to have the Langdon genetic constitution for all other chromosomes. Homoeologous group 1 chromosomes carrying marker genes for the seed protein markers in this cross are coded by *Gli-D1*, *Gli-A1* and *Gli-B1* loci (1D and 1A ω-gliadins and the 1B γ-42 or γ-45 gliadin bands), which could be easily identified in Acid-PAGE separations (Autran *et al*, 1989a; Joppa *et al*, 1983a).

Altogether 266 $F_2$ seeds were analysed and approximately 20% of the progeny were homozygous for 1B and homozygous or hemizygous for 1A (or 1D) (Fig. 7.1 and Table 7.2). Except for the consequences of segregation of allelic differences between chromosome 1B of the parental lines, the selected $F_2$-derived lines type-I, type-II and type-III were equivalent to the parental lines Langdon, Ldn (Edmore-1B) and Ldn 1D(1A) substitutions, respectively. Type-IV represented a new substitution type, combining the presence of chromosome 1D along with the Edmore alleles (LMW-2 or γ-45) on chromosome 1B. Thus the selected $F_2$ plants were grown in the glasshouse and their $F_3$ families were checked for homogeneity with respect to protein markers (glutenins and gliadins) as well as for chromosome constitution to select those with 28 chromosomes and to exclude those with 27 (Table 7.2).

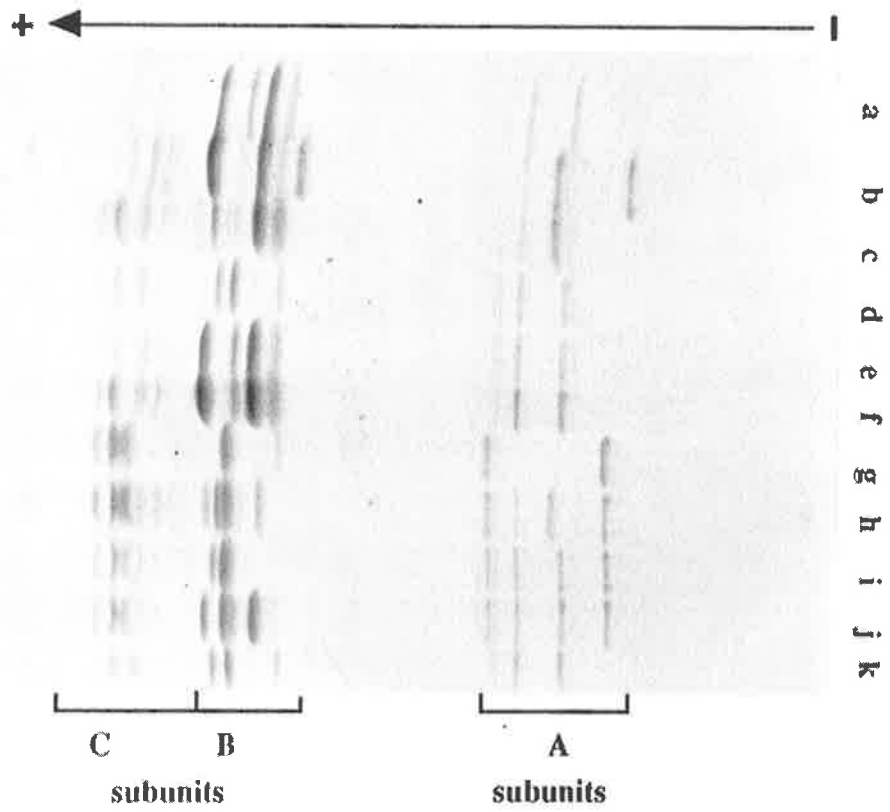
**TABLE 7.2** Number of $F_2$ progeny (homozygous or hemizygous) selected having required genotypes and the number of surviving and homogeneous $F_3$ lines from the cross between Ldn 1D(1A) and Ldn (Edmore-1B) substitution lines

| Genotypes | No. of $F_2$ progeny observed | No. of $F_3$ Harvested | No. of Homogeneous $F_3$ lines |
|---|---|---|---|
| **Type-I** | 18 | 15 | 14 |
| **Type-II** | 17 | 11 | 11 |
| **Type-III** | 10 | 6 | 6 |
| **Type-IV** | 7 | 5 | 3 |

The HMW and LMW glutenin subunit composition of these types is shown in Fig. 7.2.

**Figure 7.2** SDS-PAGE banding patterns of the $F_2$-derived progeny lines/parents, and the controls.

(a, e), Edmore; (b), Kharkof-5; (c), Langdon (Kharkof-5-1B); (d, k), Type-I/Langdon; (f), Type-II/Langdon (Edmore-1B); (g), Langdon 1D(1B); (h), Chinese Spring; (i), Type-III/Langdon 1D(1A); (j), Type-IV.


+
-
a b c d e f g h i j k
C
B
A
subunits
subunits

### 7.3.2 Grain yield and quality characters of the $F_2$-derived progeny and the parental types

The $F_2$-derived genotype lines were grown with the respective parents in a birdcage in rows and the general appearance of a replicate is shown in Fig. 7.3. Subsequently, a series of quality tests were performed after harvest. SDS sedimentation test and mixograph traces of one replicate lines are shown in Figs 7.4 and 7.5 respectively.

The mean values for yield and all the quality attributes measured on the $F_2$-derived progeny and parent lines are given in Table 7.3 and Fig. 7.6. Analyses of variance showed that there were significant genotypic effects for all characters, except grain protein concentration (PC) and bandwidth breakdown (BBD) in the mixograph data (Table 7.3). The durum 1D substitution lines [Langdon 1D(1A), phenotypes III and IV], in general, had lower yield and seed hardness index (HI) ($P < 1\%$) than normal type durums except parent Langdon. The grain PC remained relatively constant, indicating that the yield changes in these $F_2$-derived lines did not influence the grain protein concentration.

The quality differences between the $F_2$-derived lines and parents with similar prolamin phenotype [eg. type-I vs. Langdon, type-II vs. Ldn (Edmore-1B)] were found to be non-significant (Table 7.3 and Fig. 7.6).

The $F_2$-derived lines having the gliadin marker γ-45 substituted for γ-42 (or LMW markers, LMW-2 and LMW-1), gave significantly higher values for gluten strength as measured by SDSS, $P_1\%$, $P_1/P_2$, $P_{1\text{-}1}/P_2$, MT and TPBW values (Fig. 7.6 and Table 7.4), both in the absence of chromosome 1D [i. e. Ldn/type-I vs. Ldn (Edmore-1B)/type-II] and in its presence [i. e. Ldn 1D(1A)/type-III vs. type-IV] (Table 7.3 and Fig. 7.6). This agreed with previous results showing that gliadin band γ-45 of durum wheat was associated with stronger gluten quality than plants having γ-42 (Damidaux *et al*, 1978; Pogna *et al*, 1990).

However, substituting chromosome 1D from Chinese Spring had an even more pronounced effect (about two-fold) on quality parameters than the introduction of *Glu-B3/Gli-B1* alleles. There were larger increases in SDSS, $P_1\%$, $P_1/P_2$, $P_{1\text{-}1}/P_2$, MT, PR, BWAP, TPBW and PBW (considered to be positive quality parameters) and larger decreases in $P_2\%$ and a dough breakdown character RBD (negative quality parameters) in the $F_2$-derived lines and these differences were all significant to highly significant (5% to 0.01%) (Tables 7.4 and 7.5). Comparison of the quality attributes of the four different types of $F_2$-derived progeny lines showed that type-IV (1D with LMW-2) had the highest values for all parameters contributing positively to dough strength and lowest values for all negative parameters except for RBD (Table 7.3 and Fig. 7.6), indicating that there were cumulative effects on the quality characters. The interactions between the effects of chromosomes 1B and 1D for SDSS values, the HPLC fractions (i. e. $P_1\%$, $P_2\%$ etc) and MT were nearly additive whereas PR, RBD, BWAP and PBW exhibited significant negative interactions as shown in Table 7.5.

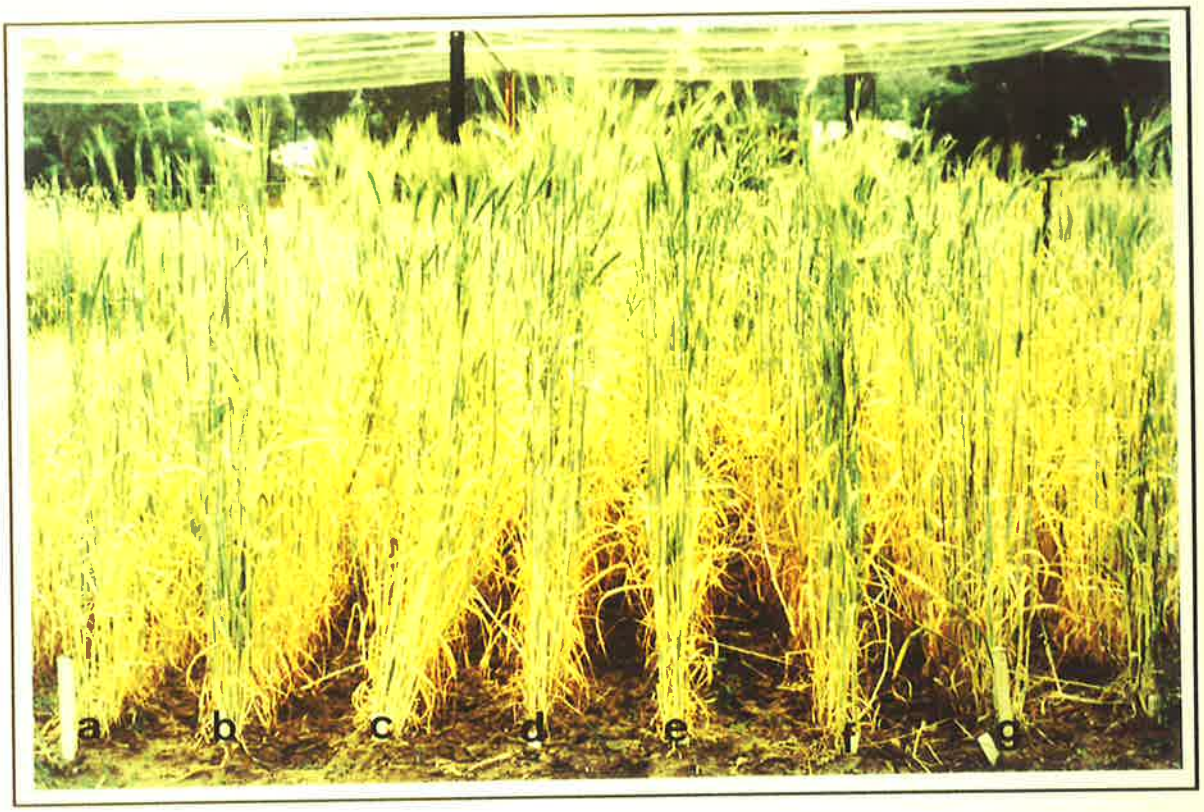



**Figure 7.3** General appearance of the $F_2$-derived progeny lines, and the parents growing in a bird-proof enclosure.

(a), Type-IV; (b), Langdon (Edmore-1B); (c), Langdon 1D(1A); (d), Langdon; (e), Type-III; (f), Type-I; (g), Type-II.

**Table 7.3** Mean values for grain yield and certain quality characteristics in $F_2$-derived progeny of durum wheat 1D and 1B substitution lines and their respective parents[a]

| Lines[b] | Grain yield (g) | PC | HI | SE-HPLC analysis | | | | Mixograph parameters | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | $P_{1-1}$% | $P_2$% | $P_1/P_2$ | $P_{1-1}/P_2$ | BWAP | RBD | BBD | TPBW | PBW |
| **Langdon** | 57.1 | 13.0 | 83.5 | 28.2 | 52.6 | 0.8 | 0.54 | 130 | 23.3 | 24.1 | 126 | 131 |
| Type-I | 73.8 | 14.2 | 88.0 | 30.8 | 49.7 | 0.9 | 0.62 | 123 | 20.9 | 25.6 | 101 | 136 |
| **Ldn (Edmore-1B)** | 85.0 | 13.7 | 87.0 | 37.2 | 44.5 | 1.1 | 0.84 | 146 | 6.9 | 23.3 | 199 | 180 |
| Type-II | 75.7 | 14.0 | 87.8 | 35.6 | 45.1 | 1.1 | 0.79 | 137 | 8.8 | 20.7 | 184 | 177 |
| **Ldn 1D(1A)** | 44.8 | 13.4 | 82.7 | 38.1 | 42.3 | 1.3 | 0.90 | 183 | 9.3 | 25.5 | 330 | 238 |
| Type-III | 37.4 | 14.0 | 83.5 | 37.8 | 41.8 | 1.3 | 0.92 | 181 | 7.8 | 24.8 | 294 | 227 |
| Type-IV | 36.0 | 13.7 | 82.7 | 42.8 | 37.2 | 1.6 | 1.16 | 239 | 8.6 | 17.0 | 417 | 246 |
| Grand mean | 58.5 | 13.7 | 85.0 | 35.8 | 44.8 | 1.2 | 0.82 | 163 | 12.2 | 22.6 | 236 | 191 |
| PLSD[c] | 24.6 | 0.9 | 4.4 | 2.4 | 1.4 | 0.1 | 0.08 | 15.1 | 4.3 | 8.4 | 43 | 14 |
| Significant level[c] | *** | n.s. | * | **** | **** | **** | **** | **** | **** | n.s. | **** | **** |

[a] = Symbols used in this table are bracketed. Protein concentration (PC) (in %); Hardness index (HI) (in %); Percentage of highly aggregating glutenin, total glutenin and gliadin ($P_{1-1}$%, $P_1$% and $P_2$%); Bandwidth at peak resistance (BWAP), peak bandwidth (PBW), resistance break down (RBD), bandwidth break down (BBD) (in mixograph Bradender units); time to peak bandwidth (TPBW) (in sec);

[b] = The parents are given in bold type;

[c] = Multiple comparisons were performed by Tukey Compromise test and significant difference groups were similar to the Fishers' Protected LSD test. Therefore, the latter one is shown for simplicity. *, **, ***, **** were significant at 5%, 1%, 0.1% and 0.01% probability level, respectively. n.s. = non-significant.

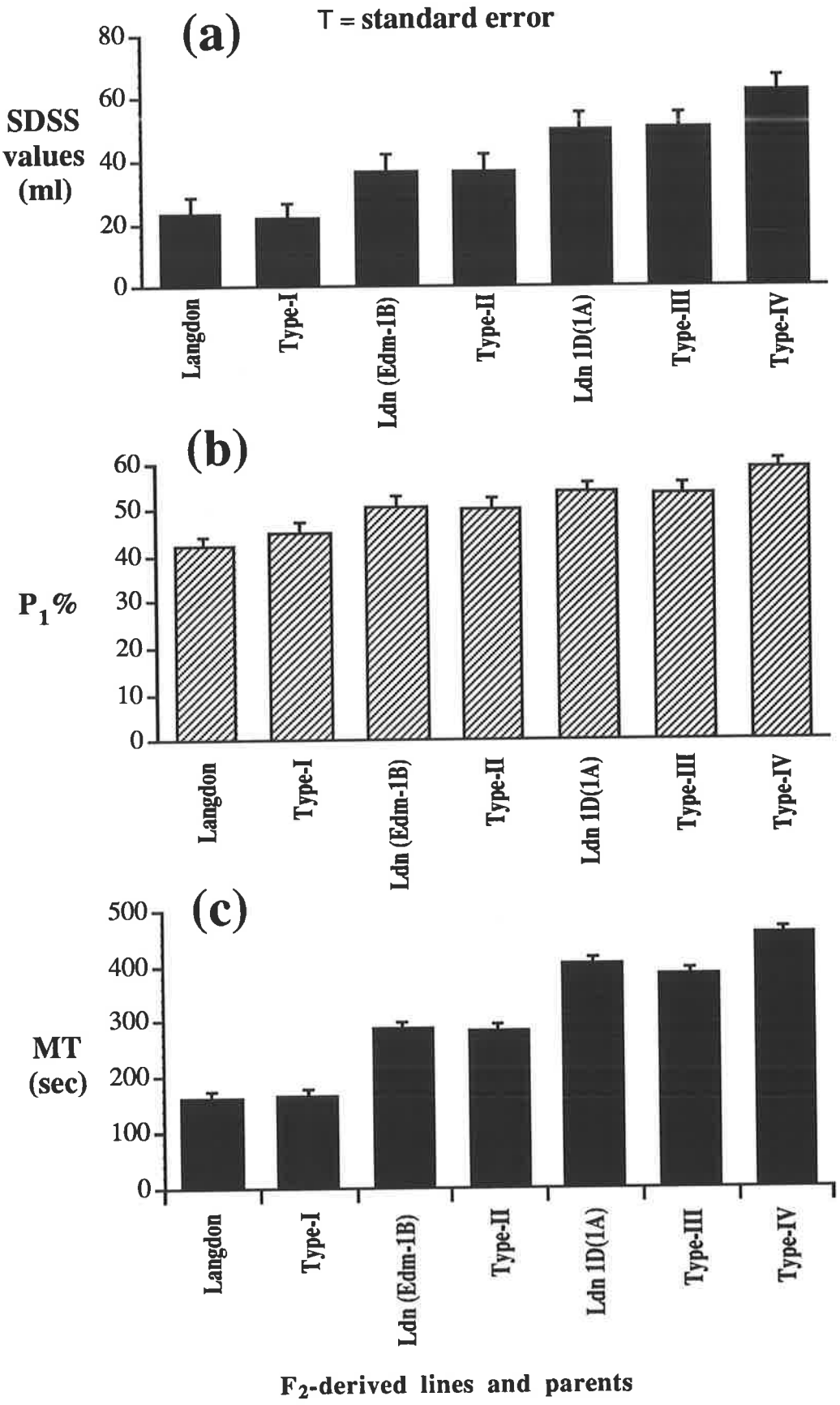



**Figure 7.6** Mean values of (a) SDS-sedimentation values, (b) proportion of glutenin ($P_1$%) and (c) mixograph mixing time of four $F_2$-derived progeny lines and parents.

**Table 7.4** Comparison of the effects of major LMW glutenin subunits and chromosomes on certain quality characteristics in the $F_2$-derived progeny of durum wheat 1D and 1B substitution lines[a]

| | Yield | PC | HI | SDSS | SE-HPLC parameters | | | | Mixograph parameters | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | $P_1$% | $P_2$% | $P_1/P_2$ | $P_{1\text{-}1}/P_2$ | MT | PR | BWAP | RBD | BBD | TPBW | PBW |
| *Glu-B3/Gli-B1* compared | | | | | | | | | | | | | | | |
| + *Glu-B3b'* | 55.6 | 13.8 | 85.3 | 49.7 | 54.5 | 41.2 | 1.35 | 0.97 | 369 | 263 | 188 | 8.73 | 18.8 | 300 | 212 |
| - *Glu-B3b'* | 55.8 | 14.1 | 85.8 | 36.0 | 49.4 | 45.7 | 1.10 | 0.77 | 273 | 217 | 152 | 14.3 | 23.7 | 198 | 182 |
| Significant level | n.s. | n.s. | n.s. | * | * | * | * | * | * | n.s. | n.s. | * | n.s. | * | n.s. |
| Chromosomes compared | | | | | | | | | | | | | | | |
| + 1D | 36.7 | 13.8 | 83.1 | 56.3 | 56.2 | 39.5 | 1.43 | 1.03 | 419 | 297 | 210 | 8.19 | 20.9 | 355 | 237 |
| - 1D | 74.8 | 14.1 | 87.9 | 29.4 | 47.7 | 47.4 | 1.01 | 0.71 | 224 | 184 | 130 | 14.9 | 21.6 | 143 | 157 |
| Significant level | **** | n.s. | ** | **** | **** | **** | **** | **** | **** | **** | **** | * | n.s. | **** | **** |

[a] = Symbols as in Table 7.3. SDS sedimentation volume (SDSS) (in ml); Mix time (MT) (in sec); Peak resistance (PR - in mixograph Brabender units), *, **, ***, **** were significant at 5%, 1%, 0.1% and 0.01% probability level, respectively. n.s. = non-significant.

**Table 7.5** Mean effects of different chromosome constitutions on major quality characteristics in the $F_2$-derived progeny of durum wheat 1D and 1B substitution lines[a]

| | Yield | PC | HI | SDSS | SE-HPLC parameters | | | | | Mixograph parameters | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | $P_{1-1}$% | $P_1$% | $P_2$% | $P_1/P_2$ | $P_{1-1}/P_2$ | MT | PR | BWAP | RBD | BBD | TPBW | PBW |
| Effect of chromosome 1B (*Glu-B3* locus) | 0.11 | -0.12 | -0.25 | 6.81 | 2.47 | 2.65 | -2.28 | 0.13 | 0.10 | 47.9 | 23.2 | 17.9 | -2.8 | -2.42 | 51.4 | 15.0 |
| | n.s. | n.s. | n.s. | *** | *** | *** | *** | *** | *** | *** | *** | *** | *** | n.s. | *** | *** |
| Effect of chromosome 1D | -19.03 | -0.12 | -2.42 | 13.48 | 3.53 | 4.30 | -3.93 | 0.21 | 0.17 | 97.3 | 56.5 | 40.1 | -3.34 | -0.38 | 106 | 39.8 |
| | *** | n.s. | ** | *** | *** | *** | *** | *** | *** | *** | *** | *** | *** | n.s. | *** | *** |
| Interaction | -0.85 | -0.01 | -0.17 | -0.89 | 0.06 | -0.01 | -0.02 | 0.02 | 0.02 | -10.8 | -7.7 | 11.0 | 3.2 | -1.5 | 10.2 | -5.5 |
| effects | n.s. | n.s. | n.s. | n.s. | n.s. | n.s. | n.s. | n.s. | n.s. | n.s. | * | *** | *** | n.s. | * | * |

[a] = Symbols as in Tables 7.3 and 7.4. *, **, *** were significant at 5%, 1% and 0.1% probability level, respectively. n.s. = non-significant.

The simple correlation coefficients between the different quality characters and grain yield calculated on the phenotypic means for all pair-wise comparisons, are presented in Table 7.6. Although the meal protein concentration (PC) ranged from 11.6 to 16.1%, it was not significantly correlated with grain yield nor with $P_1$% and $P_2$%, indicating that PC did not reflect yield changes, nor did the relative amounts of protein components. PC was also not significantly correlated with other quality characters including SDSS and all mixograph parameters, simply because there were no significant differences of PC among the phenotypes studied (Table 7.3). On the other hand, SDSS, a good predictor of gluten strength (Dexter *et al*, 1981), was correlated with all of the other quality parameters at the 1% or 0.01% levels, except BBD, indicating a high degree of interrelationship among these quality characters. $P_1$% was strongly inversely correlated with $P_2$% ($r = -0.999$, $P < 0.01$%) and also highly significantly correlated with the other major quality attributes (Table 7.6). This demonstrated that the quality characters were mainly influenced by the proportion of HPLC fractions ($P_1$% and $P_2$%) rather than the total protein amounts, which agreed with the earlier finding of Singh *et al* (1990b). The first peak for aggregated protein in the SE-HPLC profile could be further sub-divided into two fractions, representing glutenin of high and intermediate size aggregates ($P_{1-1}$ and $P_{1-2}$, and see Fig. 6.2, Chapter 6). $P_{1-1}$% was found to be highly correlated with the most important parameters of mixing properties, along with $P_1$%, ratio of aggregates to monomeric proteins ($P_1/P_2$) and $P_{1-1}/P_2$, indicating that the glutenin quantity, especially the proportion of highly aggregated glutenins ($P_{1-1}$%), rather than gliadin quantity, was the causal factor influencing the variation of dough quality and gluten strength.

### 7.3.3 Comparisons of the grain yield and quality of the $F_2$-derived progeny lines with normal tetraploid and hexaploid wheats

A separate field trial similar to the above experiment was conducted including four tetraploid and four bread wheat as controls, along with the $F_2$-derived progeny lines and their respective parents. Similar quality tests were also performed separately as in section 7.3.2.

Analyses of variance showed that there were significant genotypic effects for all characters, except for $P_{1-2}$ % and $P_{1-1}/P_{1-2}$ of the protein components (Table 7.7).

#### *7.3.3.1 Grain yield*

Cultivars Edmore and Yallaroi ranked the highest among the durum wheat lines but their yields were still well below that of all hexaploids except Chinese Spring (Table 7.8). The durum 1D substitution lines had the lowest yield of most genotypes analysed, confirming the earlier observation that chromosome 1D of hexaploid wheat did not compensate well for the removal of 1A (Chapter 6 and section 7.3.2). The grain yields of the three local hexaploid wheats were

**Table 7.6** Simple correlation coefficients between grain quality and yield characteristics (calculated on mean values) in $F_2$-derived progeny and their parents of durum wheat 1D and 1B substitution lines[a]

| Name | Yield | PC | HI | SDSS | HPLC parameters | | | | | | | | Mixograph parameters | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | $P_{1-1}$% | $P_{1-2}$% | $P_1$% | $P_2$% | $P_{1-1}/P_{1-2}$ | $P_1/P_2$ | $P_{1-1}/P_2$ | $P_3$% | MT | PR | BWAP | RBD | BBD | TPBW |
| PC | 0.22 | | | | | | | | | | | | | | | | | |
| HI | 0.90 | 0.58 | | | | | | | | | | | | | | | | |
| SDSS | -0.70 | 0.06 | -0.63 | | | | | | | | | | | | | | | |
| $P_{1-1}$% | -0.45 | 0.24 | -0.38 | 0.95 | | | | | | | | | | | | | | |
| $P_{1-2}$% | -0.86 | 0.15 | -0.64 | 0.82 | 0.67 | | | | | | | | | | | | | |
| $P_1$% | -0.54 | 0.23 | -0.45 | 0.97 | 0.99 | 0.76 | | | | | | | | | | | | |
| $P_2$% | 0.54 | -0.26 | 0.43 | -0.97 | -0.99 | -0.75 | -1.00 | | | | | | | | | | | |
| $P_{1-1}/P_{1-2}$ | 0.01 | 0.26 | -0.02 | 0.69 | 0.87 | 0.23 | 0.80 | -0.80 | | | | | | | | | | |
| $P_1/P_2$ | -0.59 | 0.20 | -0.49 | 0.98 | 0.98 | 0.77 | 0.99 | -0.99 | 0.77 | | | | | | | | | |
| $P_{1-1}/P_2$ | -0.55 | 0.21 | -0.46 | 0.97 | 0.99 | 0.73 | 0.99 | -0.99 | 0.81 | 1.00 | | | | | | | | |
| $P_3$% | 0.57 | -0.13 | 0.48 | -0.96 | -0.93 | -0.81 | -0.96 | 0.95 | -0.71 | -0.94 | -0.93 | | | | | | | |
| MT | -0.67 | 0.07 | -0.61 | 0.99 | 0.95 | 0.82 | 0.97 | -0.97 | 0.70 | 0.97 | 0.96 | -0.97 | | | | | | |
| PR | -0.67 | 0.11 | -0.59 | 0.97 | 0.93 | 0.85 | 0.96 | -0.95 | 0.66 | 0.94 | 0.93 | -0.96 | 0.99 | | | | | |
| BWAP | -0.78 | -0.03 | -0.72 | 0.95 | 0.87 | 0.81 | 0.90 | -0.90 | 0.56 | 0.94 | 0.93 | -0.84 | 0.92 | 0.89 | | | | |
| RBD | 0.17 | -0.28 | 0.12 | -0.78 | -0.88 | -0.45 | -0.85 | 0.84 | -0.90 | -0.79 | -0.81 | 0.85 | -0.81 | -0.80 | -0.56 | | | |
| BBD | 0.19 | -0.07 | 0.13 | -0.54 | -0.58 | -0.30 | -0.56 | 0.57 | -0.55 | -0.61 | -0.6 | 0.54 | -0.46 | -0.37 | -0.59 | 0.37 | | |
| TPBW | -0.76 | -0.04 | -0.72 | 0.99 | 0.91 | 0.83 | 0.94 | -0.93 | 0.62 | 0.96 | 0.94 | -0.92 | 0.98 | 0.96 | 0.98 | -0.68 | -0.51 | |
| PBW | -0.70 | 0.07 | -0.63 | 0.98 | 0.92 | 0.85 | 0.96 | -0.95 | 0.65 | 0.94 | 0.93 | -0.95 | 0.99 | 1.00 | 0.90 | -0.79 | -0.37 | 0.97 |

[a] = Symbols as in Tables 7.3 and 7.4. Values ≥ 0.75, 0.88, 0.95 and 0.98 were significant at 5%, 1%, 0.1% and 0.01% probability levels, respectively.

**Table 7.7** The mean, range and F-test values of grain yield and quality characteristics of $F_2$-derived progeny lines of durum wheat 1D and 1B substitution lines and normal tetraploid and hexaploid wheats[a]

| Characteristic | Mean | Range | | | | F tests | |
|---|---|---|---|---|---|---|---|
| | | Min. - Max. | SD[b] | SE[b] | CV[b] | Treatment | Replicate |
| Yield | 96.9 | 25.0 - 276 | 78.3 | 14.1 | 80.9 | **** | * |
| PC | 12.8 | 9.6 - 15.7 | 1.5 | 0.26 | 11.5 | *** | *** |
| HI | 78.7 | 47.0 - 92.0 | 12.1 | 2.17 | 15.4 | **** | n. s. |
| SDSS | 42.3 | 21.5 - 69.0 | 12.0 | 2.15 | 28.3 | **** | n. s. |
| $P_{1-1}$% | 36.0 | 27.0 - 47.0 | 5.0 | 1.00 | 14.8 | ** | **** |
| $P_{1-2}$% | 16.0 | 13.0 - 21.0 | 2.0 | 0.30 | 10.2 | n. s. | *** |
| $P_1$% | 52.0 | 42.0 - 61.0 | 5.0 | 1.00 | 9.8 | **** | **** |
| $P_2$% | 43.0 | 36.0 - 54.0 | 5.0 | 1.00 | 10.7 | **** | *** |
| $P_{1-1}/P_{1-2}$ | 2.3 | 1.5 - 3.5 | 0.5 | 0.08 | 20.3 | n. s. | **** |
| $P_1/P_2$ | 1.2 | 0.8 - 1.7 | 0.3 | 0.04 | 20.0 | *** | *** |
| $P_{1-1}/P_2$ | 0.9 | 0.5 - 1.3 | 0.2 | 0.04 | 24.9 | ** | **** |
| $P_3$% | 4.0 | 3.0 - 7.0 | 1.0 | 0.16 | 21.5 | *** | *** |
| MT | 276 | 124 - 468 | 89.6 | 16.1 | 32.5 | **** | n. s. |
| PR | 212 | 114 - 310 | 48.7 | 8.7 | 23.0 | **** | n. s. |
| BWAP | 156 | 106 - 250 | 36.3 | 6.5 | 23.3 | **** | n. s. |
| RBD | 13.1 | 2.0 - 31.5 | 8.4 | 1.5 | 63.8 | ** | n. s. |
| BBD | 21.7 | 2.0 - 35.0 | 9.2 | 1.7 | 42.6 | ** | n. s. |
| TPBW | 204 | 80 - 462 | 80.9 | 14.5 | 39.7 | **** | n. s. |
| PBW | 183 | 107 - 262 | 34.3 | 6.2 | 18.8 | *** | n. s. |

[a] = Symbols as in Tables 7.3 and 7.4. *, **, *** and **** were significant at 5%, 1%, 0.1% and 0.01% probability level, respectively. n. s. = non-significant;

[b] = SD (standard deviation), SE (standard error) and CV (coefficient of variation).

more than two-fold higher than that of the commercial durums (i. e. Edmore, Vic and Yallaroi), confirming that these cultivars were much better adapted to the local environment than the durums (Table 7.8).

### *7.3.3.2 Grain protein level*

The mean grain protein concentration of genotypes ranged from 10.0 to 15.2% and was generally lower in the hexaploids than the tetraploids (Table 7.8), resulting in an overall negative correlation between PC and yield. PC was also significantly negatively correlated with other quality characters including SDSS and most mixograph parameters. However within the

**Table 7.8** The average genotypic values for grain yield and certain quality characteristics of $F_2$-derived progeny of durum wheat 1D and 1B substitution lines and normal tetraploid and hexaploid wheats[a]

| Genotype[b] | Yield (g) | PC (%) | HI | $P_{1-1}$% | $P_1/P_2$ | RBD | BBD |
|---|---|---|---|---|---|---|---|
| Tetraploid | | | | | | | |
| **Langdon** | 36.6 | 13.5 | 89.0 | 27.8 | 0.82 | 20.5 | 21.8 |
| Type-I | 81.9 | 13.4 | 90.0 | 30.2 | 0.89 | 18.3 | 14.3 |
| **Ldn (Edmore-1B)** | 77.0 | 13.7 | 87.0 | 33.5 | 1.08 | 6.8 | 20.5 |
| Type-II | 91.5 | 14.5 | 89.0 | 35.9 | 1.18 | 8.5 | 21.3 |
| Edmore | 105 | 13.5 | 89.0 | 33.3 | 1.10 | 3.5 | 5.3 |
| Vic | 38.0 | 15.2 | 89.5 | 37.3 | 1.20 | 3.3 | 9.3 |
| Yallaroi | 90.2 | 12.2 | 81.0 | 41.7 | 1.49 | 23.0 | 25.3 |
| Ldn 1D(1B) | 27.7 | 12.9 | 77.0 | 32.8 | 1.03 | 23.0 | 32.8 |
| **Ldn 1D(1A)** | 50.7 | 12.2 | 79.5 | 37.3 | 1.36 | 9.3 | 22.5 |
| Type-III | 41.1 | 13.4 | 82.5 | 39.4 | 1.41 | 6.3 | 27.8 |
| Type-IV | 42.4 | 13.3 | 79.5 | 41.4 | 1.48 | 6.5 | 15.8 |
| Hexaploid | | | | | | | |
| CS | 77.8 | 12.8 | 47.0 | 36.1 | 1.14 | 19.5 | 28.8 |
| Halberd | 245 | 11.1 | 65.5 | 35.3 | 1.19 | 17.2 | 25.8 |
| Warigal | 268 | 10.0 | 63.5 | 41.0 | 1.51 | 24.2 | 34.0 |
| Gabo | 202 | 11.8 | 71.0 | 41.5 | 1.50 | 12.0 | 28.0 |
| Mean | 98.3 | 12.9 | 78.7 | 36.3 | 1.20 | 13.5 | 22.2 |
| LSD[c] | 60.5 | 1.5 | 6.9 | 5.0 | 0.20 | 10.9 | 11.5 |
| Sig. level[c] | **** | *** | **** | *** | *** | ** | ** |

[a] = Symbols as in Tables 7.3 and 7.4;

[b] = The parents are given in bold type;

[c] = Multiple comparisons were performed by Tukey Compromise test and significant difference groups were similar to the Fishers' Protected LSD test. Therefore, the latter one is shown for simplicity. **, *** and **** were significant at 1%, 0.1% and 0.01% probability level, respectively.

tetraploid wheats, there was no significant relationship between PC and yield, nor with any of the quality characters. It was concluded that the overall correlation was probably due to the high values for yield and quality characters among the hexaploids and their generally lower

protein level, resulting in spurious correlations when all data were included in the analysis. Among the hexaploid wheats themselves, on the other hand, PC was negatively correlated with yield but not with quality parameters. For the size-exclusion chromatography fractions, the proportion of highly aggregated or total glutenin fractions ($P_{1\text{-}1}$% or $P_1$%) were strongly negatively correlated with $P_2$%, $P < 0.01$%) and positively with other protein parameters ($P_1/P_2$ and $P_{1\text{-}1}/P_2$).

### *7.3.3.3 Grain hardness*

The grain hardness index (HI) of the lines tested showed much variation with Chinese Spring having the softest endosperm (HI = 47) and durum wheat the hardest type (HI > 80) (Table 7.8). The three Australian bread wheat cultivars gave intermediate grain hardness readings and were classified commercially as Australian Standard White class. The HI values for the durum 1D substitution lines were slightly higher than those of the ASW hexaploids except Gabo but lower than those of the commercial durum wheat cultivars except Yallaroi.

### *7.3.3.4 Grain quality characteristics*

#### (A) Comparison within the $F_2$-derived progeny lines and their parents

The grain quality characters of the $F_2$-derived tetraploid lines and parental types analysed showed the same features as described in the previous section (section 7.3.2). In general the genotypes could be rated for quality as follows: Langdon = type-I < Langdon (Edmore-1B) = type-II < Langdon 1D(1A) = type-III < type-IV with respect to SDSS, $P_1$% and major positive mixograph parameters (MT and PR) (Figs 7.7 and 7.8). These results confirmed that chromosome 1D had a much greater effect on quality characters than 1B prolamin alleles. Although there was an apparent cumulative effect on quality when the 1D substitution was combined with improved 1B prolamin alleles (Figs 7.7 and 7.8), as observed earlier (section 7.3.2), but the increments were small and not significant in the current experiment except MT, probably because two replicates were used instead of six earlier (section 7.3.2).

#### (B) Comparison with tetraploid wheats

All of the commercial durums were γ-45 types. Edmore and Vic were very similar for all quality parameters and Australian durum Yallaroi gave significant higher $P_1$% and major differences in mixing properties (MT, RBD and BBD), except SDSS and PR (Table 7.8 and Figs 7.7 and 7.8).

It was apparent that type-I $F_2$ progeny lines (with γ-42 and LMW-1) had poorer quality and SDSS values when compared to the commercial durums (Table 7.8 and Fig. 7.7a). However, the type-II $F_2$ progeny lines (with γ-45 and LMW-2) had similar quality characteristics to Edmore and Vic in most respects, which were also γ-45 types. These durum

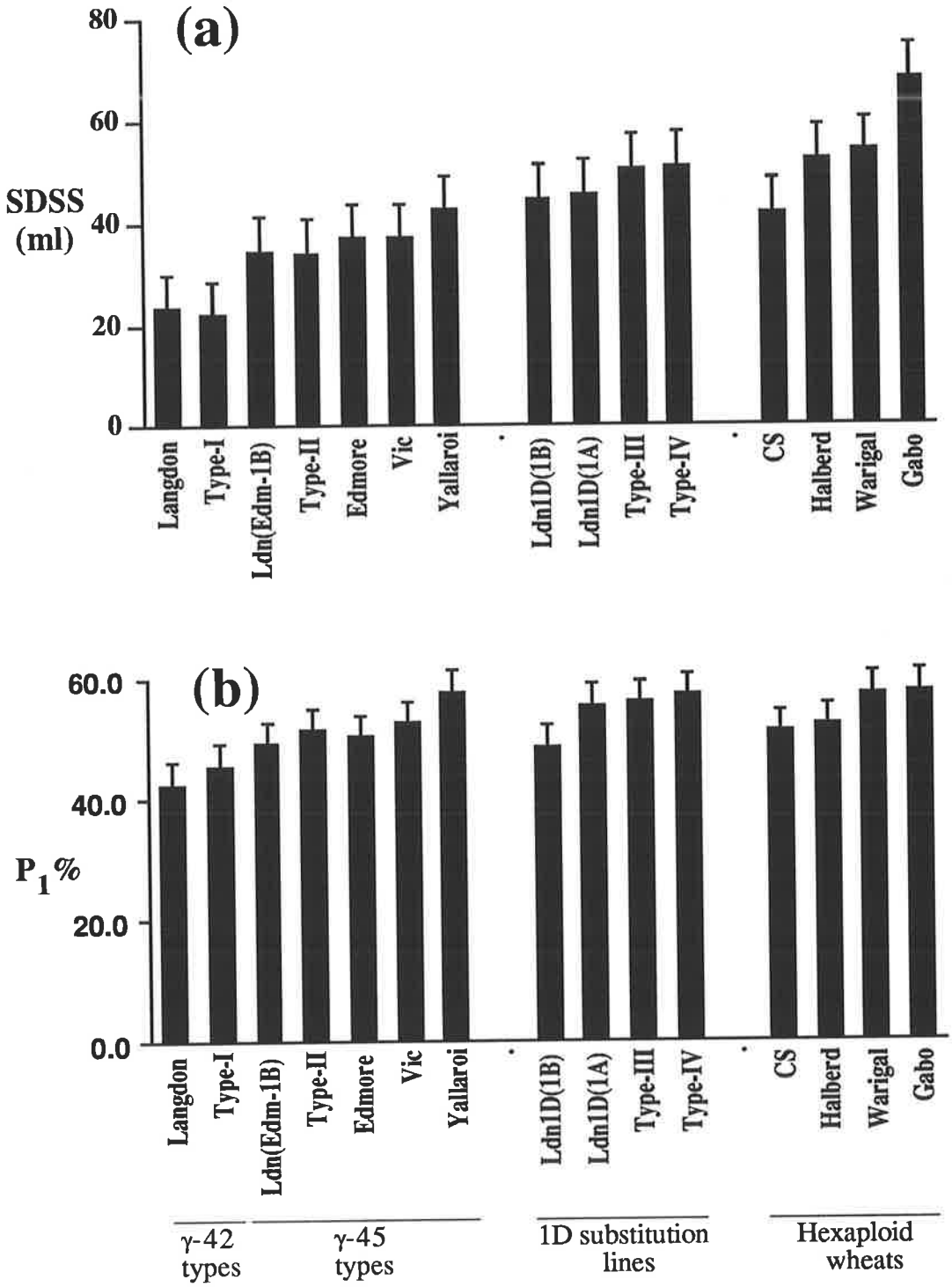



**Figure 7.7** Mean values for (a) SDSS and (b) $P_1$% (SE-HPLC) of $F_2$-derived progeny lines of durum wheat 1D and 1B substitution lines and normal tetraploid and hexaploid wheats (CS, Halberd, Warigal and Gabo). The error bar of each column represents the standard error of the mean.

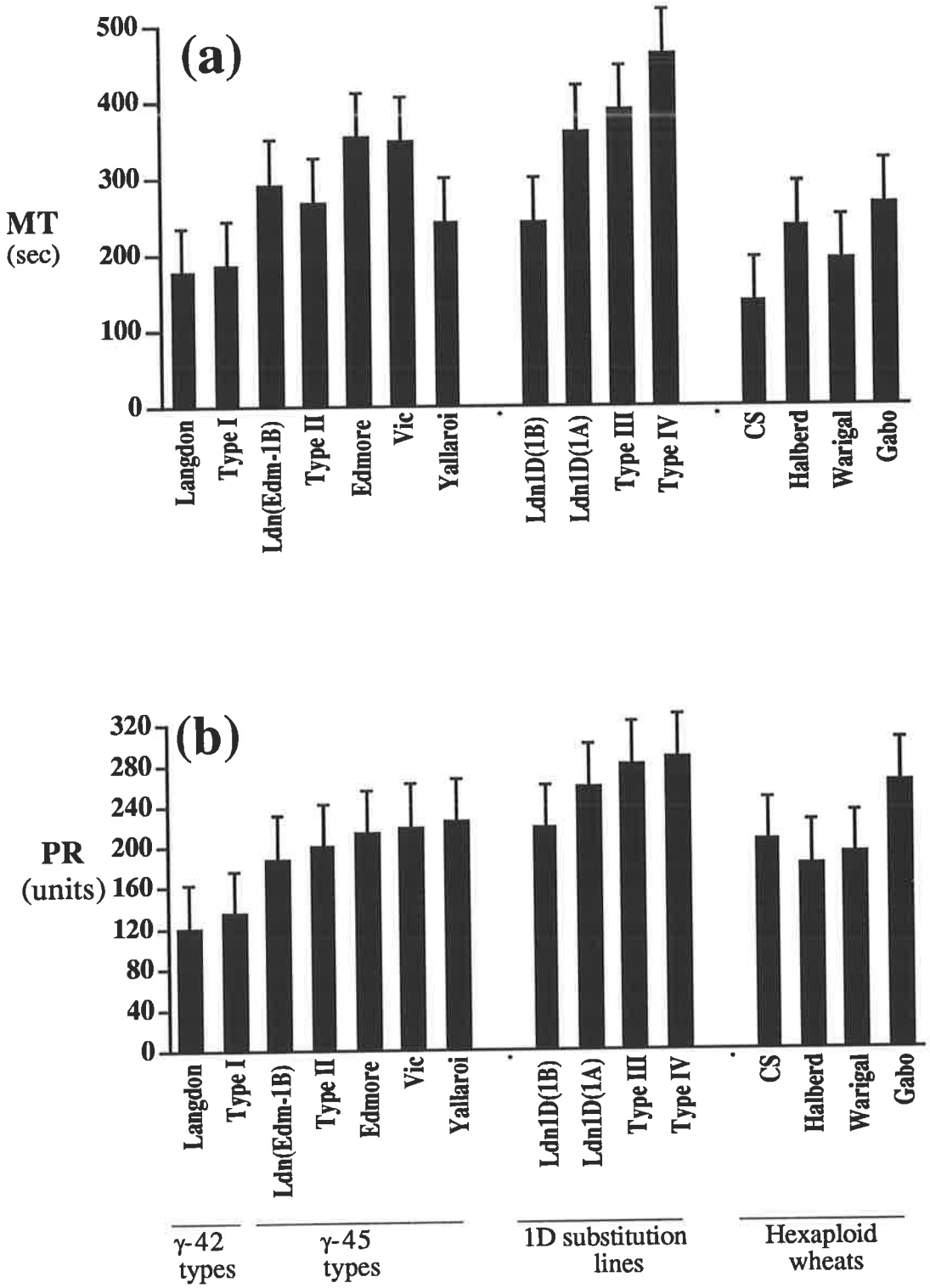



**Figure 7.8** Mean values of (a) dough mix time and (b) peak resistance of $F_2$-derived progeny lines of durum wheat 1D and 1B substitution lines and normal tetraploid and hexaploid wheats (CS, Halberd, Warigal and Gabo). The error bar of each column represents the standard error of the mean.

wheat cultivars were known to have high dough strength (Joppa *et al*, 1983b; Quick and Crawford, 1983).

Substitution of chromosome 1D for chromosomes 1A and 1B had a large effect on the quality parameters of the derived durum wheats. The Langdon 1D(1A) substitution lines gave similar SDSS and BBD values to Langdon 1D(1B) but significantly higher values for $P_1$%, MT and PR and lower values for RBD (Table 7.8 and Figs 7.7 and 7.8). The quality differences between Langdon 1D(1A) and 1D(1B) substitution lines were attributed to chromosome 1B having a large influence on quality characters compared to chromosome 1A. In fact, the Ldn 1D(1B) line showed similarities to the γ-45 type durums with respect to some quality characters (Table 7.8 and Figs 7.7 and 7.8).

The best of the 1D substitutions (types-III and -IV) were superior to the commercial durum with respect to SDSS and $P_1$% values, except for Yallaroi which had a similar $P_1$% (Fig. 7.7). In addition, type-IV was superior to most of durums for MT and PR (Fig. 7.8)

### (C) Comparison with bread wheat cultivars

Chinese Spring, the donor of chromosome 1D to Ldn 1D(1A) and 1D(1B) substitutions, is a Soft Red Spring hexaploid wheat and had poor bread-making quality. It was inferior to all or most of other hexaploids with respect to SDSS, $P_1$% and MT but not for PR, RBD and BBD (Table 7.8 and Figs 7.7 and 7.8). Halberd and Warigal, representing medium dough quality, were similar to each other for all characters except significantly less $P_1$% in Halberd. Gabo gave largest values for all positive characters and significantly more than other hexaploids for SDSS and PR.

The γ-42 types were inferior to hexaploids in all characters except some mixing properties including MT, RBD and BBD (Table 7.8 and Figs 7.7 and 7.8). The γ-45 types, including commercial durums had less SDSS than all hexaploids except for Chinese Spring, but similar in $P_{1\text{-}1}$%, $P_1$% and $P_1/P_2$ values, and equal or better mixing characters (MT and PR) in nearly all cases.

The best of substitution lines, type-IV (and usually type-III also) were equal or better in SDSS (except for Gabo) and $P_1$%, but significantly higher in MT and PR and less in RBD (Table 7.7 and Fig. 7.8).

## 7.4 DISCUSSION

### 7.4.1 Comparisons among the durum wheat progeny lines

It is important to note that since the progeny analysed in the first experiment (section 7.3.2) were closely related genetically, the genetic basis of the quality differences could be ascribed only to the segregation of genes on homoeologous group 1 chromosomes. This allowed the association of glutenin subunit alleles (or gliadin alleles) with quality attributes to be more precisely measured.

The Langdon type plants i. e. type-I (γ-42 and LMW-1) produced on average a much lower amount of the highly-aggregated glutenin ($P_{1-1}$%) compared with Langdon (Edmore-1B) type plants i. e. type-II (γ-45 and LMW-2) in the current study. This difference in the amount of highly-aggregated protein (49.4 vs. 54.5%, $P < 0.05$) was due mainly to the allelic variation at the *Glu-B3/Gli-B1* loci, which was consistent with previous results (Damidaux *et al*, 1978; du Cros *et al*, 1982; Joppa *et al*, 1983b; Autran *et al*, 1987; Autran and Galterio, 1989a, 1989b). Since the monomeric type gliadins (γ-42 and γ-45 types) could not account for the differences in gluten characteristics of durum wheat observed earlier (Autran *et al*, 1987; Feillet *et al*, 1989), it is likely that the quality effects observed in the present study were due to the LMW glutenin subunits. In the earlier study (Autran *et al*, 1987), it was shown that there were large differences in the relative amounts of these two groups of proteins (14% for LMW-1 vs. 27% for LMW-2).

Substituting chromosome 1D for 1A in the $F_2$-derived progeny incorporated the HMW glutenin subunit allele *Glu-D1a* (bands 2+12) as well as the LMW glutenin subunit allele and gliadin genes (i. e. *Glu-D3/Gli-D1*) in place of the corresponding alleles from chromosome 1A. This resulted in much higher values for $P_1$% and much lower $P_2$% values as expected, confirming that relatively more aggregated proteins have been synthesized in the presence of chromosome 1D in a durum wheat background with a relatively stable grain protein level. The increased amount of $P_1$% or $P_{1-1}$% was associated, perhaps causally, with increases in gluten strength as measured by SDSS, MT, PR. The values for some of these characters were doubled compared to incorporating the LMW-2 genes. However, incorporation of both chromosome 1D together with gliadin band 45 (or LMW-2) of chromosome 1B, resulted in the largest amounts of highly aggregated glutenins, and also the highest/lowest values for some other positive/negative quality parameters. This demonstrated the cumulative effects of these gene products on quality parameters, as found earlier with other genes affecting quality characteristics in durum and bread wheats (Payne *et al*, 1988a; Gupta *et al*, 1989; Pogna *et al*, 1990). The cumulative effects were additive with respect to SDSS values and the HPLC fractions whereas the mixograph parameters PR and RBD showed significant negative interactions.

The application of sonication to allow complete extraction of total wheat endosperm proteins combined with the SE-HPLC technique, has revolutionized wheat quality prediction at the micro-level (Singh *et al*, 1990a). It is now possible to use this technique to compare the seed protein molecular weight distributions among different genotypes, since it was rapid, reproducible and accurate (Singh *et al*, 1990b). High positive correlations were observed between $P_1$%, $P_{1-1}/P_2$, $P_1/P_2$ indicating that any of these parameters could be used for quality prediction. Variation in quality characters was found to be closely related to the percentage of the large-sized aggregated glutenins ($P_{1-1}$%), regardless of total protein content. Therefore, it is possible that the influence on gluten quality was actually caused by the variation in the total amounts of highly-aggregated glutenin. In general, the larger the $P_{1-1}$%, $P_1$%, or greater the

$P_1/P_2$ ratio, the better the quality, as demonstrated earlier (Feillet *et al*, 1989; Dachkevitch and Autran, 1989; Singh *et al*, 1990b).

The current results suggested that improving gluten strength in durum wheat could be achieved by introducing favourable protein genes from hexaploid wheat, especially those on chromosome 1D, which control the production of seed proteins with higher aggregating ability. It has been observed that removal of the *Glu-D1* locus reduced the dough rheological parameters of hexaploid wheats dramatically (Payne *et al*, 1987a, 1988c; Lawrence *et al*, 1988) whereas inclusion of this locus, especially the allele codes for the 5+10 subunits, resulted in a large increase in bread-making quality (Burnouf and Bouriquet, 1980; Payne and Lawrence, 1983; Moonen *et al*, 1983). This locus has been shown to have the largest effect on the baking characteristics of common wheats as well as on other synthesized hexaploid wheats (Kerber and Tipples, 1969; Moonen and Zeven, 1985; Lagudah *et al*, 1987). Compared to hexaploid wheats, durum wheats usually lack functional *Glu-A1* genes and of course the *Glu-D1* genes (Bietz and Wall, 1972; Orth and Bushuk, 1973b; Bietz *et al*, 1975; Payne *et al*, 1981b, 1984b; Vallega, 1986). Thus, combining the better known alleles such as *Glu-D1d* (coding for HMW bands 5+10) from hexaploid wheat with the positive alleles from chromosome 1B of durum wheat (eg. LMW-2) would be a feasible way to further improve gluten quality potential of durum wheats, and this possibility is now being subjected to detailed investigation in this laboratory and elsewhere (Ceoloni *et al*, 1993; Tsunewaki and Matsuda, 1993).

### 7.4.2 Comparisons between durum and bread wheats

Historically, the yield of durum wheats has been lower than bread wheats (Quick and Crawford, 1983) but modern breeding has narrowed the difference and in some growing areas durum wheat cultivars now equal hexaploid wheats in yield (Breth, 1984 and see Chapter 6 for details). The three local bread wheat cultivars included in the trial gave relatively high yields reflecting their better adaptation to the local growing conditions than the tetraploids. The Langdon chromosomal derivatives were produced in the USA and were genetic stocks rather than commercial varieties. In the USA the 1D substitution line [i. e. Ldn 1D(1B)] incurred a 10% yield loss compared to Langdon (Joppa *et al*, 1983b) whereas in the present experiment more than a 50% yield reduction was observed, indicating that these lines were poorly adapted to the South Australian environment. Protein concentration and seed hardness index were distinctively different between the tetraploid and hexaploid wheats, whereas the 1D substitution lines were similar to the commercial durum wheats, for PC and other characters including plant morphology and seed colour etc.

Durum wheat has been used traditionally for pasta-making. The quality differences between durum and bread wheat have not been subjected to detailed investigation. In the present study, a comparison was set up to investigate the differences in gluten strength between durum and bread wheats as well as 1D substitution lines.

On the basis that SDSS values reflect dough strength, it was evident that γ-42 type durums gave the weakest doughs of all wheats tested whereas γ-45 type had better strength than the poorest quality bread wheats. The substitution of chromosome 1D for 1B retained dough strength at the level of γ-45 durums while when chromosome 1A was substituted along with a γ-42 type (i. e. Langdon 1D(1A) and type-III), the dough quality was almost equivalent to the medium strength bread wheats. The tetraploid type-IV, possessing γ-45 with 1D substitution did not show any significant increase in SDSS values in the latter experiment, unlike in the previous experiment (section 7.3.2). In contrast, if these lines were rated by their mixing properties, it appeared that all the 1D(1A) lines were the strongest mixing types, indicating large technological quality differences among the three types of wheat studied.

The large differences in quality characteristics among the derived Langdon lines could be related to changes in the proportion of glutenins ($P_1$%) or the highly aggregated glutenin ($P_{1\text{-}1}$%) fraction of each genotype, irrespective of the total protein concentration, as found in section 7.3.2. For example, the weak extensible gluten normally found in durum wheats has been ascribed to the high proportion of gliadins present (Dexter and Matsuo, 1977a, 1977b, 1978a), as observed in the present γ-42 durum types, which possessed the highest $P_2$% values. The highly aggregated glutenin fraction ($P_{1\text{-}1}$) corresponded to residue protein reported earlier (Orth *et al*, 1976), because sonication used here extracted almost all of the endosperm proteins (Singh *et al*, 1990a). The residue protein was the fraction normally left in flour after dilute acetic acid or urea extraction (Bushuk, 1985a; Lee and MacRitchie, 1971), and was known to possess larger molecular size aggregates than the other classes of wheat protein (Dexter and Matsuo, 1978a). The amount of residue protein has been found to be strongly positively correlated with most farinograph parameters and gluten strength (Dexter and Matsuo, 1980) and was negatively correlated with gluten viscosity and the proportion of albumins, globulins and soluble glutenins (Dexter and Matsuo, 1978a). Thus, the present results obtained with HPLC analyses were consistent with the previous investigations. Nevertheless, these criteria alone could not account for the variation in mixograph parameters observed in the diverse materials examined in the present study. For example, the medium and medium-strong quality bread wheat cultivars had the $P_1$% values higher than 57% and $P_1/P_2$ ratios larger than > 1.4, equivalent to type-IV, but showed significant large differences in their mixing time.

The anomalies in mixograph properties between the three types of wheat could not be explained by the proportion of their different protein components, since the glutenin to gliadin ratios were similar at the same level of gluten strength in both durum and bread wheats, as classified by SDSS values. The mixing characteristics measured in the farinograph have been used to classify gluten type (i. e. strong, medium or weak) (Bendelow, 1967; Dexter *et al*, 1980). Strong durum gluten showed a tendency for long MT, low RBD and wide PBW. Large PBW values were characteristic of a dough which possessed strong nonsticky gluten (Dexter and Matsuo, 1980). The observation that all the bread wheats except Gabo exhibited lower MT and PR and higher SDSS, as compared to the tetraploid wheats (except for γ-42 types), suggesting that bread wheats possessed inferior mixing properties to durums except for

the γ-42 types. In durum wheats, MT and PR were increased by the substitution of γ-45 (or LMW-2) for γ-42 (or LMW-1) phenotypes and from substituting chromosome 1D in place of 1A, consistent with the expectation that improved rheological characters were associated with improvements to the types of prolamin present. In contrast, the hexaploids with weak to medium strong dough strength, did not show a similar association between dough strength and mixing properties. All tetraploid wheats with the exceptions of Langdon 1D(1B) and Yallaroi were lower in RBD than the hexaploids, but there was no consistent trend for BBD (see section 7.3.1). The quality of the Australia durum wheat check cultivar Yallaroi was more like a bread wheat than the other durums. This line had moderate grain yield, desirable amber flour colour, and higher SDS-sedimentation values than Edmore and type-II but it exhibited a much shorter mixing time and larger dough breakdown (i. e. mixing tolerance index) than Vic and Edmore.

The uniqueness of the functional properties of durum gluten were associated with its traditional utilization in pasta-making and generally a medium gluten strength produced optimum spaghetti quality (Matsuo and Irvine, 1970). Semolina from γ-45 type durums generally gave better cooking quality compared to semolina from γ-42 types (Damidaux *et al*, 1978). Cooking quality of durum wheat has been shown to be influenced by many factors, including protein amount (Dexter and Matsuo, 1977a), gluten quality (Grzybowski and Donnelly, 1977, 1979; Kosmolak *et al*, 1980), gluten protein solubility (Dexter and Matsuo, 1978b), ratio of glutenin to gliadin (Wasik and Bushuk, 1975a; Dexter and Matsuo, 1977b ), SDSS and farinograph mixing characteristics (Matsuo and Irvine, 1970; Dexter and Matsuo, 1980). Therefore, no single quality parameter could satisfactorily predict cooking quality. For example, high ratios of glutenins to gliadins in durum might possess superior cooking qualities to those with a low ratio, but it was considered to be a prerequisite for good cooking quality not a guarantee (Wasik and Bushuk, 1975a; Dexter and Matsuo, 1977a, 1977b), since gluten quality was not only governed by the quantity of specific proteins but also by their quality. Mixing characteristics have also been inconsistent in predicting cooking quality of durum flours (Matsuo and Irvine, 1970; Walsh and Gilles, 1971; Dexter *et al*, 1981). The amount of residue protein was considered as the only seed protein fraction best representing cooking quality and overcooking quality (Dexter and Matsuo, 1980). The Ldn 1D(1A) substitution lines had very strong mixing properties, suggesting that it might have potential for better cooking quality.

The poorer baking quality of durum wheats (Quick and Crawford, 1983) has been ascribed to the absence of the D-genome chromosomes (Welsh and Hehn, 1964; Morris *et al*, 1966; Kerber and Tipples, 1969), although some durum wheats producing good loaves of bread have been reported (Quick and Crawford, 1983; Boggini and Pogna, 1989). The basic differences in quality traits required for good bread-making and pasta-making are still largely unknown. In a reconstitution study, it was found that addition of the gliadin-rich fraction of durum wheat resulted in a shorter MT and reduced cooking quality, whereas addition of the glutenin-rich fraction improved the cooking quality of durum wheat. However, addition of the glutenin-rich fraction of bread wheat to another bread wheat cv. Manitou, even at greater

proportion, resulted in little improvement in cooking quality (Dexter and Matsuo, 1978a). On the other hand, when a soluble protein fraction of bread wheat (cv. Manitou) was replaced by a durum wheat gluten fraction (cv. Wascana) at 30% level, the hexaploid wheat product resulted in fairly good overcooking quality but the reciprocal replacement of a soluble protein fraction of bread wheat into durum wheat, the durum product resulted in no tolerance to overcooking (Dexter and Matsuo, 1978a). Furthermore, when Hard Red Spring wheat farina having comparable dough properties (i. e. similar PC and MT) was added to durum wheat semolina, spaghetti cooking quality deteriorated significantly (Dexter *et al*, 1981). The data available clearly indicated that durum wheat was unique in cooking quality. Qualitative rather than quantitative differences of the gluten proteins might account for the dough rheological differences between durum and bread wheat (Dexter *et al*, 1981).

In general, durum doughs exhibit greater viscosity but less elasticity than the doughs from bread wheat. In the processing of spaghetti, dough is not fully developed and the gluten elasticity (not the viscosity) is the only requirement. The poorer bread-making quality of durums might in fact be due to their poorer strength in the case of γ-42 types and the slightly stronger mixing properties and lower medium strength in the case of γ-45 types, since dough with a well-balanced elasticity and viscosity with medium to medium-strong strength was required in the bread-making process. The addition of chromosome 1D to the durum wheat genome improved dough strength but resulted in strong mixing characters and might lead to unbalanced viscoelastic properties. This was unexpected, since the substituted chromosome 1D of Chinese Spring has inferior HMW glutenin alleles (coding for bands 2+12) and moderate LMW glutenin alleles (Payne, 1987c; Sozinov and Poperelya, 1980), with respect to bread-making quality (Payne *et al*, 1987c).

Besides qualitative differences in gluten proteins, durum wheat semolina/flour might also differ in other vital chemical components such as lipid content, the amount of -SH plus S-S groups in glutenin (Alary and Kobrehel, 1987; Kobrehel *et al*, 1988a; Kobrehel and Alary, 1989a, 1989b), which could affect the properties of stickiness and surface state of cooked pasta (Alary and Kobrehel, 1987; Kobrehel *et al*, 1988a, 1991). Differences in grain hardness, milling characteristics including starch damage, also contribute to the different mixing properties from flour of durum and bread wheats (Lindahl and Eliasson, 1992) and the effect of these factors requires further detailed experiment.

Semolina colour was another important quality criterion for the end-products of pasta and in hexaploid wheats was mainly affected by a major gene on chromosome 5D inhibiting yellow pigmentation. However, this gene was not present in durum wheats (see Chapter 6, section 6.3.2). The amber colour of durum wheat was still well retained following the substitution of chromosome 1D into a durum wheat background. Although the grain hardness of these 1D substitution lines was significantly reduced over commercial durums, they were still harder than the hard grained *T. aestivum* cultivars.

Further detailed research must be pursued, in order to fully characterise the quality differences among the three types of wheat (i. e. normal durum and bread wheats as well as the 1D substitution lines). The present study provided an indication for future work and large scale quality tests should be applied to these genotypes to confirm the present results. It is likely that the 1D substitution lines in durum wheat may provide basic material for improving pasta/baking quality but the end-use quality must be confirmed. Moreover, the quality differences between bread and durum wheat will be further investigated once superior alleles such as the HMW glutenin subunit gene *Glu-D1d* (coding for bands 5+10) is substituted into a durum wheat background.

# CHAPTER 8. GENERAL DISCUSSION

Many advances in our understanding of the functional quality of durum and bread wheats have been achieved in the last fifteen years of extensive research in cereal chemistry and the genetics of wheat gluten proteins. The gluten proteins present in the seed endosperm have attracted most attention, not only because they are the direct products of genes controlling flour technological quality, but because they also contribute to human nutrition.

## VARIATION AND GENETICS

The extent of variability in LMW glutenin subunit and gliadin band patterns was much greater in the 280 wild and less cultivated tetraploid wheat lines (*T. dicoccoides*, *T. dicoccum* and *T. polonicum*) surveyed, when compared to durum wheats (Chapter 4). Most of the durum lines tested had one or other three frequently occurring patterns. Most commercial durum wheat cultivars and advanced breeding lines possessed the LMW-B pattern or the γ-45 gliadin type, indicating a narrow genetic variability among the modern cultivars. However, greater genetic variation in LMW glutenin subunit composition of durum wheat was observed from areas with less breeding research than areas with extensive breeding (Chapter 4). The extent of variability of LMWGS among the Israelian *T. dicoccoides* wheat was similar to that of HMWGS, but the LMW glutenin subunit group might be well underestimated by the 1-D SDS-PAGE system. Since the LMW glutenin subunit bands varied within a narrow MW range.

Most of the present work concentrated on the variability of B subunits whereas C subunits were largely ignored, mainly due to the poor resolution and weak staining. As the B and C subunits of glutenin were shown to belong to two different subfamilies (Lew *et al*, 1992), the latter group should be also worth pursuing for further study.

The present inheritance study (Chapter 5) verified earlier findings that all the B subunits in tetraploid wheat are controlled by genes on chromosome arms 1AS and 1BS and the *Gli-1* and *Glu-3* loci are closely linked. Each of the *Glu-3* loci were found to code for one to several co-inherited polypeptides, confirming previous observations that LMWGS occur as linkage-blocks, similar to the gliadin blocks on chromosome arms 1AS and 1BS (Sozinov and Poperelya, 1980, 1982; Payne *et al*, 1984d; Singh and Shepherd, 1988a, 1988b; Gupta and Shepherd, 1990a; Chapter 5). Intralocus recombination within the *Gli-1* loci has been observed very rarely (Kasarda *et al*, 1976; Sozinov and Poperelya, 1980, 1982; Payne and Lawrence, 1983; Payne *et al*, 1984d; Singh and Shepherd, 1988a, 1988b; Gupta and Shepherd, 1990a, 1990b) and two recombinants were detected between the *Glu-A3* and *Gli-A1* loci in the present study, consistent with the previous findings (Singh and Shepherd, 1988a, 1988b). Moreover, recombination was observed to occur within the 'block' of major B subunits controlled by *Glu-B3* locus in durum wheat (Chapter 5), suggesting that genes coding for these 1BS-controlled bands are in fact closely linked loci. This might also apply to the

other homoeolocus (i. e. *Glu-A3*) in durum wheat and the other two homoeoloci (i. e. *Glu-A3* and *Glu-D3*) in bread wheat as well. Pogna *et al* (1993) recently showed that a group of ω-gliadins in bread wheat, which formerly were believed to be controlled by the *Gli-A1* locus, are in fact controlled by two closely linked loci (i. e. *Gli-B5* and *Gli-B1*) with 1.8% recombination between them.

Recently, a group of minor ω type gliadins coded by *Gli-3* were detected and mapped midway between the *Glu-1* and *Gli-1* loci, and 22-28 cM from the *Gli-1* loci of hexaploid wheat (Sobko, 1984; Metakovsky *et al*, 1986; Payne *et al*, 1988b). However, this locus has also been shown to code for D subunits of glutenin, depending upon the genotype (Payne *et al*, 1988b). Moreover, other minor storage protein loci have also been found such as *Tri-1*, *Gli-4* and *Gli-5* loci, coding for triticins (Singh and Shepherd, 1991a) and other minor ω-gliadins (Metakovsky *et al*, 1986; Redaelli *et al*, 1992; Pogna *et al*, 1993). Genes for these proteins (*Tri-1*, *Gli-4* and *Gli-5*) were mapped 55.2-55.8 cM, 10 cM and 1.4-1.8 cM from *Gli-1* loci, respectively (Singh and Shepherd, 1988b; Metakovsky, 1986; Redaelli *et al*, 1992; Pogna *et al*, 1993) suggesting a more complex structure of seed storage proteins on group 1 chromosomes.

In the present inheritance study, a separate locus coding for B subunits of glutenin was detected in durum wheat cv. Edmore, located apparently in a similar position to the *Gli-3* loci in bread wheat (Payne *et al*, 1988b). This locus was detected by chance, simply because the B subunits segregating in the study had different MW. Because of the high similarity of the B subunits in durum wheat, these bands are likely to be overlapped by the major *Glu-3* bands fractionated by the 1-D SDS-PAGE system. For example, Gupta and Shepherd (1990a, 1990b) failed to observe any new locus of B subunits from extensive inheritance studies of this group in hexaploid wheat and related species with the same technique. The major reasons for this would presumably be the overlapping of many bands with similar MW. Another possibility would be the inactivation of bands of interest, as it has been shown earlier for the bands coded by *Gld-B6* locus (= *Gli-3* loci), which have undergone a massive and non-random process of diploidization in the polyploid wheats (Galili and Feldman, 1983c, 1984; Payne *et al*, 1988b). This presumably applied to the present new locus (*Glu-B2*) as well, but more detailed work needs to be done on this aspect.

It is now generally agreed that the different prolamins were formed by the replication of a common ancestral gene and subsequent mutation and divergence at the molecular level (Shewry and Miflin, 1985). For instance, the similarity of amino acid sequences of B and C subunits in bread wheats suggested that they might have originated from gliadin genes during the evolutionary process (Lew *et al*, 1992). Some ω-gliadins, which are generally S-free, were recently reported to possess cysteine residues (presumably arising by mutation), clearly demonstrating the conversion of a gliadin gene to a glutenin gene (Masci *et al*, 1993). At the *Glu-B3* and *Glu-D3* loci of bread wheat, different alleles were found to code for either ω-gliadin components or D-subunits of glutenin, also indicating the divergence and complexity of these genes (Payne *et al*, 1988b; Masci *et al*, 1991a, 1991b). In conclusion, the only

structural differences between gliadin and glutenin subunit might due to their ability to form inter-molecular disulphide bonds (Kasarda, 1989; Lew *et al*, 1992). Furthermore, the extensive variation of LMWGS observed in this study could be concluded that not only due to the allelic changes at the *Glu-3* loci, but to the presumed Glu-2 and Glu-4 loci as well. The complexity of these banding patterns could be utilised to improve quality of cultivated wheats once their functional properties are determined.

## QUALITY ASSOCIATION

The work in Chapter 6 further confirmed the earlier observation that durum genotypes having gliadin band γ-45 or LMW-2 possessed stronger gluten strength than lines with band γ-42 (LMW-1), irrespective of their genetic background. It could be concluded from the present study that, except the influence of total protein amount, dough quality changes were more likely due to changes of glutenin quantity rather than gliadin quantity. Analysis of $F_2$-derived selected progeny lines of durum wheat in the present study exhibited that the glutenin : gliadin ratio, especially the higher-aggregated glutenin fraction ($P_{1-1}$), was strongly positively correlated with the SDSS values and mixing tolerance (Chapter 7), consistent with many previous results including that of Lafiandra *et al* (1991), who used RP-HPLC techniques and showed that the total amount of glutenins were responsible for dough strength as measured by alveograph and SDSS values. Of particular interest in the present study was the finding that major dough quality changes observed were not due to total protein quantitative differences but mainly due to changes in their specific protein quantity (i. e. glutenins/gliadins), because the protein concentration differences were small. This result was consistent with another study where SE-HPLC techniques were used to study a set of durums grown in several sites (McKenzie *et al*, 1991). Thus, an increase of dough mixing properties or SDSS values, was associated with an increase in the glutenin proportion, and decrease in gliadin proportion, further confirming that glutenin not the gliadin was the major factor in determining dough quality.

In bread wheat, a greater number of HMW glutenin subunits or more dark bands gave enhanced quality (Payne *et al*, 1983; Singh *et al*, 1990b) whereas fewer bands or the 'null' alleles had detrimental effects on quality (Lawrence *et al*, 1988). Similarly, in durum wheat, the number and quantity of the B subunits might be used as an indication of the quality potential of durum wheat. For example, the poor quality *Glu-B3a'* allele synthesized only two darkly stained and one weak B subunit whereas the better quality *Glu-B3b'* allele synthesized three dark-staining and one weak band (corresponding to synthesis of more aggregative proteins), and the latter allele was associated with stronger gluten strength than the former allele. Also, the LMW-2 (i. e. *Glu-B3b'*-related) types (Fig. 4.1B, Chapter 4), have been reported to have quality ranging from weak to medium strong (Damidaux *et al*, 1980b; du Cros *et al*, 1982) and the data presented in Fig. 4.1B, in which all the LMW-2-related B subunits were present, might provide an explanation. For example, Kharkof-5 which had a

greater number of LMW glutenin subunit bands than Edmore (see Fig. 4.1B), has been shown to have superior quality properties (Josephides *et al*, 1987).

The importance of B subunits coded by *Glu-3* loci to durum wheat quality might perhaps have been overestimated, since two separate loci (*Glu-B2* and *Glu-B4*) identified in the present study (Chapter 5) has not been thoroughly investigated. The LMW alleles at these new loci might also have similar effects on the dough quality property just like the major *Glu-3* loci in both tetraploid and hexaploid wheats. Furthermore, it should be remembered that the HMWGS and LMWGS alleles are not the only determinants of dough quality. Other endosperm components such as lipids, starch etc are also known to affect this property. Environmental effects have also been shown to exert a pronounced effect on gluten strength (Lafiandra *et al*, 1991).

It appeared from various previous studies that the LMWGS (B subunits) exerted the predominant influence on dough properties of durum wheats whilst the HMWGS (A subunits) appeared to have little or no influence on these properties. However, this apparent lack of association between allelic variation of HMWGS and durum quality might be due to the narrow range in genetic variability occurring among the commercial durum varieties investigated (Autran and Feillet, 1985; Vallega, 1986; du Cros, 1987a; Boggini and Pogna, 1989). The present study, however, employing a large number of world durum lines with large differences in their protein quality, demonstrated unequivocally that the presence of certain HMW glutenin subunits (eg. bands 13+16 and 7+8) were closely associated with the improved SDSS values. It could be concluded that the effect of HMWGS on durum dough quality was similar to the functional effect of these alleles in bread wheats and that the results obtained in bread wheats could be directly employed for durum wheat quality improvement.

Compared to the bread wheat check varieties, the gluten strength of durums as measured by SDSS, tended to be much poorer, even though the durum wheats had higher grain protein concentration. Recently, it has been shown that both of the HMW and LMW glutenin subunits cumulatively contributed to dough quality in durum (Boggini and Pogna, 1989; Pogna *et al*, 1990, 1992) as well as in bread wheat (Payne *et al*, 1987b, 1987c). The effect of LMWGS on viscoelastic quality was less effective than the HMWGS (Gupta *et al*, 1990). Although the relative contribution of HMWGS and LMWGS was not measured in the present study (Chapter 6 and 7), from other work this likely that both fractions are important and cumulative to contribute dough strength in durum wheats. An almost complete absence of the *Glu-A1* alleles and only the *Glu-B1* alleles having the functional effect, and the absence of the D genome could be the major cause of poor quality in durum wheat.

Many approaches are available in hexaploid wheat (Payne, 1983) to improve gluten quality by the manipulation of HMWGS, and some of these may be applicable to durum wheat. One strategy was to increase the number of HMWGS genes controlling the synthesis of quality related polypeptides such as by the introduction of the HMW 1Ay subunit from *T. monococcum*, *T. urartu* or *T. dicoccoides*, which is not expressed in bread and durum wheat

(Waines and Payne, 1987; Ciaffi *et al*, 1991). Introduction of unique genetic blocks coding for more copies of the HMW glutenins [eg. an Israelian wheat cv. TAA 36, (Galili and Feldman, 1983b; Pogna *et al*, 1992)], or introduction of genes that have increased efficiency of transcription and/or translation of the genes [eg. a Canadian cultivar Glenlea (Ng *et al*, 1989; Pogna *et al*, 1992)], are other approaches. Translocation of chromosome segments carrying quality related genes resulting from interspecific hybridization could also be effective. For instance, a 1AS/1DS translocation has been reported in the Italian cultivar, Perzivan (Metakovsky *et al*, 1990; Redaelli *et al*, 1992) but the effect of this translocation on quality remain to be measured. Conversely, Pogna *et al* (1992) has suggested that removal of gliadin genes could shift the balance of nitrogen assimilation towards synthesizing more proteins such as HMWGS, and towards this aim various spontaneous mutants with deleted *Gli-1* locus have been isolated (eg. Lafiandra *et al*, 1987, 1988).

Alternatively, improvement in dough properties in durum wheat could be achieved by replacing an A or B chromosome with a D-genome chromosome because of the larger influence of this genome on quality as demonstrated in hexaploid wheats. Using the nullisomic-tetrasomic lines of Chinese Spring, Rogers *et al* (1988, 1990) have demonstrated that increase in the number of copies of the 1D chromosome increased the suitability of the poor quality bread wheat Chinese Spring for bread-making. Similarly, in the present study, analysis of a set of D-genome disomic substitution lines in Langdon has also confirmed that the chromosome 1D substitutions had a large beneficial effect on durum wheat rheological properties, as well as the proportion of glutenin ($P_1$%) and mixing properties even in a genetic background promoting weak dough (i. e. possessing γ-42 band or null) (Chapter 6). This suggested that the introduction of the 1D chromosome could be used to improve the dough strength of tetraploid wheats. Furthermore, a follow-up experiment showed that the effect of substituting chromosome 1D for 1A had a two-fold effect on the improvement of major quality characteristics compared to changing particular alleles (i. e. *Glu-B3b'* to *a'*) on chromosome 1B (Chapter 7). These 1D substitution lines were found to have a gluten strength comparable to that of medium-strength hexaploid wheat, as measured by SDSS values.

The introduction of the HMWGS quality-related allele such as *Glu-D1d* instead of *Glu-D1a* of Chinese Spring (eg. the case in Chapters 6 and 7) from hexaploid wheat would be expected to further improve the gluten strength of durum wheat. This has resulted in a current project aiming at bringing this allele into a durum wheat background by substituting a 1D chromosome with *Glu-D1d* from a bread wheat. This work is under-way in our laboratory (see Appendix F) and has coincided with two other groups from Italy and Japan with a similar project (Ceoloni *et al*, 1993; Tsunewaki and Matsuda, 1993). The latter two groups reported recently at the Eighth International Wheat Genetics Symposium.

Recent developments in molecular biology have provided an even better chance of efficiently transferring the genes of interest to host plants. A transformation system will open up the opportunity to insert directly single genes or a small number of genes and is likely to have a significant impact on the improvement of dough quality in wheat (Anderson *et al*,

1990). The advent of these new techniques promise to shorten cultivar development time and to create new functions and products. There have been several reports of introduction of foreign genes into monocotyledonous plants, suggesting that genetic improvement of the protein quality in wheat is close to be achieved. Galili (1989) has established a high-level expression system, enabling the production of individual HMW glutenin subunit in *E. coli.* Robert *et al* (1989) reported 0.1% of the total endosperm protein was produced by a HMWGS gene in tobacco under its own promoter. Expression of particular prolamin genes (natural or synthetic) could also be altered specifically, for example by the introduction of additional gene copies, by the modification of cis-acting regulatory sequences, or by the synthesis of anti-sense mRNAs. The genes of several different storage proteins have been isolated and preliminary studies are beginning to unravel the molecular controls that subject them to developmental regulation. It is expected that transformation and *in vitro* selection methods will soon be routine in breeders' laboratories.

Economic situations have reduced the range of genetic variability among cultivars of many crops, including durum wheat. The wide variation of LMW as well as HMW glutenin subunits identified in the current extensive survey of germplasm might be helpful for plant breeders to improve the pasta-making quality as well as bread-making quality of wheat. A dual purpose durum wheat (i. e. having satisfactory bread-making quality, and acceptable pasta-making quality) would be an ideal for the future as a multi-purpose crop. In years of high production, it could be used in place of bread wheat in high quality flour blends (Boggini and Pogna, 1989). Gupta (1989) suggested that novel alleles from durum wheat might be explored and introgressed into bread wheat background for further improvement of the dough quality of bread wheats. In addition, the rich genetic pools from landraces of primitive agriculture (Payne *et al*, 1984b) or from the wild relatives of wheat (Law and Payne, 1983) also need to be explored and exploited to widen the range of variation of LMW and HMW glutenin subunits in cultivated wheat. Several breeding programs around the world are being introducing beneficial genes from wild type into cultivated varieties. Several breeding programs are introducing beneficial genes from wild type into cultivated varieties. However, for immediate results more impact should be made by the introduction of HMW glutenin subunit alleles with high *Glu-1* scores (Payne et al, 1987a) such as those coding for bands 17+18, 13+16 and 7+8, rather than introducing new LMW glutenin subunit alleles.

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**Appendix A** Classification of 240 durum wheat (*Triticum turgidum* var. *durum*) cultivars/breeding lines with respect to LMW glutenin banding patterns, allelic blocks, HMW glutenin alleles and ω-γ-gliadin composition

| Cultivar Name | Acq No./ Aus No. | Origin[a] | LMW patterns[b] | *Glu-A3* block[b] | *Glu-B3* block[b] | *Glu-A1* allele[c] | *Glu-B1* allele[c] | ω-γ-gliadins[d] |
|---|---|---|---|---|---|---|---|---|
| Abyssinia 6 | Aus 10250 | UAR | C | *a'* | *d'* | *V* | *e* | 34-36-42 |
| Adamello | Acq 11529 | ITL | B | *a'* | *b'* | *c* | *b* | 35-45 |
| Agini | Aus 11696 | ALG | B | *a'* | *b'* | *a* | *d* | 35-45 |
| Agini | Aus 11698 | ALG | B | *a'* | *b'* | *a* | *d* | 35-45 |
| Amedeo | Acq 11530 | ITL | B | *a'* | *b'* | *c* | *e* | 32-35-(42)-45 |
| Aouedj | Aus 13172 | TUN | B | *a'* | *b'* | *c* | *VIII* | 35-45 |
| Arrubiu | Aus 16524 | ITL | BF | *c'* | *k'* | *c* | *X* | 38-47 |
| Bayadi | Aus 8890 | SYA | BS(=BH?) | *b'* | *b'* | *c* | 7+18 | 35-45 |
| BD-1588 | Aus 13197 | TUN | AF | *f'* | *a'* | *c* | *e* | 38-42 |
| Beladi | Aus 7816 | UAR | DA | *f'* | *f'* | *V* | *IX* | 33-45 |
| Beladi 116 | Aus 8554 | UAR | DA | *f'* | *f'* | *V* | *IX* | 37-43.5 |
| Beladi 116 | Aus 8966 | UAR | DA | *f'* | *f'* | *V* | *IX* | 37-43.5 |
| Beladi 25 | Aus 13067 | UAR | BD | *a'* | *f'* | *V* | *IX* | 35-45 |
| Beladi 33 | Aus 13065 | UAR | BG | *a'* | *e'* | *V* | *IX* | 35-45 |
| Beladi 45 | Aus 13063 | UAR | B | *a'* | *b'* | *c* | *VIII* | 34-38-(42)-45 |
| Beladi Bouhi | Aus 8553 | UAR | AA | *g'* | *a'* | *V* | *IX* | 33-35-38-42 |
| Beladi Dakar | Aus 8555 | UAR | AG | *c'* | *g'* | *c* | *e* | 33-35-38-42 |
| Beliouni | Aus 11694 | ALG | DF(=B) | *a'* | *b'* | *c* | *d* | γ-43.5 |
| Berbern | Aus 13184 | TUN | A | *a'* | *a'* | *c* | 7+18 | 33-35-38-42 |
| Biancu Sicilianu | Aus 16510 | ITL | DB | *i'* | *i'* | *b* | *a* | 37-43.5 |
| Bidi | Aus 7925 | ALG | B | *a'* | *b'* | *c* | *e* | 35-45 |
| Bidi | Aus 9276 | ALG | B | *a'* | *b'* | *c* | *e* | 35-45 |
| Biskri | Aus 10155 | ALG | A | *a'* | *a'* | *c* | *X* | 33-35-38-42 |
| Biskri | Aus 13180 | TUN | BH | *b'* | *b'* | *c* | *e* | 35-45 |
| Biskri Glabre AC 1 | Aus 13230 | TUN | B | *a'* | *b'* | *c* | *e* | 35-45 |
| Ble Dur 250 | Aus 9050 | MOR | BP | *a'* | *o'* | *c* | *d* | 33-45 |
| Ble Dur Noh 866 | Aus 11626 | ALG | BO | *e'* | *f'* | *c* | *b* | 32-(34)-(35)-45 |
| Boyeros 's' | Acq 11531 | MEX | B | *a'* | *b'* | *c* | *d* | 35-45 |
| Bravo | Aus 20409 | ITL | B | *a'* | *b'* | *c* | *e* | 35-(42)-45 |
| Calvin | Acq 11637 | USA | A/BT | *a'* | *a'/k'* | *c* | *d* | 33-35-38-42 |
| Cando | Acq 11638 | USA | A | *a'* | *a'* | *c* | *d* | 33-35-38-42 |
| Capeiti | Aus 2065 | ITL | B | *a'* | *b'* | *c* | *f* | 35-45 |
| Capinera | Acq 15526 | ITL | E | *h'* | *a'* | *b* | *V* | γ-40 (Reference) |
| Cappelli | Aus 2068 | ITL | B | *a'* | *b'* | *c* | *e* | 35-42-45 |
| Castello | Acq 11532 | ITL | B | *a'* | *b'* | *c* | *e* | 32-35-45 |
| Chamali | Aus 8889 | SYA | B | *a'* | *b'* | *c* | *e* | 35-45 |
| Charki | Aus 8888 | SYA | B | *a'* | *b'* | *c* | *b* | 35-45 |
| Chen 's' | Aus 22622 | MEX | B | *a'* | *b'* | *c* | *b* | 35-45 |
| Chichcvlote 's' | Aus 21246 | MEX | B | *a'* | *b'* | *c* | *b* | 35-45 |
| Chili | Aus 13221 | TUN | B | *a'* | *b'* | *c* | *e* | 35-45 |
| Coulter | Acq 11640 | USA | BO | *e'* | *f'* | *c* | *d* | 35-45 |
| Creso | Acq 11639 | USA | BA | *a'* | *i'* | *c* | *d* | 35-45 |
| D-117 | Aus 13202 | TUN | B | *a'* | *b'* | *c* | *d* | 35-45 |
| D-15 | Aus 19771 | UAR | C | *a'* | *d'* | *c* | *d* | 35-45 |
| D-2 | Aus 19770 | UAR | B | *a'* | *b'* | *c* | *b* | 35-45 |
| D-240 | Aus 13204 | TUN | AG | *c'* | *g'* | *c* | *e* | 33-35-38-42 |
| D-56-89-C | Aus 13200 | TUN | B | *a'* | *b'* | *c* | *e* | 35-45 |
| Dakar 52 | Aus 8967 | UAR | EC(=C) | *a'* | *d'* | *V* | *IX* | γ-40 |
| Diabola | Acq 11641 | France | BT | *a'* | *k'* | *c* | *e* | 35-45 |
| Dommel 's' | Aus 20407 | MEX | B | *a'* | *b'* | *c* | *d* | 35-45 |
| Drago | Acq 11527 | ITL | D | *a'* | *c'* | *a* | *d* | γ-43.5 (Reference) |
| Dural | L82/89 | AUS | B | *a'* | *b'* | *c* | *e* | 35-45 |

| Cultivar Name | Acq No./ Aus No. | Origin[a] | LMW patterns[b] | *Glu-A3* block[b] | *Glu-B3* block[b] | *Glu-A1* allele[c] | *Glu-B1* allele[c] | ω-γ-gliadins[d] |
|---|---|---|---|---|---|---|---|---|
| Duramba A | Acq 11630 | AUS | BA | *a'* | *i'* | *b* | *f* | 35-45 |
| Duramba B | Acq 11631 | AUS | A | *a'* | *a'* | *c* | *f* | 33-35-38-42 |
| Duramba C | Acq 11632 | AUS | BA | *a'* | *i'* | *c* | *f* | 35-45 |
| Duramba D | L79-176 | AUS | A | *a'* | *a'* | *c* | *f* | 33-35-38-42 |
| Durati | Acq 11634 | AUS | B | *a'* | *b'* | *c* | *e* | 35-45 |
| Durum H | Aus 7909 | UAR | BG | *a'* | *e'* | *V* | *IX* | 35-45 |
| Durum H2 | Aus 7817 | UAR | DA | *f'* | *f'* | *V* | *IX* | 37-43.5 |
| Edmore | Acq 10017 | USA | B | *a'* | *b'* | *c* | *d* | 35-45 (Reference) |
| Euro | Aus 9115 | MOR | B | *a'* | *b'* | *c* | *e* | 35-45 |
| Flamingo 's' | Aus 20405 | MEX | B | *a'* | *b'* | *c* | *b* | 35-45 |
| Flemeen | Aus 10136 | ALG | BK | *c'* | *l'* | *V* | *d* | 35-45 |
| from Morocco | Aus 13128 | MOR | BL | *h'* | *h'* | *c* | *d* | 35-45 |
| Gasvi | Aus 7901 | UAR | A | *a'* | *a* | *c* | *e* | 33-35-38-42 |
| Gasvi | Aus 12922 | UAR | C | *a'* | *d'* | *V* | *IX* | 30-40 |
| Gaviota 's' | Aus 20404 | MEX | B | *a'* | *b'* | *c* | *b* | 35-45 |
| Gharbi | Aus 8886 | SYA | B | *a'* | *b'* | *c* | *e* | 35-45 |
| Ghouff | Aus 10154 | ALG | B | *a'* | *b'* | *c* | *d* | 35-38-45 |
| Ghozlanie | Aus 8877 | SYA | B | *a'* | *b'* | *c* | *e* | 35-45 |
| Gionp 1954 | Acq 10819 | ITL | BG | *a'* | *e'* | *c* | *e* | 35-45 |
| Girgeh | Aus 13029 | UAR | EB | *g'* | *f'* | *V* | *IX* | γ-40 |
| Grazia | Acq 11533 | ITL | B | *a'* | *b'* | *c* | *e* | 35-45 |
| Guillemot 's' | Aus 21142 | MEX | B | *a'* | *b'* | *c* | *e* | 35-45 |
| Hercules | Acq 11643 | CAN | A/BD | *a'* | *a'/f'* | *c* | *d/b* | γ-42/45 (Heterogeneous) |
| Ibis | Aus 18148 | MEX | B | *a'* | *b'* | *c* | *d* | 35-45 |
| J G Dickson 377 | Aus 7740 | UAR | DA | *f'* | *f'* | *c* | *d* | 34-36-44.5 |
| J G Dickson 388 | Aus 7743 | SYA | B | *a'* | *b'* | *c* | *b* | 35-45 |
| J G Dickson 388 | Aus 7744 | SYA | B | *a'* | *b'* | *c* | *e* | 37-43.5 |
| Jeidouri | Aus 8880 | SYA | A | *a'* | *a* | *c* | *VIII* | 33-35-38-42 |
| Jenna Khetifa AP 10 | Aus 13187 | TUN | BO | *e'* | *f'* | *c* | *X* | 35-45 |
| Kahala | Aus 10045 | ALG | AE | *i'* | *a'* | *V* | *d* | 33-35-38 |
| Kameshli | Aus 8878 | SYA | C | *a'* | *d'* | *c* | *b* | γ-47 |
| Kamilaroi | Acq 11644 | AUS | BT | *a'* | *k'* | *c* | *e* | 35-45 |
| Kharkof-5 | Acq 10019 | USA | BI | *e'* | *i'* | *a* | *b* | 33-(34)-45 |
| Koukou | Aus 8894 | SYA | A | *a'* | *a* | *c* | *b* | 33-35-38-42 |
| Kyle | Acq 11646 | CAN | BM | *a'* | *h'* | *c* | *d* | 35-45 |
| Langdon | Acq 10018 | USA | A | *a'* | *a'* | *c* | *d* | 33-35-38-42 (Reference) |
| Lauria | Aus 23934 | ITL | A | *a'* | *a'* | *c* | *e* | 33-35-38-42 |
| Lebei Pubescent Bas | Aus 13233 | TUN | B | *a'* | *b'* | *c* | *e* | 35-45 |
| Lebei Pubescent Bas | Aus 13234 | TUN | B | *a'* | *b'* | *c* | *e* | 35-45 |
| Leeds | Acq 11636 | USA | A | *a'* | *a'* | *c* | *e* | 33-35-38-42 |
| Lenah Khetifa | Aus 13186 | TUN | BO | *e'* | *f'* | *c* | *e* | 35-45 |
| Lioyd | Acq 11645 | CAN | B | *a'* | *b'* | *c* | *e* | 35-45 |
| Lira | Aus 23935 | ITL | B | *a'* | *b'* | *c* | *e* | 35-45 |
| Macoon | Acq 11647 | CAN | BD | *a'* | *f'* | *c* | *d* | 35-45 |
| Mahmoudi AP 5 | Aus 13223 | TUN | B | *a'* | *b'* | *c* | *d* | 35-45 |
| Mahmoundi | Aus 13164 | TUN | B | *a'* | *b'* | *c* | *e* | 35-45 |
| Mahoudi Glabre AP 3 | Aus 13189 | TUN | BB | *c'* | *b'* | *c* | *e* | 35-45 |
| Mahoundi | Aus 13231 | TUN | B | *a'* | *b'* | *c* | *e* | 35-45 |
| Mahoundi | Aus 13232 | TUN | B | *a'* | *b'* | *c* | *e* | 35-45 |

| Cultivar Name | Acq No./ Aus No. | Origin[a] | LMW patterns[b] | *Glu-A3* block[b] | *Glu-B3* block[b] | *Glu-A1* allele[c] | *Glu-B1* allele[c] | ω-γ-gliadins[d] |
|---|---|---|---|---|---|---|---|---|
| Marouani | Aus 11643 | ALG | AC | *e'* | *a'* | *c* | *d* | 33-35-38-42 |
| Medea | Aus 13171 | TUN | BE | *d'* | *b'* | *c* | *e* | 35-45 |
| Medeah | Aus 10114 | ALG | B | *a'* | *b'* | *c* | *e* | 35-45 |
| Medeah | Aus 8932 | ALG | B | *a'* | *b'* | *c* | *e* | γ-42/45 |
| Medora | Acq 11648 | CAN | BD | *a'* | *f'* | *c* | *d* | 35-45 |
| Messapia | Aus 23936 | ITL | B | *a'* | *b'* | *c* | *d* | 35-45 |
| Mexicali 's' | Aus 18613 | MEX | B | *a'* | *b'* | *c* | *b* | 35-45 |
| Mexicali 75 | Aus 18149 | MEX | B | *a'* | *b'* | *c* | *d* | 35-45 |
| MG 2988 | Acq 11528 | ITL | C | *a'* | *d'* | *c* | *b* | γ-47 (Reference) |
| Minieh 72 | Aus 13033 | UAR | AA | *g'* | *a'* | *V* | *IX* | 33-35-38-42 |
| Mohamed Ben Bacher | Aus 8621 | ALG | B | *a'* | *b'* | *a* | *d* | (34)-45 |
| Mohamed Ben Balhir | Aus 11633 | ALG | AB | *c'* | *a'* | *c* | *b* | 33-35-38-42 |
| Morocco 181 | Aus 13123 | MOR | DB | *i'* | *i'* | *c* | *X* | 35-45 |
| Morocco C 10897 | Aus 13151 | MOR | BM | *a'* | *h'* | *c* | *VII* | 35-45 |
| Mouchie | Aus 8893 | SYA | B | *a'* | *b'* | *c* | *III* | 35-45 |
| No. 0180 | Aus 9574 | MOR | DB | *i'* | *i'* | *c* | *X* | 35-45 |
| No. 250 | Aus 9573 | MOR | B | *a'* | *b'* | *c* | *d* | 35-45 |
| P-66/270 | Aus 19755 | MEX | BF | *c'* | *k'* | *c* | *e* | 35-45 |
| Pavone | Aus 3144 | ITL | B | *a'* | *b'* | *c* | *d* | 35-45 |
| Peliss | Aus 11630 | ALG | B | *a'* | *b'* | *c* | *d* | 35-45 |
| Pelissier | Aus 10044 | ALG | BF | *c'* | *k'* | *c* | *d* | 35-45 |
| Pellissier d Face | Aus 10131 | ALG | BP | *a'* | *o'* | *c* | *X* | 35-45 |
| Piceno | Acq 11535 | ITL | BK | *c'* | *l'* | *c* | *e* | 35-45 |
| Pinguino | Aus 19548 | MEX | B | *a'* | *b'* | *c* | *X* | 35-45 |
| Pinguino | Aus 19756 | MEX | B | *a'* | *b'* | *c* | *X* | 35-45 |
| Quadruro | Acq 11536 | ITL | A | *a'* | *a'* | *c* | *II* | 33-35-38-42 |
| Qued Zenati 368 | Aus 12901 | ALG | B | *a'* | *b'* | *c* | *e* | 35-45 |
| Real Forte | Aus 11737 | TUN | B | *a'* | *b'* | *c* | *d* | 35-45 |
| RHare 870156 | | AUS | BT | *a'* | *k'* | *c* | *f/e* | 35-45 |
| RHare 880008 | | AUS | BT | *a'* | *k'* | *c* | *f* | 35-45 |
| RHare 880009 | | AUS | BT | *a'* | *k'* | *c* | *f* | 35-45 |
| RHare 880011 | | AUS | BT | *a'* | *k'* | *c* | *f* | 35-45 |
| RHare 880033 | | AUS | BT | *a'* | *k'* | *c* | *f* | 32-35-45 |
| RHare 880095 | | AUS | BT | *a'* | *k'* | *c* | *b* | 32-35-45 |
| RHare 880096 | | AUS | BT | *a'* | *k'* | *c* | *b* | 32-35-45 |
| RHare 880156 | | AUS | BT | *a'* | *k'* | *c* | *f* | 32-35-45 |
| Riehi | Aus 10046 | ALG | AB | *c'* | *a'* | *c* | *b* | 33-35-38-42 |
| RM01 | Acq 11649 | CAN | BA | *a'* | *i'* | *c* | *e* | 35-45 |
| Rodeo | Acq 11537 | ITL | B | *a'* | *b'* | *c* | *b* | 35-42-46 |
| Rokel 's' | Aus 21249 | MEX | B | *a'* | *b'* | *c* | *d* | 35-45 |
| Roulette | Acq 11650 | USA | A | *a'* | *a'* | *c* | *d* | 33-35-38-42 |
| Roussia | Aus 13166 | TUN | B | *a'* | *b'* | *c* | *X* | 35-45 |
| Roxel 's' | Aus 21135 | MEX | B | *a'* | *b'* | *c* | *b* | 35-45 |
| Ruby | Acq 11652 | USA | A | *a'* | *a'* | *c* | *d* | 33-35-38-42 |
| Sahm Jelan | Aus 8882 | SYA | C | *a'* | *d'* | *b* | *II* | 35-45 |
| Salamouni | Aus 8866 | SYA | B | *a'* | *b'* | *c* | *e* | 35-45 |
| Salapia | Aus 23941 | ITL | B | *a'* | *b'* | *c* | *e* | 35-45 |
| Sbei | Aus 13178 | TUN | B | *a'* | *b'* | *c* | *e* | 35-45 |
| Sbei 1 | Aus 11735 | TUN | DF(=B) | *a'* | *b'* | *V* | *d* | γ-43.5 |
| Seln of Beladi | Aus 13058 | UAR | AE | *i'* | *a'* | *V* | *IX* | 33-35-38-42 |
| Seville | Aus 10094 | ALG | B | *a'* | *b'* | *c* | *X* | 35-45 |
| Seville | Aus 13213 | ALG | B | *a'* | *b'* | *c* | *e* | 35-45 |
| Shousha 67 | Aus 13031 | UAR | AA | *g'* | *a'* | *c* | *e* | 33-35-38-42 |
| Sicilian | Aus 7908 | ALG | B | *a'* | *b'* | *c* | *d* | 35-45 |
| Siglawi 1 | Aus 8895 | SYA | BG | *a'* | *e'* | *V* | *e* | 35-45 |
| Siglawi 2 | Aus 8896 | SYA | BG | *a'* | *e'* | *V* | *e* | 35-45 |

| Cultivar Name | Acq No./ Aus No. | Origin[a] | LMW patterns[b] | *Glu-A3* block[b] | *Glu-B3* block[b] | *Glu-A1* allele[c] | *Glu-B1* allele[c] | ω-γ-gliadins[d] |
|---|---|---|---|---|---|---|---|---|
| Sin Jamal | Aus 8891 | SYA | C | *a'* | *d'* | *c* | *III* | γ-47 |
| Smooth Sbei | Aus 13225 | TUN | B | *a'* | *b'* | *c* | *e* | 35-45 |
| Soueidi | Aus 8885 | SYA | B | *a'* | *b'* | *c* | *IV* | 35-45 |
| Stewart | Acq 11487 | USA | A | *a'* | *a'* | *c* | *b* | 33-35-38-42 |
| Stewart 63 | Acq 11593 | CAN | A | *a'* | *a'* | *c* | *b* | 33-35-38-42 |
| Surgul | Aus 8892 | SYA | EA | *i'* | *f'* | *c* | 7+18 | γ-40 |
| T.d 10139 (Aus#) | Aus 10139 | MOR | B | *a'* | *b'* | *c* | *e* | 35-45 |
| T.d 10251 (Aus#) | Aus 10251 | UAR | B | *a'* | *b'* | *c* | *VIII* | 35-45 |
| T.d 10252 (Aus#) | Aus 10252 | UAR | B | *a'* | *b'* | *c* | *b* | 35-45 |
| T.d 10253 (Aus#) | Aus 10253 | UAR | DE | *a'* | *q'* | *c* | *e* | 37-43.5 |
| T.d 10254 (Aus#) | Aus 10254 | UAR | AD | *h'* | *g'* | *c* | *V* | 33-35-38-42 |
| T.d 10255 (Aus#) | Aus 10255 | MOR | B | *a'* | *b'* | *c* | *d* | 38.5-45 |
| T.d 10256 (Aus#) | Aus 10256 | MOR | DC | *g'* | *j'* | *c* | *e* | 37-43.5 |
| T.d 10257 (Aus#) | Aus 10257 | MOR | BL | *h'* | *h'* | *c* | 7.1+8 | 35-45 |
| T.d 10258 (Aus#) | Aus 10258 | MOR | B | *a'* | *b'* | *c* | *e* | 35-45 |
| T.d 10259 (Aus#) | Aus 10259 | MOR | B | *a'* | *b'* | *c* | *e* | 35-45 |
| T.d 10260 (Aus#) | Aus 10260 | MOR | B | *a'* | *b'* | *c* | *VIII* | 35-45 |
| T.d 10261 (Aus#) | Aus 10261 | MOR | B | *a'* | *b'* | *c* | *d* | 35-45 |
| T.d 10262 (Aus#) | Aus 10262 | MOR | BL | *h'* | *h'* | *c* | *VI* | 35-45 |
| T.d 10263 (Aus#) | Aus 10263 | MOR | BD | *a'* | *f'* | *c* | *e* | 38-45 |
| T.d 10264 (Aus#) | Aus 10264 | MOR | BN | *g'* | *n'* | *c* | *X* | 35-45 |
| T.d 10265 (Aus#) | Aus 10265 | MOR | BR | *f'* | *b'* | *c* | *X* | 35-45 |
| T.d 10266 (Aus#) | Aus 10266 | MOR | C | *a'* | *d'* | *c* | *b* | 33-35-38-41-44 |
| T.d 10267 (Aus#) | Aus 10267 | MOR | DD | *i'* | *m'* | *c* | *d* | 37-43.5 |
| T.d 10268 (Aus#) | Aus 10268 | MOR | C | *a'* | *d'* | *c* | *b* | 33-35-38-42 |
| T.d 11624 (Aus#) | Aus 11624 | MOR | B | *a'* | *b'* | *c* | *VIII* | 35-45 |
| T.d 11645 (Aus#) | Aus 11645 | UAR | BO | *e'* | *f'* | *c* | *X* | 35-45 |
| T.d 11700 (Aus#) | Aus 11700 | ALG | B | *a'* | *b'* | *c* | *d* | 38-45 |
| T.d 13038 (Aus#) | Aus 13038 | UAR | BL | *h'* | *h'* | *c* | *d* | 35-45 |
| T.d 13060 (Aus#) | Aus 13060 | UAR | AD | *h'* | *g'* | *c* | *V* | 33-35-38-42 |
| T.d 13062 (Aus#) | Aus 13062 | UAR | CB | *h'* | *d'* | *c* | *B* | γ-47 |
| T.d 13099 (Aus#) | Aus 13099 | MOR | A | *a'* | *a'* | *c* | *e* | 33-35-38-42 |
| T.d 13100 (Aus#) | Aus 13100 | MOR | DD | *i'* | *m'* | *c* | *d* | 37-43.5 |
| T.d 13101 (Aus#) | Aus 13101 | MOR | C | *a'* | *d'* | *c* | *II* | 33-35-38-44.5 |
| T.d 13102 (Aus#) | Aus 13102 | MOR | C | *a'* | *d'* | *c* | *II* | 33-35-38-44.5 |
| T.d 13103 (Aus#) | Aus 13103 | MOR | BQ | *g'* | *p'* | *c* | *X?* | 37-45? |
| T.d 13104 (Aus#) | Aus 13104 | MOR | BR | *f'* | *b'* | *c* | *X* | 35-45 |
| T.d 13105 (Aus#) | Aus 13105 | MOR | BC | *f'* | *o'* | *c* | *X* | 35-45 |
| T.d 13106 (Aus#) | Aus 13106 | MOR | BJ | *f'* | *h'* | *a* | *I* | 35-45 |
| T.d 13108 (Aus#) | Aus 13108 | MOR | B | *a'* | *b'* | *c* | *e* | 35-45 |
| T.d 13109 (Aus#) | Aus 13109 | MOR | C | *a'* | *d'* | *c* | *d* | 33-35-38-44.5 |
| T.d 13113 (Aus#) | Aus 13113 | MOR | B | *a'* | *b'* | *c* | *e* | 35-45 |
| T.d 13114 (Aus#) | Aus 13114 | MOR | B | *a'* | *b'* | *c* | *VIII* | 35-45 |
| T.d 13115 (Aus#) | Aus 13115 | MOR | DA | *f'* | *f'* | *V* | *IX* | 37-43.5 |
| T.d 13116 (Aus#) | Aus 13116 | MOR | B | *a'* | *b'* | *c* | *d* | 34-36-39-43-45 |
| T.d 13117 (Aus#) | Aus 13117 | MOR | BK | *c'* | *l'* | *c* | *e/II* | 35-45 |
| T.d 13118 (Aus#) | Aus 13118 | MOR | B | *a'* | *b'* | *c* | *VIII* | 35-45 |
| T.d 13119 (Aus#) | Aus 13119 | MOR | B | *a'* | *b'* | *c* | *e* | 35-45 |
| T.d 13120 (Aus#) | Aus 13120 | MOR | B | *a'* | *b'* | *c* | *b* | 35-45 |
| T.d 13140 (Aus#) | Aus 13140 | MOR | CA | *f'* | *d'* | *c* | *X* | γ-47 |
| T.d 13142 (Aus#) | Aus 13142 | MOR | BH | *b'* | *b'* | *c* | *e* | 35-45 |
| T.d 13163 (Aus#) | Aus 13163 | TUN | C | *a'* | *d'* | *c* | *b* | 38-44.5 |
| T.d 13168 (Aus#) | Aus 13168 | TUN | C | *a'* | *d'* | *c* | *b* | γ-47 |
| T.d 13175 (Aus#) | Aus 13175 | TUN | B | *a'* | *b'* | *c* | *e* | 35-45 |
| T.d 13176 (Aus#) | Aus 13176 | TUN | B | *a'* | *b'* | *c* | *e* | 35-45 |
| T.d 13953 (Aus#) | Aus 7911 | ALG | B | *a'* | *b'* | *c* | *e* | 35-45 |
| T.d 19762 (Aus#) | Aus 19762 | UAR | A | *a'* | *a'* | *c* | *d* | 33-35-38-42 |
| T.d 19764 (Aus#) | Aus 19764 | UAR | A | *a'* | *a'* | *c* | *b* | 33-35-38-42 |
| T.d 19768 (Aus#) | Aus 19768 | UAR | B | *a'* | *b'* | *c* | *e* | 35-45 |
| T.d 368 | Aus 7910 | ALG | B | *a'* | *b'* | *c* | *e* | 35-45 |

| Cultivar Name | Acq No./ Aus No. | Origin[a] | LMW patterns[b] | *Glu-A3* block[b] | *Glu-B3* block[b] | *Glu-A1* allele[c] | *Glu-B1* allele[c] | ω-γ-gliadins[d] |
|---|---|---|---|---|---|---|---|---|
| T.d 58-25-A | Aus 13195 | TUN | B | *a'* | *b'* | *c* | *e* | 35-45 |
| T.d 7p 2 | Aus 12932 | UAR | AA | *g'* | *a'* | *V* | *IX* | 33-35-38-42 |
| T.d 9456 (Aus#) | Aus 9456 | SYA | BG | *a'* | *e'* | *c* | *II* | 35-45 |
| T.d 9457 (Aus#) | Aus 9457 | SYA | B | *a'* | *b'* | *c* | *b* | 35-42-45 |
| T.d 9722 (Aus#) | Aus 9722 | UAR | BH | *b'* | *b'* | *c* | *d* | 35-45 |
| T.d 9803 (Aus#) | Aus 9803 | SYA | A | *a'* | *a'* | *c* | *b* | 33-35-38-42 |
| Tamilia | Aus 1368 | ITL | B | *a'* | *b'* | *c* | *b* | 34.5-42-44.5 |
| Touggourt | Aus 11742 | ALG | BD | *a'* | *f'* | *c* | *d* | 35-45 |
| Touggourt | Aus 10153 | ALG | B | *a'* | *b'* | *c* | *d* | 35-45 |
| Trinakria | Acq 11539 | ITL | B | *a'* | *b'* | *c* | *e* | 35-42-45 |
| Tripolino | Aus 1432 | ITL | B | *a'* | *b'* | *c* | *e* | 35-45 |
| Tripshiro | Aus 13098 | LBY | A | *a'* | *a'* | *c* | *III* | 33-35-38-42 |
| Valnova | Aus 22133 | ITL | B | *a'* | *b'* | *c* | *b* | 35-42-45 |
| Valriccardo | Acq 11541 | ITL | B | *a'* | *b'* | *c* | *b* | 35-45 |
| Vespro | Aus 23943 | ITL | B | *a'* | *b'* | *c* | *e* | 35-45 |
| Vic | Acq 11633 | USA | B | *a'* | *b'* | *c* | *d* | 35-45 |
| Wakooma | Acq 11653 | CAN | BT | *a'* | *k'* | *c* | *d* | 35-45 |
| Ward | Acq 11635 | USA | A | *a'* | *a'* | *c* | *d* | 33-35-38-42 |
| Wascana | Acq 11651 | CAN | A | *a'* | *a'* | *c* | *d* | 33-35-38-42 |
| Yallaroi | Acq 11654 | AUS | BT | *a'* | *k'* | *c* | *h*? | 35-45 |
| Yararos | Aus 20784 | MEX | B | *a'* | *b'* | *c* | *e* | 35-42-45 |
| Zaraa | Aus 8876 | SYA | AE | *i'* | *a'* | *c* | *b* | 33-35-38-42 |
| Zeramek | Aus 9345 | MOR | B | *a'* | *b'* | *c* | *e* | 35-45 |

[a] = Origin: Algeria (ALG), Australia (AUS), Canada (CAN), France (FRA), Italy (ITL), Libya (LBY), Mexico (MEX), Morocco (MOR), Syria (SYA), Tunisia (TUN), United of Arab Republic (UAR) and the United States (USA).

[b] = LMW glutenin patterns: The designation of the patterns refers to methods in Chapter 4; *Glu-A3* and *Glu-B3*: The designation of the linkage blocks refers to the result section 4.3.1.3 in Chapter 4.

[c] = *Glu-A1* and *Glu-B1*: The designation of the alleles refers to the system of Vallega (1986, 1988b).

[d] = *Gli-B1*: The number designation of the bands refers to the system of Bushuk and Zillman (1978) and these band(s) were presumed to be controlled by Gli-B1 genes. Where a bracket used denotes a weak-staining band.

**Appendix B** The LMW and HMW glutenin subunit patterns, suggested allelic forms of *Glu-3* and *Gli-B1* types of the AWCC at Tamworth and US supplied *T. dicoccum* lines deduced from gels[a]

| Australian Number | Origin | LMW banding pattern | HMW banding pattern | Suggested linkage blocks | | | | *Glu-B1*[c] | *Glu-A1*[c] |
|---|---|---|---|---|---|---|---|---|---|
| | | | | *Glu-A3* | *Glu-B3* | *Gli-B1* (Acid)[b] | *Gli-B1* (SDS)[b] | | |
| **AWCC supplied** | | | | | | | | | |
| Aus 3717 | Unknown | A1 | A1 | *a1* (*g'*) | *a1* | *a1* | *a1* | | |
| Aus 3738 | Unknown | A1 | A1 | *a1* (*g'*) | *a1* | *a1* | *a1* | | |
| Aus 17641 | USSR | B1 | B1 | *b1* (*f'*) | *a1* | *b1* | *b1* | | |
| Aus 19591 | Unknown | B1 | B1 | *b1* (*f'*) | *a1* | *b1* | *b1* | | |
| Aus 3446 | Unknown | B1 | B1 | *b1* (*f'*) | *a1* | *b1* | *b1* | | |
| Aus 17968 | USSR | B1 | B1 | *b1* (*f'*) | *b1* | *b1* | *c1* | | |
| Aus 3735 | Unknown | C1 | C1 | *c1* (*b'*) | *a1* | *b1* | *b1* | | |
| Aus 3740 | Unknown | D1 | D1 | *d1* (*c'*) | *c1* | *c1* (*b'*) | *d1* | | |
| Aus 3706 | Unknown | D1 | D1 | *d1* (*c'*) | *c1* | *c1* (*b'*) | *d1* | | |
| Aus 3731 | Unknown | E1 | E1 | *e1* (*h'*) | *d1* | *d1* | *e1* | | |
| Aus 11434 | Unknown | E1 | E1 | *e1* (*h'*) | *d1* | *d1* | *e1* | | |
| Aus 20222 | Unknown | F1 | F1 | *f1* (*a'*) | *e1* | *e1* | *f1* | | |
| Aus 11435 | Unknown | G1 | G1 | *b1* (*f'*) | *f1* | *f1* | *g1* | | |
| Aus 4037 | Unknown | G1 | G1 | *b1* (*f'*) | *f1* | *f1* | *g1* | | |
| Aus 19385 | Iran | E1 | H1 | *a1* (*g'*) | *g1* | *g1* | *h1* | | |
| Aus 3734 | Unknown | H1 | I1 | *g1* (*i'*) | *h1* | *h1* | *i1* | | |
| **USA supplied** | | | | | | | | | |
| CI 12213 | India | A2 (LMW-DD) | A2 | *a2* (*g'*) | *a2* | *a2* | *a2* | *VI* | *a* |
| PI 94683 | USSR | B2 | B2 | *b2* (*h'*) | *b2* (*h'*) | *b2* | *b2* | *II* | *I* |
| PI 94640 | Iran | C2 (LMW-DD) | C2 | *c2* (*f'*) | *c2* | *c2* | *c2* | *I* | *a* |
| PI 355505 | Germany | D2 | D2 | *b2* (*h'*) | *d2* (*m'*) | *d2* | *d2* | *II* | *a* |
| PI 94633 | Morocco | E2 | E2 | *c2* (*f'*) | *b2* (*h'*) | *b2* | *b2* | *V* | *a* |
| PI 94669 | Bulgaria | F2 (LMW-DD) | F2 | *c2* (*f'*) | *e2* | *c2* | *c2* | *VI* | *c* |
| PI 94668 | USSR | G2 | G2 | *c2* (*f'*) | *e2* | *c2* | *c2* | *I* | *c* |
| PI 349045 | USSR | F2 (LMW-DD) | G2 | *c2* (*f'*) | *e2* | *c2* | *c2* | *I* | *c* |
| PI 94665 | Ethiopia | G2 | H2 | *a2* (*g'*) | *f2* | *e2* | *e2* | *I* | *a* |

[a] = Patterns different from each other were assigned a capital letter followed by a population number "1" and "2". Patterns similar to those of durum wheats in bracket;

[b] = *Gli-B1* patterns deduced from Acid-PAGE and SDS-PAGE, respectively;

[c] = *Glu-B1 and Glu-A1* alleles cited from Vallega and Waines (1987).

**Appendix C** The LMW and HMW glutenin subunit patterns, suggested allelic forms of *Glu-3* and *Gli-B1* types of the AWCC at Tamworth supplied *T. polonicum* lines deduced from gels[a]

| Australian Number | Origin | LMW banding pattern | HMW banding pattern | Suggested linkage blocks | | | |
|---|---|---|---|---|---|---|---|
| | | | | *Glu-A3* | *Glu-B3* | *Gli-B1* (Acid)[b] | *Gli-B1* (SDS)[b] |
| Aus 22341 | Unknown | A3 | A3 | *a3* | *a3* | *a3 (b')* | *a3 (b')* |
| Aus 3824 | Unknown | B3 | B3 | *b3* | *a3* | *a3 (b')* | *a3 (b')* |
| Aus 3825 | Unknown | B3 | B3 | *b3* | *a3* | *a3 (b')* | *a3 (b')* |
| Aus 3826 | Unknown | B3 | B3 | *b3* | *a3* | *a3 (b')* | *a3 (b')* |
| Aus 22342 | Unknown | B3 | B3 | *b3* | *a3* | *a3 (b')* | *a3 (b')* |
| Aus 22345 | Unknown | B3 | B3 | *b3* | *a3* | *a3 (b')* | *a3 (b')* |
| Aus 22346 | Unknown | B3 | B3 | *b3* | *a3* | *a3 (b')* | *a3 (b')* |
| Aus 3817 | Unknown | C3 | C3 | *c3* | *a3* | *a3 (b')* | *a3 (b')* |
| Aus 11445 | Unknown | C3 | C3 | *c3* | *a3* | *a3 (b')* | *a3 (b')* |
| Aus 17970 | Unknown | C3 | C3 | *c3* | *a3* | *a3 (b')* | *a3 (b')* |
| Aus 22340 | Unknown | C3 | C3 | *c3* | *a3* | *a3 (b')* | *a3 (b')* |
| Aus 22344 | Unknown | C3 | C3 | *c3* | *a3* | *a3 (b')* | *a3 (b')* |
| Aus 22347 | Unknown | C3 | C3 | *c3* | *a3* | *a3 (b')* | *a3 (b')* |
| Aus 3823 | Unknown | D3 | D3 | *d3* | *d3* | *a3 (b')* | *a3 (b')* |
| Aus 22343 | Unknown | E3 | C3 | *e3 (e')* | *b3* | *a3 (b')* | *a3 (b')* |
| Aus 3816 | Unknown | E3 | C3 | *e3* | *b3* | *a3 (b')* | *a3 (b')* |
| Aus 11444 | Unknown | E3 | C3 | *e3* | *b3* | *a3 (b')* | *a3 (b')* |
| Aus 3822 | Unknown | E3 | C3 | *e3* | *b3* | *a3 (b')* | *a3 (b')* |
| Aus 17945 | Unknown | F3 | C3 | *f3* | *c3* | *a3 (b')* | *a3 (b')* |
| Aus 3814 | Unknown | D3 | E3 | *g3* | *d3 (a')* | *b3 (a')* | *b3 (a')* |
| Aus 3815 | Unknown | G3 | E3 | *g3 (b')* | *d3 (a')* | *b3 (a')* | *b3 (a')* |

[a] = Patterns different from each other were assigned a capital letter followed by a population number "3". Patterns similar to those of durum wheats in bracket;

[b] = *Gli-B1* patterns deduced from Acid-PAGE and SDS-PAGE, respectively.

**Appendix D** The LMW and HMW glutenin subunit patterns, suggested allelic forms of *Glu-3* and *Gli-B1* types of the AWCC at Tamworth supplied *T. dicoccoides* lines deduced from gels[a]

| Australian Number | Origin | LMW banding pattern | HMW banding pattern | Suggested linkage blocks | | | |
|---|---|---|---|---|---|---|---|
| | | | | *Glu-A3* | *Glu-B3* | *Gli-B1* (Acid)[b] | *Gli-B1* (SDS)[b] |
| Aus 21255 | Israel | A4 | A4 | *a4* | *a4* | *a4* | *a4* |
| Aus 21256 | Israel | A4 | A4 | *a4* | *a4* | *a4* | *a4* |
| Aus 21266 | Israel | A4 | A4 | *a4* | *a4* | *a4* | *a4* |
| Aus 21268 | Israel | A4 | A4 | *a4* | *a4* | *a4* | *a4* |
| Aus 21258 | Israel | A4 | A4 | *a4* | *a4* | *a4* | *a4* |
| Aus 21259 | Israel | A4 | B4 | *a4* | *a4* | *a4* | *a 4* |
| Aus 21260 | Israel | A4 | B4 | *a4* | *a4* | *a4* | *a4* |
| Aus 21261 | Israel | A4 | B4 | *a4* | *a4* | *a4* | *a4* |
| Aus 21257 | Israel | B4 | B4 | *b4* | *a4* | *b4* | *a4* |
| Aus 21267 | Israel | C4 | C4 | *c4* | *a4* | *a4* | *a4* |
| Aus 21262 | Israel | D4 (LMW-C) | D4 | *d4* | *a4 (d')* | *b4* | *a4* |
| Aus 21269 | Israel | D4 | D4 | *d4* | *a4* | *a4* | *a4* |
| Aus 21263 | Israel | E4 | D4 | *e4* | *a4* | *a4* | *a4* |
| Aus 21264 | Israel | E4 | D4 | *e4* | *a4* | *a4* | *a4* |
| Aus 21265 | Israel | F4 | A4 | *f4* | *a4 (d')* | *a4* | *a4* |
| Aus 21270 | Israel | G4 | E4 | *g4* | *a4* | *a4* | *a4* |

a = Patterns different from each other were assigned a capital letter followed by a population number "4". Patterns similar to those of durum wheats in bracket;

b = *Gli-B1* patterns deduced from Acid-PAGE and SDS-PAGE, respectively.

**Appendix E** The LMW and HMW glutenin subunit patterns of the *T. dicoccoides* lines obtained from Dr E. Nevo (Nevo and Payne, 1987; Nevo and Beiles, 1989), Israel[a]

| Population | Line | LMWGS | HMWGS | Population | Line | LMWGS | HMWGS |
|---|---|---|---|---|---|---|---|
| Achihood | 13–07 | AR | A1, 20 | Qazrin | A–21 | A | A2, 6+8 |
| | 13–12 | BC | A1, 13+20 | | A–27 | BB | A2, 20 |
| | 13–17 | AQ | A1, 13+20 | | A–37 | B | A1, 6+18? |
| | 13–20 | AQ | A1, 13+20 | | A–41 | A | A2, 6+8 |
| | 13–25 | AQ | A1, 13+20 | | A–51 | A | A2, 6+8 |
| | 13–30 | AQ | A1, 13+20 | | A–57 | A | A2, 6+8 |
| | 13–33 | BC | A1, 13+20 | | A–80 | BB | A2, 20 |
| | 13–34 | BC | A1, 13+20 | | A–82 | A | A2, 6+8 |
| | 13–35 | BC | A1, 13+20 | | A–96 | A | A2, 6+8 |
| | 13–53 | AQ | A1, 20 | | | | |
| Amirim | 14–41 | AH | A3, 7+18? | Yehudiyya | B–06 | R | 0, (20+21)'/(=B–15) |
| | 14– 2 | BE | A1, 6+8?/(=19–27?) | | B–08 | R | 0, (20+21)'/(=B–15) |
| | 14– 9 | AX | A3, 7+18 | | B–14 | S' | A5, (20–21)' |
| | 14–14 | AX | A2', 20 | | B–15 | S | 0, (20+21)'/(=B–40) |
| | 14–17 | BE | A1, 6+8?/19–27 | | B–16 | S | 0, (20+21)' |
| | 14–22 | AH | A3, 7+18? | | B–19 | S | 0, (20+21)' |
| | 14–31 | AH | A3, 7+18? | | B–32 | R | 0, (20+21)'/(=B–15) |
| | 14–35 | AH | A2, 20+8? | | B–37 | R | 0, (20+21)'/(=B–15) |
| | 14–39 | AH | A3, 7+18? | | B–40 | S | 0, (20+21)' |
| | | | | | B–41 | R | 0, (20+21)'/(=B–15) |
| Beit–Oren | 15–1 | AL | A14, 20 | Rosh–Pinna | C–04 | E | A1, 7.1+8.1 |
| | 15–14 | AL | A14, 20 | | C–09 | D | A2, 6+8 |
| | 15–17 | AL | A14, 20 | | C–11 | C | A1, 6+8 |
| | 15–2 | AL | A14, 20 | | C–12 | C | A1, 6+10? |
| | 15–32 | AL | A14, 20 | | C–24 | J | A1, 7.1+8.1 |
| | 15–37 | AL | A14, 20 | | C–29 | J | A1, 7.1+8.1 |
| | 15–4 | AL | A14, 20 | | C–34 | J | A1, 7.1+8.1 |
| | 15–43 | AL | A14, 20 | | C–57 | J | A1, 7.1+8.1 |
| | 15–44 | AL | A14, 20 | | C–73 | F | A1, 20 |
| | 15–52 | AL | A14, 20 | | C–74 | J | A1, 7.1+8.1 |
| Givat–Koach | 17–13 | Q | A4, 6+20 | Sanhedriyya | E–04 | I | A3', 20 |
| | 17–14 | Q | A4, 6+20 | | E–12 | H | A3, 7 |
| | 17–21 | Q | A4, 6+20 | | E–18 | G | A2, 6+18 |
| | 17–32 | Q | A4, 6+20 | | E–19 | H | A3, 7 |
| | 17–34 | Q | A4, 6+20 | | E–30 | H | A3, 7 |
| | 17–39 | Q | A4, 6+20 | | E–36 | K | A3,7.1 |
| | 17–44 | Q | A4, 6+20 | | E–42 | H | A3, 7 |
| | 17–8 | Q | A4, 6+20 | | E–43 | H' | A3,7.1 |
| | 17–9 | Q | A4, 6+20 | | | | |
| Gitit | 18–21 | AN | A3, 20 | Bet-Meir | F–07 | M | A2, 6+18 |
| | 18–27 | AN | A3, 20 | | F–10 | N | A4, 6+18 |
| | 18–28 | AS | A3, 20+8 | | F–18 | M | A2, 6+18 |
| | 18–35 | AO | A1, 10 | | F–25 | M | A2, 6+18 |
| | 18–43 | AM | A3, 20 | | F–28 | M | A2, 6+18 |
| | 18–46 | BQ | A1, 20 | | F–37 | L | A2, 20 |
| | 18–48 | BQ | A1, 20 | | F–54 | L | A2, 18? |
| | 18–52 | AP | A3, 20+1 band | | F–67 | M | A2, 6+18 |
| | 18–56 | AM | A3, 20 | | F–82 | L | A2, 20 |
| | | | | | F–03 | M | A2, 6+18 |

| Population | Line | LMWGS | HMWGS | Population | Line | LMWGS | HMWGS |
|---|---|---|---|---|---|---|---|
| J'aba | 19–13 | BH | A3, 6+14 | Mt. Dov | G–01 | Z | A11?, 7.1+20 |
| | 19–14 | BH | A3, 6+14 | | G–03 | Z | A11?, 7.1+20 |
| | 19–26 | AJ | A3, 7+18?/(=14–22) | | G–08 | AB(~W) | 2*, 6+8 |
| | 19–27 | BD | A3, 6+8?/(=14–2) | | G–22 | AB(~W) | 2*, 6+8 |
| | 19–36 | BH | A3, 6+14 | | G–42 | AB(~W) | 2*, 6+8 |
| | 19–40 | BD | A3, 6+8?/(=14–2) | | G–46 | Y | 2*, 7.1+20 |
| | 19–41 | BH | A3, 6+14 | | G–47 | Y | 2*, 7.1+20 |
| | 19–46 | BH | A3, 6+14 | | G–60 | BO | A11, 6+18? |
| | 19–5 | BH | A3, 6+14 | | G–61 | AB(~W) | 2*, 6+8 |
| | 19–6 | BH | A3, 6+14 | | G–68 | U | A12, (7.1+20)' |
| Daliyya | 20–11 | BP | A2, 13+20 | Tabigha | H–02 | O | 0, 21+22 |
| | 20–20 | BP | A2, 13+20 | | H–03 | O | 0, 21+22 |
| | 20–22 | BP | A2, 13+20 | | H–04 | O | 0, 21+22 |
| | 20–31 | BP | A2, 13+20 | | H–05 | BL | 0, 20+21 |
| | 20–33 | BP | A2, 13+20 | | H–07 | BL | 0, (20+21)' |
| | 20–34 | BP | A2, 13+20 | | H–08 | BL | 0, (20+21)' |
| | 20–38 | BP | A2, 13+20 | | H–13 | O | 0, 21+22 |
| | 20–4 | BP | A2, 13+20 | | H–14 | O | 0, 21+22 |
| | 20–42 | BP | A2, 13+20 | | H–15 | BL | 0, 20+21 |
| | 20–8 | BP | A2, 13+20 | | H–54 | BL | 0, 20+21 |
| Yabad | 24–14 | BS | A3', 20+21? | Bat | I–02 | AE | A2, 7+18 |
| | 24–18 | BS | A3', 20+21? | Shelomo | I–04 | AE | A2, 7+18 |
| | 24–2 | BG | A2, 6+18 | | I–07 | P | A3', 7+20?/(=M32) |
| | 24–23 | BS | A3', 20+21? | | I–08 | P | A3', 7+20?/(=M32) |
| | 24–28 | BG | A2, 6+18 | | I–11 | AD | A4, 7+18 |
| | 24–31 | BG | A2, 6+18 | | I–14 | P | A3', 7+20?/(=M32) |
| | 24–41 | BG | A2, 6+18 | | I–17 | P | A3', 7+20?/(=M32) |
| | 24–48 | BG | A2, 6+18 | | I–22 | P | A3', 7+20?/(=M32) |
| | 24–50 | BG | A2, 6+18 | | I–27 | AF | 0, (20+21)' |
| | 24–61 | BG | A2, 6+18 | | | | |
| Nesher | 27–11 | BI | 0, 6+8 | Taiyiba | J–11 | AT | A2, 7+18/(=M–32) |
| | 27–14 | BI | 0, 6+8 | | J–12 | BN | A1+A4', 8? |
| | 27–15 | BI | 0, 6+8 | | J–14 | BR | A3', (7+18)' |
| | 27–18 | BI | 0, 6+8 | | J–17 | BR | A3', (7+18)' |
| | 27–26 | BJ | A3', 20+8 | | J–30 | AG | A2+A4, ? |
| | 27–27 | BK | A3, 18+10? | | J–37 | AG | A2', (6+18)' |
| | 27–31 | BJ | A3', 20+8 | | J–43 | BN | A1+A4', 8? |
| | 27–33 | BI | 0, 6+8 | | J–09 | BR | A3', (7+18)' |
| | 27–38 | BK | A3', 20+8 | | | | |
| | 27–41 | BK | A3', 20+8 | | | | |
| | 27–9 | BI | 0, 6+8 | | | | |
| Nahef | 32–02 | AY | A2, 6+18 | Kokhav | K–23 | BF | A2, 20+21? |
| | 32–03 | AY | A2, 6+18 | Hashohar | K–30 | W | A7, (20+21)'/(=K–20) |
| | 32–07 | AY | A2, 6+18 | | K–36 | T | A7, 6+18 |
| | 32–17 | AK | (A13',7+18?/=14–22) | | K–45 | V | A8, 6+18 |
| | 32–19 | AK | (A13',7+18?/=14–22) | | K–46 | T | 2*, 7? |
| | 32–31 | AK | (A13',7+18?/=14–22) | | K–50 | T | A7, 6+18 |
| | 32–37 | AK | (A13',7+18?/=14–22) | | K–09 | T | 2*, 7? |
| | 32–44 | AK | (A13',7+18?/=14–22) | | K–14 | BF | A2, 20+21? |
| | 32–45 | AK | (A13',7+18?/=14–22) | | K–18 | BF | A2, 20+21? |
| | 32–46 | AK | A3, (7+18?/=14–22) | | K–20 | W | A2, 20+21? |

| Population | Line | LMWGS | HMWGS | Population | Line | LMWGS | HMWGS |
|---|---|---|---|---|---|---|---|
| Gamla | 36–03 | AU | A2, 7+8.1?+2 bands | Mt. Gilboa | L–35 | X | A9, (7.1+8.1?/=L–10) |
| | 36–12 | AW | A11+?, 6+8.1+8 | | L–4.2 | W | A9, (7.1+8.1?/=L–16) |
| | 36–25 | AU | A2, 7+8.1?+2 bands | | L–40 | X | A9, (7.1+8.1)' |
| | 36–26 | AU | A2, 7+8.1?+2 bands | | L–43 | W | A9, (7.1+8.1?/=L–10) |
| | 36–28 | AU | A2, 7+8.1?+2 bands | | L–61 | F | A10, (7.1+8.1) |
| | 36–29 | AV | A9', 7+8.1?/(=36–39=36–25) | | L–10 | W | A9, (7.1+8.1?/=L–16) |
| | 36–33 | AU | A2, 7+8.1?+2 bands | | L–17 | W | A9, (7.1+8.1?/=L–16) |
| | 36–39 | AU | A2, 7+8.1?+2 bands | | L–21 | W | A9, (7.1+8.1?/=L–16) |
| | 36–40 | AU | A2, 7+8.1?+2 bands | | L–16 | X | A9, (7.1+8.1)' |
| Grizim | M–12 | BM | A3', 20' | | | | |
| | M–14 | AZ | A3', (7+18/=M–32) | | | | |
| | M–18 | AZ | A3', (7+18/=M–32) | | | | |
| | M–31 | AC | A11, 6+18?/=G–46 | | | | |
| | M–32 | BA | A3, (7+18/=J–11) | | | | |
| | M–35 | AZ | A3', (7+18/=M–32) | | | | |
| | M–41 | W | A3', (7+8)' | | | | |
| | M–61 | AC | A11, 6+18?/=G–46 | | | | |
| | M–64 | AZ | A3', (7+18/=M–32) | | | | |
| | M–7.1 | AZ | A3', (7+18/=M–32) | | | | |

[a] = The designation of LMWGS pattern was given a capital letter from A to Z, AA to AZ etc. The designation of HMWGS was denoted for *Glu-A1* and *Glu-B1* bands by a comma. Among the *Glu-A1* variants, a capital letter 'A' was given followed by a Arabic number, and among the *Glu-B1* alleles, they were denoted as a band similar to the standard of hexaploid with a '.1' or in bracket with a prime. If same pattern was identified among the whole population, it was denoted within a bracket plus a '/=' or '/(=) and the line's name.

# Appendix F Substitution of *Glu-D1* alleles on chromosome 1D of hexaploid wheat cultivars to durum wheat Langdon

## AIM

The major aim was to improve dough quality of durum wheats by introducing the quality HMW glutenin alleles, *Glu-D1d* (coding for bands 5+10) or/and *Glu-Bi* (bands 17+18), or *Glu-A1b* (band 2*) from hexaploid wheat. The dual purpose durum would be a desirable goal as future food source for the world's increasing population.

## INTRODUCTION

The allelic pairs 1Dx5+1Dy10 (coded by *Glu-D1d*) of the HMW glutenin subunits in bread wheat have been consistently shown to be superior in bread-making quality when compared to its allelic counterparts *Glu-D1a* (for bands 2+12) and other storage genes at different loci (Payne *et al*, 1979; Payne, 1987c). However, the *Glu-D1* genes do not exist in the tetraploid durum wheat. Substitution of chromosome 1D from hexaploid wheat Chinese Spring with the *Glu-D1a* allele into durum wheat Langdon has been shown a substantial increase in SDS sedimentation values and baking quality (Josephides *et al*, 1987), suggesting that transfer of chromosomes containing even better quality alleles such as *Glu-D1d* might produce a durum with both satisfactory baking properties and acceptable pasta-making quality. Also, the *Glu-B1i* (for bands 17+18) could also be substituted into 1D(1A) type while *Glu-A1b* (for band 2*) into 1D(1B) type. The following paragraph summaries the progress of this project started early in 1989.

## BREEDING PROCEDURES

### *Windebri cross - Development of a durum wheat 1D (1B) substitution line*

Durum wheat disomic 1D substitution line Langdon 1D(1B) was crossed to an Australian hexaploid wheat Windebri early 1989. Their glutenin subunit components are presented in Table 1 and the selection procedure in Fig. 1. The selection was started at $BC_1F_1$ and 52 $BC_1F_3$ seeds identified were homozygous at *Glu-D1* (Fig. 1).

### *Festiguay cross - Development of durum wheat 1D (1B) and 1D(1A) substitution lines*

Two crosses were made between Langdon 1D(1B), Langdon 1D(1A) and an Australian hexaploid Festiguay (Table 2). Similar procedures were adopted with the previous cross (Fig. 2).

**Table 1** Glutenin alleles present in the parents Langdon 1D(1B) and Windebri on homoeologous group 1 chromosomes

| Cultivar | *Glu-1* loci[1] | | | *Glu-3* loci[2] | | |
|---|---|---|---|---|---|---|
| | *Glu-D1* | *Glu-B1* | *Glu-A1* | *Glu-D3* | *Glu-B3* | *Glu-A3* |
| Langdon 1D(1B) | *a* (2+12) | - | *c* (0) | *a* | - | *a'* (*e*) |
| Windebri | *d* (5+10) | *i* (17+18) | *b* (2*) | *a* | *c* | *a'* (*e*) |

Note: [1] = common designation of the HMW glutenin subunit bands included in parentheses;
[2] = *Glu-3* allele symbols from Liu and Shepherd (1991), the equivalent symbol of Gupta and Shepherd (1988) included in parentheses.

**Table 2** Glutenin alleles present in the parents Langdon 1D(1B), Langdon 1D(1A) and Festiguay on homoeologous group 1 chromosomes[1]

| Cultivar | *Glu-1* loci | | | *Glu-3* loci | | |
|---|---|---|---|---|---|---|
| | *Glu-D1* | *Glu-B1* | *Glu-A1* | *Glu-D3* | *Glu-B3* | *Glu-A3* |
| Langdon 1D(1A) | *a* (2+12) | *d* | - | *a* | *a'* (*e*) | - |
| Langdon 1D(1B) | *a* (2+12) | - | *c* (0) | *a* | - | *a'* (*e*) |
| Festiguay | *d* (5+10) | *i* (17+18) | *b* (2*) | *b* | *b* | *a'* (*e*) |

Note: [1] = symbols as in Table 1.

## METHODS

The selection was started after the first backcross. A modified one-step gradient SDS-PAGE procedure was employed to monitor the HMW glutenin subunit markers all through the selection procedure (see Chapter 3). To test for homogeneity, six $BC_1F_4$ seeds were analysed for their glutenin composition (HMW and LMW). Cytological analysis was used to analyse the chromosome configurations at metaphase-I of meiosis in pollen mother cells as well as of mitosis in root tip preparations (see Chapter 5, section 5.2.6 and Chapter 7, section 7.2.4.2). For each plant, four to six cells were checked for their chromosome constitution for the last selection before the multiplication.

## GLASSHOUSE EXPERIMENT

All the 52 and 182 selected $BC_1F_3$ seeds from the first and second sets of crosses and the appropriate parents were planted in a glasshouse (2 seeds per 8 inch pot). The plant materials were sown in the same day and all the agronomic practices were kept same to reduce the environmental effect as well as the inter-plant differences. Plants having more than ten grams of $BC_1F_4$ seeds were subjected to the homogeneity study by electrophoresis,

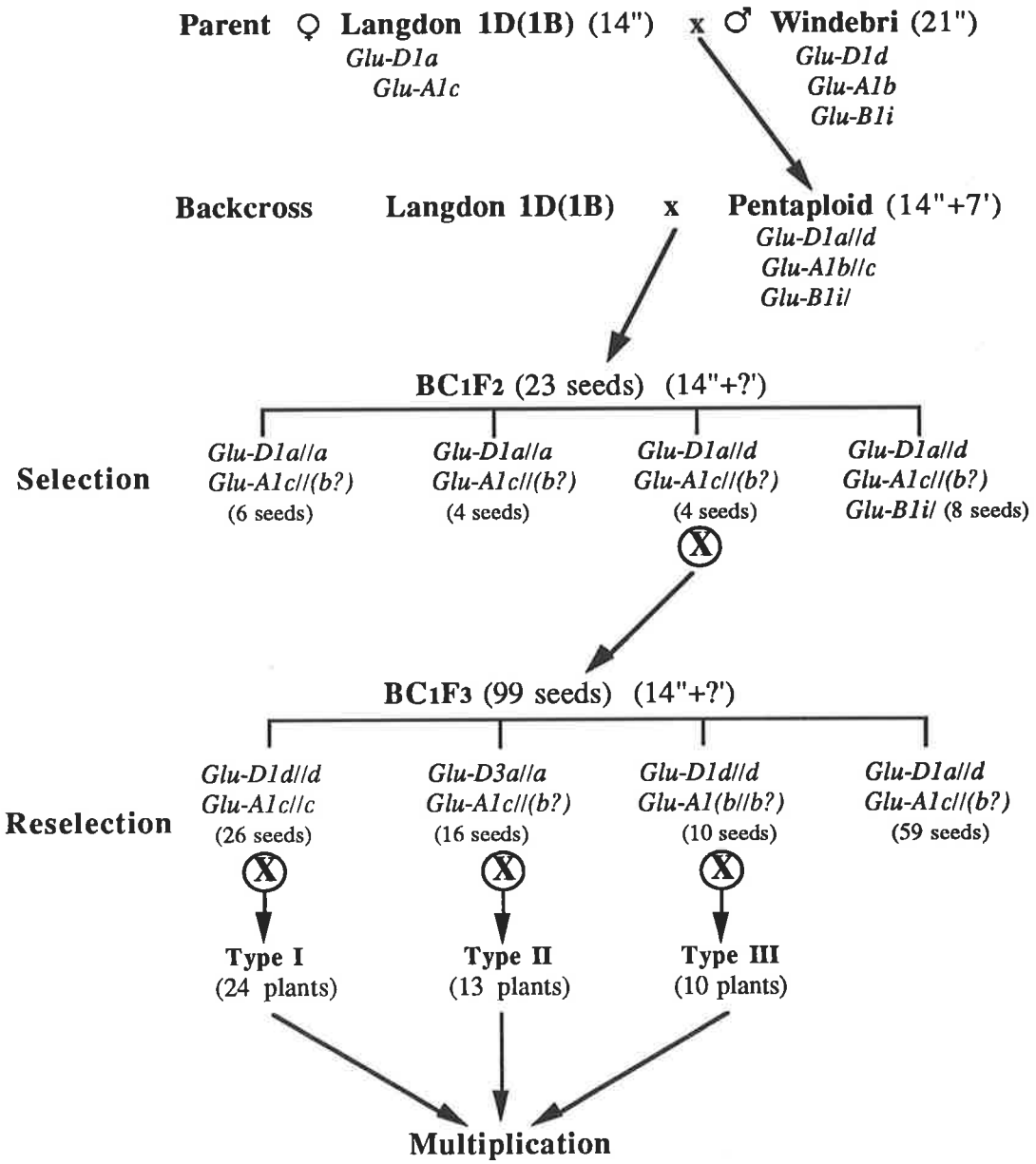


**Figure 1** Procedure used to select $BC_1F_4$ genotypes from the cross between a tetraploid Langdon 1D(1B) and the hexaploid Windebri. Bracket with a question mark denotes that the protein markers were unreliable or undetectable during the process of selection. // and / denote disomic, monosomic status chromosomes of interest, respectively.

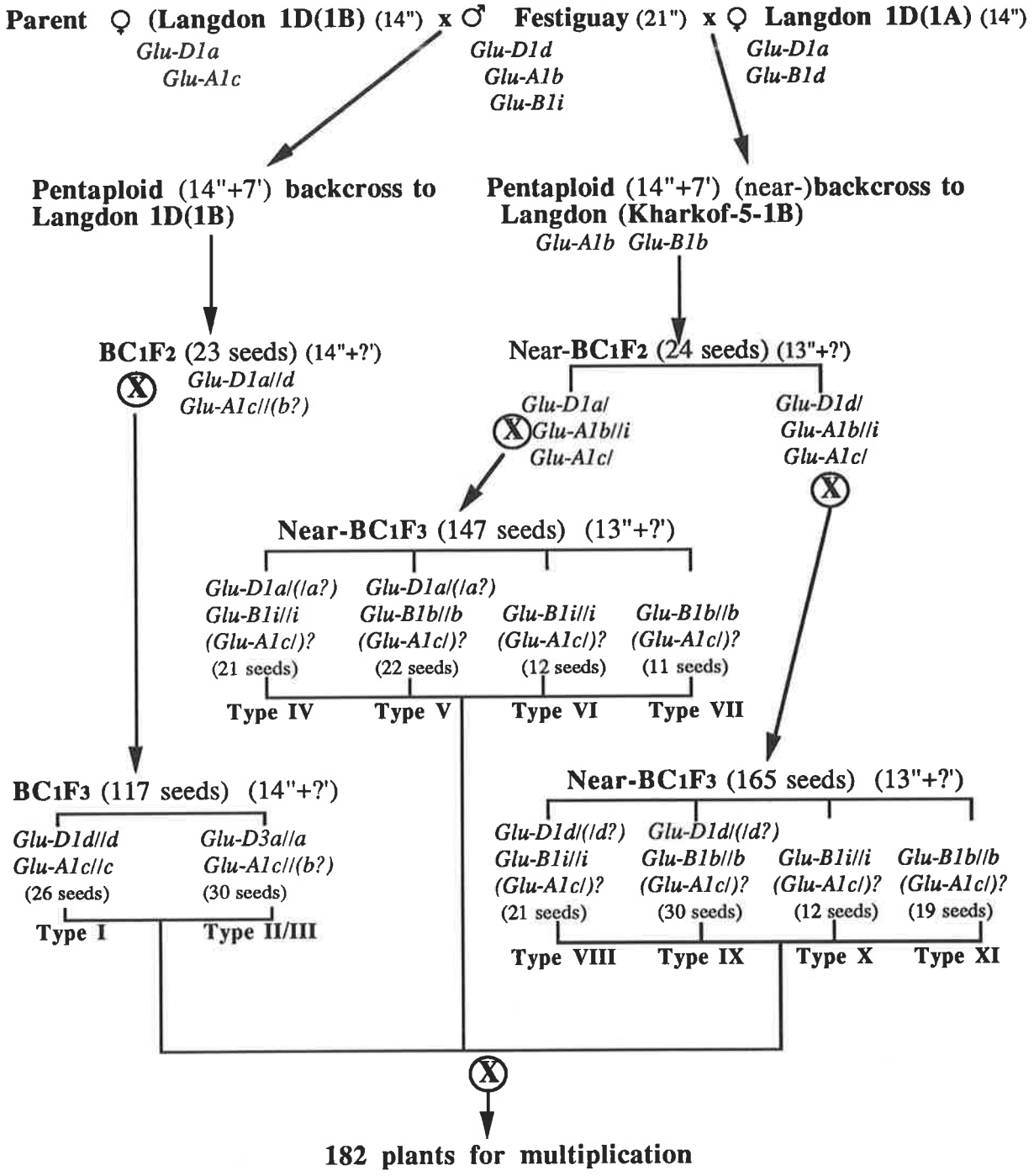


**Figure 2** Procedure used to develop $BC_1F_4$ or near-$BC_1F_4$ families from two crosses between tetraploid parents Langdon 1D(1B), Langdon 1D(1A) and hexaploid wheat cultivar Festiguay. At $BC_1F_2$ generation, only the phenotypes of interest were shown. Bracket with a question mark denotes that the protein markers were unreliable or undetectable during the process of selection. //, / denote disomic, monosomic status chromosomes of interest, respectively.

cytology and grain hardness index as described below.

## MILLING AND HARDNESS MEASUREMENT

Wholemeal samples were used to measured the grain hardness index same as in section 6.2.3.3B, Chapter 6.

## RESULTS

### *Production of durum wheat Langdon 1D(1B) $BC_1F_3$ progeny lines with Glu-D1 alleles from hexaploid cv. Windebri*

$BC_1F_3$ plants having about ten grams of $BC_1F_4$ seeds were screened electrophoretically for the homogeneity of the HMW glutenin subunit constitutions. These were then checked cytologically for their chromosome constitution and 20 plants with six aneuploids retained (Table 3).

**Table 3** Agronomic characters of the $BC_1F_4$ plants and the parental types (Mean-range)[a]

| Genotypes | No. of plants | Height (Min-Max) | Grain weight (Min-Max) | Hardness index (Min-Max) |
|---|---|---|---|---|
| **Parent** | | | | |
| Langdon 1D(1B) | 1 | 172 | 16.9 | 92 |
| Windebri | 1 | 128 | 2.7 | 70.6 |
| **Progeny** | | | | |
| Type I (5+10, 0) | | | | |
| Euploid | 8 | 136.6 (117-185) | 13.3 (7.9-23.1) | 87 (78-98) |
| Aneuploid | 4 | 140.5 (118-168) | 14.4 (9.1-19.3) | 91.5 (84-107) |
| Type II (2+12, 0) | 3 | 130.0 (117-140) | 16.7 (7.19-24.5) | 86.3 (79-191) |
| Type III (2+12, 2*) | | | | |
| Euploid | 2 | 127.5 (126-129) | 16.3 (7.1-25.4) | 92.5 (83-102) |
| Aneuploid | 1 | 119 | 8.4 | 95 |
| Heterozygous for *Glu-D1* | 2 | 147(130-164) | 10.6 (5.4-15.7) | 86.5 (82-92) |

[a] = Progeny types refer to those in Fig. 1.

Since the wheat endosperm proteins are inherited co-dominantly, progeny lines with heterozygous protein types could be easily identified at $F_2$. Four protein loci (*Glu-D1*, *Glu-A1*, *Gli-D1/Glu-D3* and *Gli-A1/Glu-A3*) were involved in the present breeding lines. As the *Glu-D3* and *Glu-A3* alleles of two parents were same, only two HMW glutenin loci were

segregating. Further electrophoretic work revealed that these plants at $BC_1F_4$ were homogeneous for α-, β- and γ-gliadin bands but heterogeneous for ω-gliadin, coded by genes at *Gli-2*, *Gli-A1* and *Gli-D1*-loci. Since the progeny lines were selected only by HMW glutenin subunits during the isolation process, it was obvious that the *Gli-D1* alleles were heterogeneous to various degrees among the progeny lines.

A great difficulty was encountered for the separation of the HMW glutenin subunits bands 2 and 2* in SDS-PAGE because of their co-migration (eg. Payne *et al*, 1981a). These two bands were only unequivocally identified at the last survey by changing electrophoretic conditions. Therefore, phenotypes with 2+12 had band 2* were observed to be heterogeneous to some extent in the $BC_1F_4$ seeds. Meanwhile the bands at the position 10 and 12 were not reliably identified by the short-run gel system. Therefore, when 2 bands present in an early segregating progeny plants, it was difficult to distinguish what protein phenotypes the plant actually possessed, whether it had bands 2+12 with 5+10, or 2* with 5+10, or 2*, 5+10 with 2+12. Furthermore, *Glu-A1c* is a null form and it was difficult to determine whether band 2* (coded by *Glu-A1b*) was in heterozygous or homozygous state when band 2* was present. All these added the complexity and difficulty of selection.

Grain weight of individual plants varied from 0.4 to 25.4 g. The plant height of the $BC_1F_3$ progeny was generally shorter than the tetraploid parent and similar to the hexaploid parent. The shorter plant and poor grain yield might due to the poor early growth, as the hexaploid parent Windebri was also low yielding because of its winter habit (Table 3). These progeny lines, in general, had hard kernels, similar to the tetraploid parent. The progeny lines were similar to the tetraploid parent in most agronomic traits such as seed size, seed colour and awns (data not shown).

Cytological results showed that most progeny had chromosome 14" or 28, five had 14"+1', or 14"/14"+1' (Table 3). Only one plant had 14"+2' and 14"+3'. This indicated that one backcross was sufficient to bring most progeny lines back to normal tetraploid chromosomal constitution.

### *Production of Langdon 1D(1B) and 1D(1A) typed plants from Festiguay*

Similar isolation procedure was used as the above cross and summaries in Table 4A and 4B.

Except for large variation in plant height and grain yield among these three populations, it was noted that some of lines had low grain hardness index, suggesting that these lines might have recombined the soft-grain allele from chromosome 5D of hexaploid parent. In general, after one backcross to the tetraploid parent, most agronomic characters (such as plant height, morphology, kernel size and texture etc) of the progeny lines were similar to the tetraploid parent Langdon. Furthermore, the cytological results showed that about 75% of lines were euploid with 28 chromosomes.

## FUTURE WORK

This project is still in its progress. Currently, $BC_1F_5$ plants were grown in the field as short plot experiment and the results will be available shortly.

**Table 4A** Agronomic characters of the $BC_1F_4$ plants and the parental types (Mean-range). Langdon 1D(1B) types[a]

| Genotypes | No. of plants | Height (Min-Max) | Grain weight (Min-Max) | Hardness index (Min-Max) |
|---|---|---|---|---|
| **Parent** | | | | |
| Langdon 1D(1B) | 1 | 172 | 16.9 | 92 |
| Festiguay | 1 | 120 | 10.4 | 77.9 |
| **Progeny** | | | | |
| Type I (5+10, 0) | 15 | 147.2 (124-177) | 16.3 (8.2-29.3) | 85.2 (54-99) |
| Euploid | 13 | 145.5 (124-164) | 16.6 (8.2-29.3) | 83.2 (54-95) |
| Aneuploid | 2 | 157 (137-177) | 14.4 (13.9-14.8) | 98.5 (98-99) |
| Type II (2+12, 0) | 10 | 151.5 (127-180) | 18.6 (8.6-35.8) | 83.7 (66-96) |
| Euploid | 9 | 150.4 (127-180) | 18.5 (8.6-35.8) | 84.1 (66-96) |
| Aneuploid | 1 | 161 | 19.3 | 80 |
| Type III (2+12, 2*) | 2 | 160 (148-172) | 10.4 (8.6-12.1) | 90 (88-92) |
| Heterozygous for *Glu-D1* | 1 | 121 | 15.2 | 57 |

[a] = Progeny types refer to those in Fig. 2.

**Table 4B** Agronomic characters of the $BC_1F_4$ plants and the parental types (Mean-range). Langdon 1D(1A) types[a]

| Genotypes | No. of plants | Height (Min-Max) | Grain weight (Min-Max) | Hardness index (Min-Max) |
|---|---|---|---|---|
| **Parent** | | | | |
| Langdon 1D(1A) | 1 | 201 | 29.6 | 82 |
| Festiguay | 1 | 120 | 10.4 | 77.9 |
| **Progeny** | | | | |
| Type IV (5+10, 17+18) | 2 | 135.5 (135-136) | 24.8 (23.9-25.6) | 85 (82-88) |
| Type V (2+12) | 7 | 133.4 (110-150) | 20.4 (12.7-27.3) | 86.3 (71-96) |
| Euploid | 4 | 124.3 (110-135) | 23.6 (20.2-27.3) | 83.3 (73-93) |
| Aneuploid | 1 | 144 | 17.8 | 93 |
| Euploid, heterogeneous for *Glu-B1* | 2 | 146.5 (143-150) | 15.3 (12.7-17.8) | 89 (82-96) |
| Type VI (0, 17+18) | 6 | 132.7 (121-147) | 21.3 (11.6-30.5) | 88.8 (71-104) |
| Type VII (0, 7+8) | 5 | 134 (125-144) | 29.3 (22.2-35.7) | 82.8 (76-94) |
| Type VIII (2+12, 17+18) | 6 | 162.3 (136-182) | 12.6 (7.7-18.4) | 87.3 (77-94) |
| Euploid | 4 | 165.8 (136-182) | 12.5 (7.7-18.4) | 85.5 (77-92) |
| Aneuploid | 2 | 155.5 (146-165) | 12.8 (11.5-14) | 91 (88-94) |
| Type IX (2+12, 7+8) | 6 | 165.5 (141-183) | 16.9 (10.2-27) | 94.5 (83-102) |
| Euploid | 3 | 179.5 (176-183) | 18.6 (10.2-27) | 91.7 (83-100) |
| Aneuploid | 3 | 154.7 (141-165) | 15.2 (12-18.3) | 97.3 (91-102) |
| Type X (0, 17+18)[b] | 7 | 148.9 (131-169) | 24.2 (10.7-35.5) | 94.7 (88-104) |
| Type XI (0, 7+8)[b] | 6 | 164.8 (161-173) | 10.4 (9.4-11.4) | 87 (81-96) |
| Euploid | 5 | 165.6 (162-173) | 10.6 (9.9-11.4) | 88.2 (82-96) |
| Aneuploid | 1 | 161 | 9.4 | 81 |

[a] = Progeny types refer to those in Fig. 1;

[b] = Type X* vs. type VI, XI* vs. type VII, are same in HMW glutenin subunit phenotype.