LOW-MOLECULAR-WEIGHT SUBUNITS OF GLUTELIN IN WHEAT AND RELATED SPECIES

Their characterization, genetics and relation to bread-making quality

by

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Dedicated to my beloved mother

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SUMMARY

The principal storage proteins of the wheat endosperm (gliadins, glutenins) are known to be important determinants of the bread- and pasta-making qualities of wheat flours. During the last decade, with the advent of improved electrophoretic techniques of protein fractionation, the structure and genetic control of gliadins and HMW subunits of glutenin have been studied extensively. In the present work, the variation and genetic control of the LMW subunits of glutenin, which constitute approximately 30% of the total seed proteins (Payne *et al.*, 1982a), have been studied in wheat and its related species and an association between some of the LMW subunits and bread-making quality has been demonstrated.

A two-step one-dimensional (1-D) SDS-PAGE method developed by Singh and Shepherd (1985) to separate disulphide-linked protein aggregates into their constituent polypeptides was used to fractionate the LMW subunits (B and C components) of glutenin, after solubilizing in Tris-HCl buffer (pH 6.8) containing 4% SDS. Additional components, including the subunits of triticin (globulins) and albumins, were also separated with the glutenin subunits in this two-step electrophoretic procedure. Because some of the triticin and albumins overlapped the LMW subunits of glutenin, 70% ethanol was introduced as the protein extractant which prevented the solubilization of the triticin and albumins (collectively called non-prolamins) but this extracted the glutenin aggregates containing HMW and LMW subunits when used at high temperature. These modifications allowed the LMW subunits of glutenin to be analysed in a background free from both gliadins and the non-prolamins.

This modified two-step electrophoresis procedure was used to analyse 222 bread wheat cultivars. Forty different LMW subunits (28 B subunits and 12 C subunits) of glutenin were detected and they formed 20 different LMW subunit banding patterns. These patterns could be divided into three mutually exclusive groups consisting of 6, 9 and 5 different patterns, respectively. The chromosomal location of the genes controlling these patterns was determined by using substitution lines and a 1DL-1RS wheat rye translocation line and the different patterns within a group were found to be controlled by genes on the same chromosome. That is, the 6, 9 and 5 patterns in bread wheat were controlled by genes on

chromosomes 1A, 1B and 1D, respectively. Subsequent analysis showed that they were controlled by the short arms of these chromosomes.

Several different accessions of cultivated and wild tetraploid and diploid wheat and related species were also analysed for LMW subunits of glutelin and a considerable genetic variation was found. Some of the patterns resembled those found in hexaploid wheat but other unique patterns were also observed. The Edmore and Kharkof-5 chromosome 1B substitutions in Langdon and the Chinese Spring chromosome 1D substitution for chromosomes 1A and 1B in Langdon were analysed to determine the genetic control of the LMW subunits of glutenin in these durum wheat cultivars. The extent of variation and number of LMW bands controlled ~~be~~ by chromosomes 1A, 1B and 1D in wheats and diploid *Triticum* species revealed that genes on chromosome 1B controlling these subunits exhibit the maximum variation. The genes coding for the LMW subunits have undergone non-random diploidization during the evolution of wheat as shown by the minimum number of subunits, including null phenotypes, being coded by genes on chromosome 1A.

By analysis of wheat-alien substitution lines, the genes controlling LMW subunits of glutelin in *Triticum longissimum*, *T. umbellulatum* and *Elytrigia elongata* (2x) were located on chromosomes $1S^l$, 1U and 1E, respectively. Similarly, the genes controlling LMW subunits in polyploid *Elytrigia elongata* (10x) were located on two different chromosomes (arbitrarily termed 1E' and 1E"). Wild rye (*Secale montanum*) possessed one LMW glutelin band but five cultivated rye (*Secale cereale*) varieties did not show any obvious LMW glutelin bands. The chromosomal location of the genes controlling this band in *S. montanum* could not be determined. However, data obtained later from some special genetic stocks termed "Triple and Double translocations" revealed that chromosome arm 1RS in rye cultivar Imperial codes for a LMW glutelin band.

The Triple translocation stock was produced to facilitate the inheritance studies of different LMW glutenin subunits in bread wheat cultivars by using test-cross seeds, as this stock was expected to lack all the LMW subunits of glutenin. It was produced by replacing all chromosome arms 1AS, 1BS, 1DS of wheat by 1RS of rye. Concurrently, three double translocation stocks having only two of these short arm homoeologues (1AS, 1BS; 1AS, 1DS; 1BS, 1DS) replaced by 1RS were also isolated. The cytological and agronomic

characteristics of these stocks have been described. They formed multivalents at meiosis but were moderately fertile and showed good vegetative vigour indicating that the loss of chromosome arms 1AS, 1BS and 1DS was well compensated by 1RS. Analysis of their protein phenotypes by two-step electrophoresis showed that all of the B subunits and most of the C subunits of glutenin in Chinese Spring and Gabo are controlled by the short arms of group 1 chromosomes. The genes controlling one of the two remaining ethanol-soluble bands in the C subunit region was located on chromosome arm 6DS. These as well as a band controlled by 1RS of rye have been considered to be LMW subunits of glutelin.

Three different F_1 hybrids between bread wheat cultivars (Chinese Spring × Orca, Chinese Spring × Norin-61, Gabo × Insignia) possessing contrasting LMW subunit patterns were crossed to the triple translocation stock to produce test-cross seeds. Analysis of these test-cross seeds revealed that three, five and four of the patterns controlled by chromosome arms 1AS, 1BS and 1DS, respectively, segregated as alternative units in inheritance. Consequently, it was concluded that these patterns are controlled by different alleles at the *Glu-A3*, *Glu-B3*, and *Glu-D3* loci. It is likely that the other 3, 4 and 1 patterns controlled by 1AS, 1BS and 1DS will also be controlled by alleles at the same loci and thus alleles *a* to *f*, *a* to *i*, and *a* to *e* were assigned to the *Glu-A3*, *Glu-B3* and *Glu-D3* loci respectively. No recombination was detected within these loci, indicating that the LMW glutenin bands are inherited in groups and are coded by tightly linked genes. Similarly, no recombination was detected between the *Glu-3* and *Gli-1* (coding for gliadins) loci.

The association between certain *Glu-3* alleles (LMW glutenin) and the dough properties of wheat flours and the extent of the interaction between *Glu-3* and *Glu-1* (HMW glutenin) alleles in determining dough properties were also examined, using extensograph tests. Two types of experimental materials were produced for these purposes. The first type consisted of F_2-derived lines from a cross between two bread wheats 'Kite' and MKR/111/8 which differed in LMW and HMW subunit composition. Their progeny were divided into four different protein genotypes based on two LMW glutenin (*Glu-A3c* or *Glu-A3e*) and two HMW glutenin alleles (*Glu-A1b* or *Glu-A1e*). These lines were multiplied and planted in replicated trials at two field sites and their flour was analysed in the extensograph. Even though there was an average two-fold difference in grain protein levels between the sites, the

effects of *Glu-A3c* and *Glu-A1b* alleles on dough resistance and dough extensibility were found to be positive and cumulative at both sites. The LMW allele *Glu-A3c* had a larger effect than both the HMW allele *Glu-A1b* and the LMW null allele *Glu-A3e* on dough properties.

The second set of material consisted of biotypes isolated from three bread wheat cultivars (Condor, Gamenya, BT-2288) with different *Glu-A3* alleles (*Glu-A3b, Glu-A3c, Glu-A3f*). These were isolated as single seeds, multiplied and planted at two field locations in replicated trials for quality testing. Biotypes in cultivars Condor and Gamenya having the *Glu-A3b* allele had greater dough extensibility than those with the *Glu-A3c* allele. Similarly, the biotype of BT-2288 having the *Glu-A3c* allele had greater extensibility than that with the *Glu-A3f* allele. Thus, LMW and HMW subunits of glutenin affected both dough resistance and extensibility, although the effect of the LMW glutenin alleles was confounded with linked gliadin alleles in both sets of materials.

The association between dough quality and the presence of 75 kd γ-secalins (rye glutelins) from Imperial rye was also determined in bread wheat. This was achieved by backcrossing a 2BL-2RS translocation stock, available in a heterogeneous Timgalen/Gabo background, into Gabo. Lines (chromotypes) with and without chromosome 2RS which controls 75 kd γ-secalins were developed from single seeds and multiplied at one field location in replicated trials for yield and quality tests. The results showed that the presence of secalins in 2BL-2RS lines is associated with highly significant effect on dough visco-elasticity. This translocation line had a highly significant increase in flour protein level but significant reduction in the grain yield.

The genetic control, solubility and aggregation behaviour of the non-prolamins detected in the two-step procedure and their degradation behaviour during seed germination have also been investigated. The data revealed that some of these non-prolamins are disulphide-linked albumins and that they may also be storage proteins.

The implications of the results presented in this thesis on our understanding of the genetics and evolution of the genes controlling LMW subunits of glutenin in wheat and its related species and their involvement in improving the bread-making qualities, are discussed.

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Chapter 1

GENERAL INTRODUCTION

Wheat is one of the most important cereal crops in the world because of its large annual production and wide consumption in the form of diverse food products. Thus, despite having a relatively low protein content (usually in the range of 8-15%) and a deficiency of some essential amino acids (mainly lysine), it is still a significant daily source of protein in the human diet. Common wheat is processed into many products such as leavened bread, chapatis, cakes, noodles and biscuits, and durum wheats are made into spaghetti, macaroni, lasagna and couscous; many of these products require different attributes.

The storage proteins in the endosperm of wheat constitute over 80% of the total proteins in the grain (Osborne, 1907) and are the most important determinants of the nutritional and organoleptic qualities of the flour products (Elton and Ewart, 1967; Kasarda *et al.*, 1976a). The quality of products derived from wheat depends upon both the quantity and quality of proteins in the flour (Finney and Barmore, 1948, Bushuk *et al.*, 1969). While protein quantity is precisely measured by several methods of analysis, protein quality is defined depending on whether the assessment is made for nutritional or organoleptic purposes. For example, bread-making requires flour proteins which yield a highly visco-elastic protein matrix (Dimler, 1965). Protein quality is less subject to environmental influence than protein quantity and also protein quantity is usually negatively correlated with grain yield. Thus, the present thesis relates to protein quality as this is much more amenable to genetic manipulation.

Although wheat proteins have been extensively studied by biochemists, geneticists and breeders for well over a century, our knowledge about them is limited compared with other dietary sources of proteins such as meat or milk. Only during the past 20 years have significant advances been made in the biochemical and genetic studies of individual wheat protein components (see reviews by Garcia-Olmedo *et al.*, 1982; Shewry *et al.*, 1984a) and in correlating some components in durum and bread wheat cultivars with pasta- (Damidaux *et al.*, 1978) and bread-making quality (see review by Payne *et al.*, 1984d), respectively. These correlations have been partly attributed to the better aggregating ability and greater amount of glutenin protein (Payne *et al.*, 1984b; Autran and Berrier, 1984). Further investigations are

required to understand the nature of these correlations between specific protein bands and flour quality at the molecular (primary structure of the polypeptides) and functional levels (protein–protein and protein–non-protein interactions in dough development).

Besides providing a basis for relating differences in protein type between cultivars to differences in nutritional or organoleptic quality of flour, an understanding of the structure and genetics of storage proteins in wheat and related species provide:

(a) a better knowledge of the genetic and evolutionary relationships between particular wheat chromosomes and chromosomes in related species (Lawrence and Shepherd, 1981a),

(b) a useful tool in cytogenetics where storage protein markers can be used as effective markers for the controlled transfer of useful homoeoallelic characters from alien species into wheat by induction of homoeologous pairing (Koebner and Shepherd, 1985, 1986),

(c) an efficient aid to cultivar identification, pedigree analysis and verifying the purity of seeds (see review by Wrigley *et al.*, 1982a) and

(d) linked markers for other characters e.g.disease resistance (Bartos and Bares, 1971) and dough stickiness (Zeller, 1973; Moonen and Zeven, 1984).

The information available on the fractionation and genetic control of proteins of wheat and related species together with the quality associations (including their functional and molecular basis) of these proteins in bread and durum wheats is reviewed in Chapter 2.

The main aim of the work presented in this thesis has been to analyse the variation of low-molecular-weight (LMW) subunits of glutenin in bread wheat and their chromosomal control in different cultivars, their inheritance, allelic relationships and association with bread-making quality. Some of these aspects of LMW subunits of glutelin have also been investigated in tetraploid and diploid wheats and in species related to wheat.

A two-step one-dimensional (1-D) sodium dodecyl sulphate–polyacrylamide gel electrophoresis (SDS-PAGE) procedure, developed by Singh and Shepherd (1984b), was used to fractionate the LMW subunits of glutelin. When investigations for the present thesis were initiated in October, 1985, the resolution of the LMW subunits of glutenin achieved by this procedure was inadequate to analyse their variation among cultivars due to overlap with some non-glutenin bands (referred to as non-prolamins in this thesis). Hence modifications were made to the procedure to allow the LMW subunits to be analysed in the absence of non-prolamins. These changes in procedure are described in Chapter 3 along with the data

obtained on variation and genetic control of LMW subunits of glutelin in wheat and related species.

Chapter 4 includes the data obtained from test-cross analyses designed to study the inheritance of LMW subunits of glutenin in bread wheat. A special genetic stock "Triple translocation" was produced for use as the third parent in the test-crosses. Three double translocation stocks were also isolated. These stocks have provided a new approach for determining the genetic control of the LMW subunits of glutenin in bread wheat.

The relationships between allelic variation in the LMW subunits and bread-making quality are described in Chapter 5. The chapter also includes data on the relative importance of chromosome 1A-controlled LMW and HMW subunits on physical dough properties. The effect of the addition of 75 kd γ-secalins from rye on dough properties of wheat was also investigated. The genetic and biochemical features of the non-prolamins detected in the two-step procedure are described in Chapter 6.

Chapter 2

LITERATURE REVIEW

This chapter reviews the published literature up to the commencement of the present investigation (i.e.year 1985) and the information published during the course of the present studies will be included in the results and discussion sections.

2.1 Classification and characterization of wheat proteins

The first systematic study of cereal seed proteins was done by T. B. Osborne (1907) on the basis of their sequential extraction and solubility. He classified them into four groups as albumins (soluble in water), globulins (soluble in dilute salt solutions), prolamins (soluble in aqueous alcohols, particularly 70% ethanol) and glutelins (soluble in dilute acid or alkali). Subsequently, these proteins have been classified on the basis of many other characteristics (see review by Lásztity, 1984) including (i) their location within the seed: proteins of the endosperm, the aleurone layer and the embryo, (ii) their chemical composition: simple proteins and complex proteins such as lipoproteins and glycoproteins, and (iii) their biological function: metabolically active/cytoplasmic proteins and storage proteins. Metabolically active proteins correspond principally to the albumins and the globulins whereas storage proteins comprise mainly the prolamins and the glutelins.

Recent developments in the characterization of cereal proteins have clearly indicated that each of Osborne's solubility fractions is a complex mixture of different polypeptides and also that there is much overlap in the solubility of these polypeptides. For example, a small fraction of the ethanol soluble proteins, called HMW gliadin by Beckwith *et al.* (1966), behaved like glutenin in showing a drastic decrease in viscosity after reduction of disulphide bonds, so it was called LMW glutenin by Nielsen *et al.* (1968). Later, this fraction was shown to consist of disulphide-linked aggregates of LMW glutenin subunits (Bietz and Wall, 1973, 1980; Kanazawa and Yonezawa, 1973). Since this fraction was soluble in 70% ethanol, Shewry *et al.* (1983b) preferred to classify it as aggregating gliadin. The aggregates

made up of HMW glutenin subunits alone or both HMW and LMW glutenin subunits are not easily soluble in 70% ethanol in their native state (Byers *et al.*, 1983), but after the reduction of disulphide-linkages, they as individual polypeptide chains are soluble (especially in propan-1-ol) (see review by Kreis *et al.*, 1985). Because of their solubility in alcohols and high content of proline and glutamine, the glutenins have also been regarded as prolamins. Consequently, all storage proteins of cereals (prolamins and glutelins) have been re-classified into one group, the prolamins (Kreis, loc. cit). The prolamins have been further classified on the basis of their molecular weight, amino acid composition and aggregation (disulphide linkage) properties. These are (i) HMW prolamins (HMW glutenin subunits), (ii) sulphur-poor prolamins (ω-gliadins) and (iii) sulphur-rich prolamins (α-, β-, γ-gliadins and LMW glutenin subunits). In this thesis, the more traditional terms given in the above parentheses have been adopted to specify the individual fractions. Moreover, since native HMW glutenin contains aggregates of both HMW and LMW subunits (Bietz and Wall, 1973, 1980), it is considered that the term LMW glutenin subunits refers not only to the subunits of LMW glutenin but also to the LMW subunits of native HMW glutenin. Hence, the terms LMW and HMW glutenin subunits are most accurately referred to as the LMW and HMW subunits of glutenin. Alternatively, they can be referred to as the groups of A (HMW subunits), B and C (LMW subunits) subunits of glutenin (Payne and Corfield, 1979; Graveland *et al.*, 1982). Furthermore, it is inappropriate to use the the terminology B and C subunits of LMW glutenin; they should be referred to as the B and C subunits of glutenin. All of these disulphide-linked subunits are hereafter generally referred to as polymeric prolamins when described with similar proteins from other cereals, e.g. HMW and 75 kd γ-secalins from rye. Similarly the gliadins and ω- and 40 kd γ-secalins are called monomeric prolamins.

The storage proteins are deposited in protein bodies during endosperm development (Miflin *et al.*, 1981, 1983) and are normally thought to have no other function than to act as a source of nitrogen, carbon and sulphur for the germinating seed and early seedlings (Murray, 1984). Prolamins fulfill these requirements and hence they have been classified as storage proteins (see review by Shewry and Miflin, 1985). However, because of conserved interspecific differences, Ladizinsky (1983) suggested that seed storage proteins have adaptive value subject to selection and are involved in speciation. The distinction between 'storage'

and 'metabolic' proteins is also obscured in certain enzymes which appear to have a storage function. A miscellaneous group of proteins, soluble in chloroform/methanol and called CM-proteins (Salcedo *et al.*, 1978), are trypsin/α-amylase inhibitors (Shewry *et al.*, 1984e) and yet have a secondary role as minor storage proteins (c.f. Kreis *et al.*, 1985). Recently, salt-soluble triplet protein in wheat, also have been shown to have the characteristic properties of a storage protein (Singh, 1985). These observations suggest that the traditional assumption of an exclusively non-storage function for wheat globulins and albumins needs to be reassessed.

Graham *et al.* (1963) isolated two types of protein bodies from developing wheat seed on the basis of size. The amino acid composition of the large protein bodies was comparable to that of gliadins and glutenins although these protein bodies also contained 15-20% structural proteins. The amino acid composition of the small protein bodies resembled that of albumins and globulins (Jennings and Morton, 1963). These results indicate that even though the majority of storage proteins are deposited in protein bodies, the storage proteins are not easy to define and many criteria need to be taken into account to classify proteins as storage proteins.

2.2 Fractionation of the seed proteins of hexaploid wheat and their chromosomal control

The original Osborne extraction procedure has been considerably modified to give better extraction of the seed proteins from wheat (Chen and Bushuk, 1970; Bietz and Wall, 1975). Albumins and globulins have been extracted with several solvents, e.g. 1M or 2M urea, 25% aqueous 2-chloroethanol, and aqueous dimethyl formamide. Gliadins have been extracted with 2-6 M urea solution or aluminium lactate buffer. Many solvents have been used for extracting total seed proteins, e.g. acetic acid–urea–cetyltrimethylammonium bromide (AUC) (Meredith and Wren, 1966), Sodium-dodecyl-sulphate (SDS; Danno *et al.*, 1974), sodium salts of fatty acids (Kobrehel and Bushuk, 1977) and mixtures of propan-1-ol with 2-mercaptoethanol (Miflin *et al.*, 1983). Gel filtration, acid precipitation and high speed centrifugation have also been used for further separation of the complex mixtures of proteins and these fractions have been characterized by electrophoretic separation.

Albumins and globulins migrate well ahead of the gliadins when separated electrophoretically in a starch gel at acid pH. They have not been studied systematically in much detail, possibly because they are not the major determinants of bread-making quality.

Gliadins were first classified into α, β, γ and ω components based on their electrophoretic mobility in moving boundary electrophoresis (Jones *et al.*, 1959). The application of starch gel electrophoresis at acid pH (first used by Elton and Ewart, 1960 to wheat proteins) to fractionate seed protein extracts from compensating nullisomic-tetrasomic and ditelosomic stocks of Chinese Spring wheat produced by Sears (1954, 1966) was the foundation for studies of the genetics of wheat proteins (Boyd and Lee, 1967; Shepherd, 1968). Since then electrophoretic procedures have been refined to give much better resolution of bands and more detailed information on their genetic control. Especially important has been the introduction of various 2-D procedures, including the combination of isoelectricfocusing (IEF, pH 5-9) and acid starch gel electrophoresis (Wrigley, 1970), polyacrylamide gel electrophoresis (PAGE) at pH 3.2 followed by PAGE at pH 9.2 (Mecham *et al.*, 1978; Lafiandra *et al.*, 1984), combined IEF with SDS-PAGE (O'Farrell, 1975; Brown *et al.*, 1979; Brown and Flavell, 1981), combined IEF with Acid-PAGE (Bushuk and Zillman, 1978; Payne *et al.*, 1982a) and combined IEF/non-equilibrium pH gradient electrophoresis (NEPHGE) with SDS-PAGE (O'Farrell *et al.*, 1977; Jackson *et al.*, 1983).

Glutenin was first fractionated by Jones *et al.* (1959) into heterogeneous molecules ranging from about 50,000 to several millions in size using moving boundary electrophoresis. Starch gel electrophoresis was also used to try to separate the components of native glutenin of wheat but they were too large to enter the gel (Woychik *et al.*, 1961). After reduction of the disulphide bonds, however, glutenin could be separated into about 20 components (Woychik *et al.*, 1964) but the resolution of these bands was not good enough to allow genetic studies. Improved resolution of these subunits occurred when the SDS-PAGE method of Shapiro *et al.* (1967) was introduced simultaneously by Bietz and Wall (1972) and Hamauzu *et al.* (1972). The best resolution of the HMW subunits of glutenin occurred when the discontinuous system of one-dimensional SDS-PAGE (Laemmli, 1970) was introduced to wheat independently by Payne *et al.* (1979) and Lawrence and Shepherd (1980).

None of these one-dimensional SDS-PAGE methods provided adequate resolution of the LMW subunits of glutenin as they overlapped the gliadins. They were resolved by Jackson *et al.* (1983) into B, C and D subunits by applying the two different 2-D methods of O'Farrell (1975) and O'Farrell *et al.* (1977). Singh and Shepherd (1984b) introduced a simple two-step one-dimensional SDS-PAGE for separating LMW subunits of glutenin from gliadins.

The electrophoretic methods described above were applied to seed protein extracts from nullisomic-tetrasomic and ditelocentric stocks (Sears, 1954, 1966; Sears and Sears, 1978) and intervarietal substitution lines (Sears *et al.*, 1957; Morris *et al.*, 1966; Law *et al.*, 1978) in Chinese Spring wheat to locate the genes controlling certain protein bands. The nullisomic-tetrasomic (NT) stocks (where a chromosome pair is deleted and its loss is compensated by an extra pair of homoeologous chromosomes, e.g. NT 1A-1B) allowed to locate the genes controlling particular protein bands to specific chromosomes (e.g. any bands controlled by 1A will be missing in NT 1A-1B stock). Subsequent use of ditelocentric (Dt) stocks allowed genes to be located to a particular arm of the chromosomes (e.g. bands controlled by short arm 1AS will be missing in Dt 1AL). Similarly, inter-varietal substitution lines (in which a pair of chromosomes from a recipient variety is replaced by another pair of chromosomes from a donor variety, e.g. Chinese Spring-Hope 1A substitution) have proven useful in studying the chromosomal control of inter-varietal variation in protein composition. The findings obtained from using these stocks and the electrophoretic procedures have made a significant contribution towards understanding the genetics of these protein fractions and these are reviewed briefly below.

2.2.1 Albumins and Globulins

Shepherd (1971) located genes controlling some albumins on chromosome arms 5AL, 5BL and 5DL by analysing protein patterns in starch gels of urea extracts from nullisomic-tetrasomic and ditelocentric stocks in Chinese Spring. Using 2-D electrophoresis (IEF, pH 4–9 and starch gel electrophoresis), genes controlling 14 of the 25 protein components identified in dilute salt extracts were assigned to chromosomes 1B, 3B, 3D, 4A, 4B, 5B, 6B, 6D, and 7D (Fra-Mon *et al.*, 1984). Most of the salt-soluble proteins controlled by chromosomes 4

and 7 were also soluble in a chloroform/methanol mixture and thus correspond to CM proteins (Salcedo *et al.*, 1978). Singh and Shepherd (1985) located the genes controlling triplet proteins (salt soluble globulins) on the short arms of chromosomes 1A and 1D in Chinese Spring wheat. Payne *et al.* (1985) have also analysed the globulins (including triplet proteins) in two-dimensional systems and showed the chromosomal control of some of the globulins by 5AL, 5BL, 5DL, 4BL and 4DS. They found three HMW albumin streaks and confirmed that the slowest of them was controlled by chromosome arm 4DL. However, this 4DL-controlled band has earlier been classified as a HMW glutenin subunit (Bietz *et al.*, 1975) and a globulin band (Brown and Flavell, 1981).

2.2.2 Gliadins

Boyd and Lee (1967) published the first report on the chromosomal control of gliadin bands and showed that two slow-moving bands (ω-gliadins) were controlled by genes on one arm of chromosome 1D. The first comprehensive genetic study was made by Shepherd (1968) who located genes controlling 9 out of 17 major gliadin bands of Chinese Spring wheat on chromosome arms 1AS, 1BS, 1DS, 6AS and 6DS. The remaining eight bands were thought to be overlapping gliadin bands controlled by duplicate or triplicate genes on more than one chromosome. Later, Wrigley and Shepherd (1973) resolved this question by fractionating wheat gliadins into 46 spots by 2-D separation and were able to assign the genetic control of each of the 33 components to one of chromosomes 1A, 1B, 1D, 6A, 6B, or 6D. These findings have since been confirmed by many other workers using different methods of two-dimensional separation of proteins in Chinese Spring (Mecham *et al.*, 1978; Brown *et al.*, 1979; 1981; Brown and Flavell, 1981; Payne *et al.*, 1982a; Lafiandra *et al.*, 1984) and in other cultivars using intervarietal substitution lines (Kasarda *et al.*,1976b; Brown *et al.*, 1981; Lafiandra *et al.*, 1984; Payne *et al.*, 1984c; Galili and Feldman, 1985). Shepherd (1968) and Brown and Flavell (1981) found that certain fast moving gliadin bands which were deleted in nulli-tetra stocks 6D-6A and 6D-6B were also absent in nulli-tetra 2D-2A and tetra 2A lines. It was suggested that the structural genes for these proteins lie on chromosome 6D and 4 doses of chromosome 2A may result in the inhibition of these bands,but it is still not known whether this aberrant pattern was due to dosage related regulatory effects of chromosome 2A

or to the loss of genes coding for these bands on 6D during the production of the group 2 related stocks. Except for this aberrant pattern, the other results have clearly shown that gliadins are controlled by genes located on the short arms of group 1 and group 6 chromosomes of genomes A, B and D in hexaploid wheat.

2.2.3 HMW subunits of glutenin

Orth and Bushuk (1974) were the first to report on the chromosomal control of glutenin subunits and concluded that genes encoding four subunits with apparent molecular weights of 152,000, 112,000, 60,000 and 45,000 in SDS-PAGE were located on the long arm of chromosome 1D of Chinese Spring wheat. The faster moving bands (60,000 and 45,000) were, however, present in the streaky portion of the gels and it is not clear from re-examination of their gel photographs, whether these bands were actually present or absent in the ditelocentric 1DL stock. Using single kernel analysis and SDS-PAGE, Bietz *et al.* (1975) located the genes controlling two glutenin subunits (MW 104,000 and 93,000) on the chromosome arm 1BL and two other subunits (MW 133,000 and 86,000) on the long arm of chromosome 1D. Brown and co-workers (Brown *et al.*, 1979, 1981; Brown and Flavell, 1981) used 2-D electrophoresis to fractionate the glutenin subunits and obtained similar results except that the subunits coded by chromosome 1B were not detectable in their system.

Using a discontinuous system of 1-dimensional SDS-PAGE, Lawrence and Shepherd (1980, 1981b), Payne *et al.* (1980, 1981a) and Galili and Feldman (1983a, b, 1985) improved the resolution of the HMW subunits of glutenin and confirmed the chromosomal control of these four HMW subunits of glutenin of Chinese Spring by 1DL and 1BL. They also determined the chromosomal location of additional HMW subunits of glutenin in several other cultivars by using intervarietal substitution lines and showed that chromosome arm 1AL of some wheat cultivars also carried genes controlling HMW glutenin subunits. In summary, these results have shown that each cultivar contains 2 HMW subunits of glutenin controlled by chromosome 1D, 1 or 2 subunits encoded by chromosome 1B and 0 or 1 subunit controlled by 1A. The subunit pairs controlled by chromosome 1B and 1D were divided into x and y subunits on the basis of their electrophoretic mobility suggesting that there are two closely linked genes controlling these subunits (Payne *et al.*, 1981a). In some cases, three

HMW subunits of glutenin designated 1Bx, 1By and 1Bz (Holt *et al.*, 1981) or Glt-B1, Glt-B2 and Glt-B3 controlled by chromosome 1B and two subunits (Glt-A1, Glt-A2) controlled by chromosome 1A (Galili and Feldman, 1983b, 1985) have been reported. The subunits 1Bz or Glt-B3 and Glt-A2 were faint, however, and the position of the minor 1Bz band, determined by 2-D electrophoresis, was correlated with the position of the major 1By components (Holt *et al.*, 1981). It was suggested that the minor bands could be the products of the same gene and derived by post-translational modification from the major subunits.

2.2.4 LMW subunits of glutenin

The first report on the genetic control of LMW subunits of glutenin was published by Jackson *et al.* (1983) who located the genes controlling all B and D and some C subunits of Chinese Spring on the short arm of chromosomes 1A, 1B and 1D by separating proteins from nulli-tetra and ditelocentric stocks in 2-D gels. Singh and Shepherd (1985) confirmed the genetic control of five LMW subunits of glutenin of Chinese Spring by separating proteins from these genetic stocks in two-step SDS-PAGE. The genetic control of these subunits in other cultivars had not been documented when the present study was initiated.

2.3 Variation and inheritance of seed proteins in hexaploid wheat

The electrophoretic patterns of wheat seed proteins have proven useful in species and cultivar identification because they are the characteristics of the genotype of the plant and are not greatly influenced by the environmental conditions under which the plant was grown. The albumin and globulin patterns are useful for distinguishing between different wheat species because they do not exhibit much variation among cultivars of the same species (Johnson, 1972). Gliadin band patterns, however, vary substantially among cultivars and thus they have been used most often for discriminating between them (Wrigley *et al.*, 1982a; review by Lásztity, 1984). Variation in HMW glutenin subunit composition in SDS-PAGE has provided an additional means for the identification of wheat cultivars (Lawrence and Shepherd, 1980). Electrophoretic variation in gliadins and HMW subunits of glutenin has been associated with differences in the pasta- and bread-making qualities of wheat flours (Damidaux *et al.*, 1978; Payne *et al.*, 1979; Wrigley *et al.*, 1981; Moonen *et al.*, 1982, 1983)

and thus has benefitted wheat breeders and generated further interest. Electrophoretic patterns have also been useful in studying the genetic similarity between wheat and its relatives such as homoeologous relationships between group 1 chromosomes of wheat, 1R of rye and 1U of *T. umbellulatum* (Shepherd, 1973; Lawrence and Shepherd, 1980, 1981a).

2.3.1 Albumins and globulins

Johnson (1972) analysed more than 30 accessions of each of *T. aestivum*, *T. turgidum* cv. group *dicoccum* and *T. tauschii* and found very similar albumin patterns within species. Similarly, minimal variation in albumin patterns of different varieties within species was also recorded by others (Orth and Bushuk, 1972; Cole *et al.*, 1981; Wrigley, 1982). However, Caldwell and Kasarda (1978) detected considerable intraspecific variation for albumins and globulins in diploid *Triticum* species. Recently, Singh and Shepherd (1985) observed five different patterns of globulin proteins (triplet proteins) among 135 bread wheat cultivars. These results thus indicate that there is limited variation in the electrophoretic patterns of globulin and albumin within a species.

2.3.2 Gliadins

Doekes (1973) analysed the inheritance of intervarietal gliadin differences and established that gliadin patterns of different varieties were inherited as unaltered 'blocks'. Baker and Bushuk (1978) studied the segregation of gliadins in 78 backcross lines and found the 'block' inheritance of three groups of bands, two of which were alternatives to each other. The inheritance of the different gliadin bands in three cultivars was investigated by Mecham *et al.* (1978) in F_2 populations and they observed that groups of gliadin bands controlled by chromosomes 1B and 6A were always inherited as a unit and it was postulated that these groups of bands might be controlled by several closely linked genes.

The most extensive analysis of 'block' inheritance of gliadins has been carried out by workers in the U.S.S.R. (Sozinov and Poperelya, 1980; Metakovsky *et al.*, 1984) who analysed F_2 seeds from 36 crosses involving 39 winter wheat cultivars and about 80 different sets of bands were detected. Tests for allelism showed that gliadin blocks could be grouped

into six series (loci) named *Gld1A* (15 alleles), *Gld1B* (18 alleles), *Gld1D* (8 alleles), *Gld 6A* (13 alleles), *Gld 6B* (11 alleles) and *Gld 6D* (10 alleles).

Allelic variation in the gliadin patterns of bread wheat has also been recorded by other researchers. Wrigley *et al.* (1981, 1982b) analysed over 150 bread wheat cultivars in starch gels at pH 3.1 and divided them into 13 groups on the basis of similarities in their gliadin patterns. Galili and Feldman (1983b) analysed 109 cultivars by 1-D SDS-PAGE and also found considerable variation in gliadin composition. Nine subgroups of gliadins were detected, 6 of which appeared to be controlled by chromosome 1B (Gld-B1 to Gld-B6), three by chromosome 1D (Gld-D1 to Gld-D3) and none was controlled by chromosome 1A.

2.3.3 HMW subunits of glutenin

Bietz *et al.* (1975) analysed HMW glutenin subunit patterns of 80 hexaploid wheat cultivars and recorded only limited variation because the resolution of these bands was inadequate in the continuous SDS-PAGE system used. Lawrence and Shepherd (1980) analysed 98 wheat cultivars using a discontinuous gel system and found much more variation in the HMW subunits of glutenin with three to five subunits in each cultivar and at least 34 different banding patterns were observed. Based on their mutual exclusiveness (allelic nature), these patterns were divided into three groups controlled by genes on chromosomes 1A, 1B and 1D. Wrigley *et al.* (1982b) analysed the same 98 cultivars and detected 14 different HMW subunits of glutenin and a null form. These subunits occurred in 15 different banding patterns, 4, 9 and 2 of which were controlled by chromosome arms 1AL, 1BL and 1DL, respectively. Payne *et al.* (1980, 1981a) surveyed 192 cultivars and found 3, 9 and 4 different patterns controlled by chromosomes 1A, 1B and 1D, respectively. Later on, Payne and Lawrence (1983) listed 3 (*a-c*), 11(*a-k*) and 6 (*a-f*) distinct allelic variants of HMW subunits of glutenin controlled by *Glu-A1*, *Glu-B1* and *Glu-D1* loci, respectively in a total of 300 cultivars. Moonen and Zeven (1984) subsequently reported that the allele *Glu-B1j* for HMW glutenin subunit 21 (Payne and Lawrence,1983) was erroneous as band 21 represented rye proteins. Additional alleles have since been reported (Payne *et al.*,1983; Payne *et al.*, 1984d). Other bread wheat cultivars from Israel (Galili and Feldman,1983b), the Netherlands (Moonen *et al.*, 1982) and France (Branlard and Le Blanc, 1985) have been surveyed for

HMW glutenin subunit patterns and a similar extent of variation was observed. Branlard and Le Blanc (1985) also reported a new allele possibly coded by *Glu-D1* in their study.

Presumably, the variation in band patterns occurring in different cultivars is due to differences among the genes (allelic genes) coding for them and the proteins inherited as a block are the products of a cluster of structural genes that have arisen from a single ancestral gene by duplication and mutation (Wrigley, 1982). Evidence from terminal amino acid sequence of gliadins and LMW subunits of glutenin (Bietz *et al.*, 1977; Autran *et al.*, 1979; Shewry *et al.*, 1983b, 1984a; Okita *et al.*, 1985) supports the hypothesis that the different genes in each block were derived from one ancestral locus. Furthermore, it has been postulated that all storage protein genes may be derived from a single ancestral gene (see review by Kreis *et al.*, 1985).

These studies have also shown that banding patterns of HMW subunits of glutenin and gliadins controlled by chromosome 1A exhibit minimal variation among different cultivars and some of these cultivars do not contain any HMW glutenin subunit and gliadin band controlled by this chromosome. Thus, Galili and Feldman (1983a, b, c) have postulated that genes controlling HMW subunits of glutenin and gliadins in common wheat have undergone a high degree of non-random diploidization i.e.some of the duplicated genes for storage proteins have become silent through mutations or elimination of the structural genes.

2.3.4 LMW subunits of glutenin

Until 1985, only very limited studies had been made to examine the electrophoretic variation in LMW subunits of glutenin and their inheritance in bread wheat. Payne *et al.* (1984e) and Singh and Shepherd (1984b) detected some differences in LMW glutenin subunit patterns among bread wheat cultivars and used them in the linkage mapping of genes controlling these subunits. Thus, the major aim of the present project was to obtain information on the variation and genetic control of the LMW subunits of glutenin and their inheritance and allelic relationships with each other in bread wheat.

2.4 Linkage mapping of genes controlling seed proteins in hexaploid wheat

Much effort has been made to determine the linkage relationships among genes controlling seed proteins in wheat and to map them on the chromosomes. The genes controlling gliadins (*Gli-1*) on the short arms and HMW subunits of glutenin (*Glu-1*) on the long arms of group 1 chromosomes have been shown to segregate independently (Lawrence and Shepherd, 1981b; Chojeki *et al.*, 1983) or to exhibit very loose linkage (Payne *et al.*, 1982b). Using the telocentric mapping procedure of Sears (1962), Rybalka and Sozinov (1979) measured 42% recombination between the centromere and the gliadin locus on chromosome arm 1BS. Singh and Shepherd (1984b) also found similar recombination values and suggested that genes controlling gliadins are located distally on the short arms of chrmosomes 1A, 1B and 1D. Furthermore, from cytogenetic and biochemical analyses of a stock with a terminal deficiency of the short arm of chromosome 1B (Payne *et al.*, 1984c), it is clear that the *Gli-B1* locus is on the satellite region of chromosome arm 1B and linkage studies (Snape *et al.*, 1985) show it to be far away from the nucleolar organiser region.

Payne *et al.* (1982b) mapped the genes controlling the HMW subunits of glutenin at the *Glu-A1*, *Glu-B1* and *Glu-D1* loci with respect to the centromere on chromosomes 1A, 1B and 1D and measured recombination values of 7.6%, 9.2% and 10.1%, respectively. The rare recombination (1/1000) within the *Glu-B1* locus (Payne *et al.*, 1984a) and within the *Glu-D1* locus (1/720; Singh, 1985) has provided evidence that the genes controlling x and y type subunits are tightly linked. A similar low level of recombination has also been reported within the *Glu-A1* locus (Lawrence and Shepherd, 1981b).

In an analysis of 136 F_2 seeds, Mecham *et al.* (1978) detected a low level of recombination between two pairs of ω-and γ-gliadins controlled by chromosome 1B, viz. 0.007% between ω2 and γ4 and 0.04% between ω5 and γ6. Subsequently, these authors refused to interpret the observed patterns as recombinants. Payne *et al.* (1984e) did not detect any recombinant between genes controlling some gliadins (ω- and γ-gliadins) and the LMW subunits of glutenin in the 693 test-cross progeny involving genes on chromosomes 1A nor in the 203 progeny relating to chromosome 1B. They concluded that these genes occur as tightly

linked groups at two complex loci, named *Gli-A1* and *Gli-B1*, respectively. Singh and Shepherd (1984b) observed complete linkage between these genes on chromosome arms 1AS and 1DS but detected 1.7% recombination between genes controlling gliadins and LMW subunits of glutenin on 1BS. They also mapped the genes (*Tri-1*) controlling triplet proteins to a position midway between the *Gli-1* and *Glu-1* loci on chromosomes 1A and 1D and thus proximal to the centromere. They deduced the following gene order: centromere – triplet – LMW subunits of glutenin – gliadins and designated the genes controlling LMW subunits as *Glu-3* (Singh, 1985). All of these results have shown clearly that genes controlling ω- and γ-gliadins and LMW subunits of glutenin are tightly linked on the short arms of the group 1 chromosomes.

Other workers have reported genes coding for gliadin which are located away from this main cluster and which recombine with it. Branlard (1983a,b) analysed 312 F_2 seeds from a cross and showed that band 44 of a particular block of bands controlled by 1DS in variety Joss consisted of two bands whose genes were located at a distance of 24.8 recombination units from each other. Sobko (1984) found that certain gliadins were inherited in a monofactorial ratio in cultivar Bezostaya-1 and the gene was located at a separate locus *Gld-2-1A*, 31 recombination units apart from the main cluster of gliadin genes at the *Gld-1A* locus on chromosome 1A. Galili and Feldman (1984b) found that the gene (*Gld-B6*) controlling a gliadin component B-30 in CS-Thatcher 1B substitution line recombined frequently with other gliadin genes (25.5 % recombination) and with the HMW glutenin genes (23.5 % recombination) on chromosome 1B. Jackson *et al.* (1985) found that the genes controlling the D subunits of glutenin (*Glu-B2*) were located 22 cM away from the *Gli-B1* locus and 17 cM away from the *Glu-B1* locus on chromosome 1B. Although the *Glu-B2* and *Gld-B6* loci map in a similar position, it is not known whether they are the same locus (Jackson *et al.*, 1985).

The chromosome location of the *Gli-2* loci coding for α- and β-gliadins is not so well documented. It has been shown that *Gli-B2* occurs on the satellite segment of the short arm of chromosome 6B, about 22 recombination units from the ribosomal RNA genes in the nucleolar organiser region (Dvorak and Chen, 1984). The *Gli-A2* locus has been located 35% recombination units away from the centromere on 6AS (Payne *et al.*, 1984d). Nucleotide sequencing data have shown close homology between the genes at the *Gli-1* and *Gli-2* loci,

for example between γ- and α/β-gliadins (Okita *et al.*, 1985) and this suggests that they arose from the duplication and divergence of a common ancestral gene as proposed by Shepherd and Jennings (1971). Rare recombination within the *Gli-2* loci, unlike those at *Gli-1*, has not been reported.

In summary, it appears that there are at least 11 distinct loci controlling gliadins and glutenins in hexaploid wheat. *Glu-A1*, *Glu-B1* and *Glu-D1* are located on the long arm of chromosomes 1A, 1B and 1D; *Gli-A1*, *Gli-B1*, *Glu-B2*, *Gld-B6*, and *Gli-D1* are located on the short arm of group 1 chromosomes and *Gli-A2*, *Gli-B2* and *Gli-D2* are located on the short arm of group 6 chromosomes.

2.5 Variation, genetic control and inheritance of seed proteins in tetraploid wheat

Damidaux *et al.* (1980) analysed aneuploids in durum wheat (*Triticum turgidum* cv. group *durum*) by Acid-PAGE and showed that two γ-gliadin bands, called 42 and 45 on the basis of their relative mobility, are controlled by genes on chromosome 1B. Joppa *et al.* (1983) localized these genes to the short arm of this chromosome by using a durum stock ditelocentric for 1BS and also assigned the genes controlling 17 other gliadin bands out of a total 30 to group 1 and 6 chromosomes by using disomic substitutions of D-genome chromosomes from Chinese Spring in durum cultivar Langdon. Subsequently other workers (du Cros *et al.*, 1983; Lafiandra *et al.*, 1983) have confirmed that the gliadins in durum wheats are coded by the group 1 and 6 chromosomes of the A and B genomes and also that group 2 chromosomes are not implicated in the synthesis of the gliadin proteins of durum wheat. In inheritance studies, certain ω- and γ-gliadins coded by chromosome 1B segregated as a unit in 209 progeny of a tetraploid wheat cross (Damidaux *et al*, 1980) and bands 42 and 45 behaved as though controlled by alleles on chromosome 1B (Damidaux *et al*, 1980; du Cros and Hare, 1985).

The HMW glutenin subunit patterns of tetraploid wheats have not been analysed as extensively as those in the bread wheats. Bietz *et al.* (1975) observed more variation in the HMW subunits of glutenin in 55 tetraploid wheats than in 80 hexaploid wheat cultivars.

Galili and Feldman (1983c) also recorded extensive variation in the HMW glutenin patterns of *T. turgidum* cv. groups *dicoccum* and *durum*.

A limited survey of variation in LMW glutenin subunit composition in durum wheat genotypes has also been conducted using 1-D and 2-D separation methods. Two patterns, LMW-1 and LMW-2 were detected and found to be closely linked to the known gliadin bands (42 and 45, respectively) controlled by genes on the short arm of chromosome 1B (Payne *et al.*, 1984b; Autran and Berrier, 1984).

2.6 Endosperm proteins from species related to wheat

Some progress has also been made in the genetic and biochemical characterization of seed proteins from wild diploid *Triticum* species, *Secale* species and *Elytrigia elongata* as described below.

2.6.1 Wild diploid Triticum *species*

Orth and Bushuk (1973), using 1-D SDS-PAGE, found very limited variation in the reduced glutenin patterns of 6 accessions of *T. tauschii*. Lawrence and Shepherd (1980), however, reported 8 different patterns for HMW subunits of glutenin in 28 accessions of *T. tauschii* . Similarly, Law and Payne (1983) analysed 8 species of diploid wheats and recorded extensive variation in the HMW glutenin subunit patterns. Variation in the HMW glutenin subunit patterns has also been recorded in *T. urartu* (Galili and Feldman, 1983c). Comparison between the HMW subunit patterns of *T. tauschii* and bread wheats has shown that *T. tauschii* had two pattrens similar to those controlled by the chromosome 1D in bread wheats (Lawrence and Shepherd, 1980).

Analysis of *T. umbellulatum* addition lines in Chinese Spring have shown that two of the chromosomes in *T. umbellulatum,* namely B (=1U) carried genes for three monomeric prolamin bands and chromosome A (=6U) controlled one protein band (Shepherd, 1973). Brown *et al.* (1979) using 2-D electrophoresis showed that chromosome 1U controlled 7 bands, two of them were of HMW and 4 of them were in the size range of 52-55 kd and the remaining band was of 42 kd. Lawrence and Shepherd (1981a) used 1-D separation and assigned only three prolamin bands to this chromosome, two on the long arm and the

remaining band on the short arm. Two other protein bands, in order of their increasing mobility, were found to be controlled by genes on chromosome C (=5U) and chromosome A (=6U) of *T. umbellulatum*. It is likely that bands controlled by chromosome 5U of *T. umbellulatum* are albumins and globulins as the protein bands governed by corresponding chromosomes in bread wheats are normally albumins and globulins (Shepherd, 1971).

2.6.2 Secale *species*

The prolamins or storage proteins of rye have been collectively called secalins which are further divided into HMW secalins, 75kd γ-secalins, ω-secalins and 40 kd γ-secalins on the basis of their relative electrophoretic mobility and structural relationship (Shewry *et al.*, 1982). In a survey of 6 species of *Secale*, considerable variation was observed in the band patterns of the first three fractions but the 40kd secalins appeared to be less variable (Shewry *et al.*, 1984b).

Genetic analyses revealed that monomeric prolamins (corresponding to ω-secalins and 40 kd γ-secalins) were controlled by genes on the short arms of chromosome V (=1R) in cereal rye cv. King II and chromosome E (=1R) in cv. Imperial (Shepherd, 1968; Shepherd and Jennings, 1971). Later, Lawrence and Shepherd (1981a) showed that two HMW glutelin subunits (corresponding to HMW secalin) and an intermediate MW subunit (corresponding to the 75kd γ-secalins) in rye were controlled by chromosome arm 1RL and chromosome 2R, respectively. Shewry *et al.* (1984b) also found that genes controlling 75kd γ-secalins in cereal rye are located on 2R. In *Secale montanum*, however, genes controlling these proteins were found to be located on chromosome 6R (Shewry *et al.*, 1985).

Linkage mapping has revealed that the genes controlling the HMW secalins (*Sec-3*) on chromosome arm 1RL, are loosely linked (40.8% recombination) with the genes controlling ω- and 40kd γ-secalins (*Sec-1*) on 1RS (Shewry *et al.*, 1984b). Singh and Shepherd (1984a) have shown that *Sec-3* (denoted by *Glu-R1*) is closely linked to the centromere with only 4.6 % recombination.

Biochemical and molecular studies have shown that the 75 kd γ-secalins have different amino acid composition, molecular weight and aggregation behaviour from 40 kd γ-secalins. However, since they have similar N-terminal amino acid sequences (Shewry *et al.*, 1982), it

has been suggested that genes coding for the 75kd γ-secalins may have been derived by duplication and divergence of genes controlling 40 kd γ-secalins on 1RS. It is postulated that the 40 kd γ-secalin genes were duplicated and translocated from 1RS to 6R in the ancestor of *Secale montanum* and eventually from 6R to 2R during the speciation of *S. cereale* (Shewry *et al.*, 1985). Studies have also shown that rye and wheat prolamins are chemically and structurally inter-related (Ewart, 1966; Charbonnier *et al.*, 1981; Field *et al.*, 1982; Shewry *et al.*, 1982; Kasarda *et al.*, 1983) and thus they are probably derived from a single common ancestral gene (see review by Kreis *et al.*, 1985).

2.6.3 Elytrigia elongata

Genetic analysis of seed proteins from diploid *Elytrigia elongata* has revealed that two prolamin bands were controlled by the long arm of chromosome I (=1E of Dvorak, 1980) and another band was assigned to chromosome III (=4E of Dvorak, 1980) (Lawrence and Shepherd, 1981a). The 4E controlled band has a similar mobility to a band in bread wheat controlled by 4DL (Bietz *et al.*, 1975) and it is likely that this could be an albumin or globulin band as the protein bands governed by group 4 chromosomes in bread wheats are normally albumins and globulins (Shepherd, 1971).

2.6.4 Summary

In summary, these protein markers have provided additional evidence for homoeologous relationships between chromosomes 1A, 1B and 1D of hexaploid of wheat and 1R of rye (Lawrence, 1969), 1U of *T. umbellulatum* (Athwal and Kimber, 1972) and 1E of *E. elongata* (Dvorak, 1980) and similarly between group 6 chromosome of hexaploid wheat and 6U of *T. umbellulatum* (Athwal and Kimber, 1972). These results also indicate that chromosomes belonging to homoeologous group 1 in all of these species are derived from a common ancestral chromosome, and have maintained their integrity during speciation since they all possess genes controlling monomeric prolamins on their short arms and genes controlling HMW glutelin subunits on their long arms (Lawrence and Shepherd, 1981a).

It is not known, however, whether chromosomes 1R, 1E and 1U in these species also carry genes for LMW subunits of glutelin, comparable to those on group 1 chromosomes in

bread wheat. Similarly, it is not known whether glutelins in rye (HMW and 75 kd γ-secalins), like glutenins in wheat, also have a major influence on the physical dough properties (visco-elasticity) of rye or wheat flours. Hence, the above genetic aspects and the effects of 75 kd γ-secalins on dough quality of wheat flours are investigated in the present thesis.

2.7 Influence of seed proteins on flour quality

There are many components which affect the quality of the end-use products made from wheat flours and these can be divided into protein factors (storage, non-storage) and non-protein factors (carbohydrates, lipids). Studies have shown that carbohydrates and lipids have some influence on the pasta- and bread-making properties of flours (Hoseney *et al.*, 1970; Lin *et al.*, 1974; MacRitchie, 1977; Pomeranz, 1980; Bushuk *et al.*, 1980; 1984; McMaster and Bushuk, 1983). However, it is widely accepted that the amount and composition of proteins present in the flour have the most significant effects on its quality. To produce a satisfactory commercial product, flour must have a minimum quantity and quality of protein. These requirements vary depending upon the type of products desired. In general, flours used for producing biscuits and cakes should have a low level of protein (about 10%) and should yield dough with good extensibility and low elasticity. On the other hand, strong flours (with 12% or more protein) are preferred for bread production because they are capable of producing an extensive visco-elastic matrix during dough formation which retains the gas during baking. For producing chapatis, an intermediate flour protein requirement between bread and biscuits is desirable. Durum wheat flours used for pasta products should have high protein (14% or more) and produce strong elastic doughs.

2.7.1 Assessment of flour quality

There are many tests which have been developed to evaluate the quality of bread wheat flours. Pelshenke (1933) devised a test which measures the time required for the disintegration of a heavily yeasted whole-meal dough ball in water. The Pelshenke values vary from 20 minutes for poor quality flours to more than 2 hours for good quality wheats and are insensitive to variation in grain protein content and thus reflect mainly gluten quality (Pushman and

Bingham, 1975; Branlard and Dardevet, 1985a). Monsivais *et al.* (1983) assessed the suitability of this test and concluded, in contrast, that the Pelshenke value is largely a function of the environment and has limited value for measuring the quality and quantity of flour protein.

Zeleny's (1947) test involves measurement of the sedimentation rate of flour suspended in a dilute lactic acid solution. The sedimentation value ranges from 20 or less for low protein flour of very inferior bread-baking quality to 50 or more for high protein flour with superior bread-baking quality. McDermott and Redman (1977) introduced the SDS-sedimentation test which is in principle similar to the Zeleny test except that the liquid added to the flour suspension includes SDS as well as lactic acid. It has been shown that SDS sedimentation volume is generally highly correlated with protein content, bread loaf volume (Axford *et al.*, 1979; Preston *et al.*,1982), SDS-insoluble glutenins (Moonen *et al.*, 1982) and with dough strength parameters (Blackman and Gill, 1980; Preston *et al.*, 1982). Preston *et al.* (1982) further showed that the correlation coefficient between SDS sedimentation volume and bread loaf volume was insignificant for combined samples from different environments having over 14% protein content. Moreover, since both of these tests give an overall assessment of protein quality, they are not suitable for more detailed information about the protein quality.

Tests are also available for direct evaluation of the physical properties of dough made from bread wheat flour and they have been described in detail by Kent-Jones and Amos (1967). The Chopin Alveograph involves the inflation of a bubble in dough under constant air pressure and measures tenacity, extensibility and strength of dough and is commonly used in France (Branlard and Dardevet, 1985a). The Simon Research Extensometer and the Brabender Extensograph, both of which involve direct stretching of dough until it breaks, are commonly used in the United Kingdom and Australia to record the same dough parameters as measured by the Alveograph. The Farinograph and Mixograph tests (generally used in North America) are similar and they measure the water absorption capacity of flour and its mixing tolerance (stability) during dough formation. All of these tests are very sensitive to the environment and thus require controlled conditions for reproducibility of results, but unlike the sedimentation and Pelshenke tests, they provide direct information about the extensibility, elasticity (resistance) and strength of the flour dough.

Modified forms of some of these tests have also been used to measure the quality of durum wheat flour (semolina), namely Farinograph (Irvine *et al.*, 1961), Mixograph (Bendelow 1967; du Cross *et al.*, 1982), Chopin Alveograph (Matsuo and Irvine, 1970) and SDS Sedimentation test (Quick and Donnelly, 1980, Dexter *et al.*, 1980). Another test, similar to a Viscoelastograph (Feillet *et al.*, 1977), is commonly used in France to measure the quality in terms of compressibility and elastic recovery of cooked gluten from durum wheat.

Doughs made from bread wheat flours are also directly baked into loaves and the loaf volume is used as a single index to determine the quality of flours.

2.7.2 *Protein quantity and flour quality*

There is a considerable amount of information which shows that protein quantity has a positive relationship with various parameters of flour quality, viz. sedimentation volume, (Bushuk *et al.*, 1969; Axford *et al.*, 1979) and bread loaf volume (Bailey and Sherwood, 1926; Finney and Barmore, 1948; Bushuk *et al.*, 1969) in bread wheats, and the farinograph parameters, viz. mixing tolerance index and maximum consistency (Irvine *et al.*, 1961; Matsuo *et al.*, 1972) and gluten elastic recovery (Dexter and Matsuo, 1977) in durum wheats. The effect of flour protein content on visco-elasticity of dough, however, is not well documented in bread wheats. Nevertheless, these results clearly indicate that the amount of protein plays an important role in dough rheology and the pasta- and bread-making qualities of wheat flours. Protein content, however, is highly influenced by growing conditions (hence poorly heritable) and genetic variation for this character is small so it is a difficult character to improve by plant breeding. In general terms, grain protein percentage is inversely related to grain yield and thus increasing protein level to improve flour quality usually results in lower grain yield.

2.7.3 *Protein composition and flour quality*

Like protein amount, protein composition (protein quality) is an important determinant of the quality of wheat flours (Bushuk *et al.*, 1969). Unlike protein quantity, however, protein quality is primarily a genetically controlled character and consequently much effort has been put into searching for a correlation between protein composition and flour quality.

It has long been known through reconstitution experiments that gluten protein fractions (glutenins and gliadins), which make up most of the total seed protein (about 80%), are the main determinants of pasta- (Sheu *et al.*, 1967; Matsuo and Irvine, 1970; Walsh and Gillies, 1971; Washik and Bushuk, 1975a) and bread-making (Finney, 1943; Dimler, 1965; Smith and Mullen, 1965) qualities. Albumins and globulins, which represent only about 20% of the seed protein often have had little or no effect on flour quality in bread wheat (Finney, 1943; Hoseney *et al.*, 1969; Kobrehel, 1984). Some other studies, however, revealed that ratio of albumin to globulin was significantly positively correlated with loaf volume per unit protein (Orth and Bushuk, 1972). Similarly, the addition of the water soluble fraction from hard red spring wheat to durum wheat flour reduced gluten firmness (Sheu *et al.*, 1967).

Dimler (1965) showed that purified gliadins and glutenins when hydrated formed a viscous and highly cohesive mass suggesting that they were responsible for dough extensibility and elasticity, respectively. Many workers have shown a strong positive correlation between various quality parameters and the glutenins called acetic acid insoluble glutenin, residue protein (Orth and Bushuk, 1972; Booth and Melvin, 1979), HMW aggregated glutenin (Huebner and Wall, 1976; Bottomley *et al.*, 1982), gel proteins (Jeanjean and Feillet, 1980), SDS insoluble gel protein or glutenin I (Graveland *et al.*, 1982; Field *et al.*, 1983). Baking studies on reconstituted flours showed that high ratios of glutenin to gliadins appeared to be a prerequisite for superior pasta quality (Walsh and Gillies, 1971; Matsuo *et al.*, 1972; Washik and Bushuk, 1975a) and for bread-making quality, particularly loaf volume (Orth and Bushuk, 1972; MacRitchie, 1978; Preston and Tipples, 1980). In contrast, Hoseney *et al.* (1969) indicated the greater importance of gliadins for bread loaf volume. These reconstitution methods could not be used to relate individual gliadins and glutenins with dough quality mainly because of the difficulty in purifying them individually.

Many attempts have been made to associate specific gliadins (Huebner and Rothfus, 1968; Orth and Bushuk, 1972) or glutenins (Huebner, 1970; Orth and Bushuk, 1972) with flour quality by analysing the electrophoretic patterns of bread wheat cultivars of diverse bread-making qualities but they did not succeed mainly due to insufficient resolution of these proteins in the gel. Nevertheless, several qualitative and quantitative differences in the gliadin and glutenin components of these cultivars were observed. Wasik and Bushuk (1975b)

analysed reduced glutenin purified by fractional salt precipitation of the AUC-soluble flour proteins using SDS-PAGE, and observed that the amount of glutenin subunit 6 (MW= 53 kd) was greater than that of subunit 5 (60 kd) in all durum wheats having excellent spaghetti-making quality. Orth and Bushuk (1973) compared the electrophoretic patterns of reduced glutenin from hexaploid wheat, extracted tetraploids and synthetic hexaploids based on these tetraploids and found that the loss of four glutenin subunits (152, 112, 60, 45 kd) controlled by the D genome resulted in a significant decrease in baking quality in extracted tetraploid wheat. Thus, they suggested that the presence of these bands is necessary for good bread-making quality.

Subsequently, researchers have used improved methods of protein separation and have shown that gliadin and glutenin composition are associatied with quality parameters in a range of genotypes (cultivars), segregating progeny of appropriate genetic crosses or isogenic lines. These results are described below.

2.7.3.1 Gliadins and LMW subunits of glutenin

Damidaux *et al.* (1978) were the first to demonstrate that γ-gliadin bands 45 and 42 were associated with high and low elastic recovery of gluten, respectively, among 74 durum wheat cultivars. These results have since been confirmed among Canadian (Kosmolak *et al.*, 1980) and Australian (du Cros *et al.*, 1982) durum wheat genotypes.

Sozinov and Poperelya (1980) used the SDS-sedimentation test to analyse the progeny of numerous bread wheat and determined the relative value of groups (blocks) of gliadins on flour quality. They ranked the gliadin blocks for their effect on quality as follows; (a) chromosome 1A controlled blocks; Gld 1A7 > 4 > 2 > 5 > 3 > 1 ≥ 6, (b) chromosome 1B controlled; Gld-1B1 > 2 ≥ 7 > 5 > 4 > 3 > 6, (c) chromosome 1D controlled; Gld-1D4 > 5 > 1 > 2 > 3, (d) chromosome 6A controlled; Gld 6A3 > 1, (e) chromosome 6B controlled; Gld 6B2 > 1 and (f) chromosome 6D controlled; Gld 6D2 > 1 ± 3. In one experiment, they compared the effects of Gld 1A1, Gld 1A4 and Gld 1B1, Gld 1B2 and found that the combination of blocks Gld 1A4 and Gld 1B1 gave the highest sedimentation volume whereas the combined presence of the allelic gliadin blocks Gld 1A1 and Gld 1B2, gave the lowest volume and the presence of Gld 1A1 with Gld 1B1 or Gld 1A4 with Gld 1B2 gave

intermediate values. Thus, the effect of these gliadin blocks appeared to be additive in nature. Some of these blocks have also been shown to be associated with dough strength and extensibility in French wheat cultivars when tested with the Alveograph (Branlard and Dardevet, 1985a).

Using extensograph tests, Wrigley *et al.* (1981) showed an association between certain gliadin components and dough strength. In a subsequent study, they observed that gliadin band 19 which had corresponding mobility to gliadin band 45 in durum wheats, was consistently associated with strong dough properties in over 100 Australian bread wheat lines (Wrigley *et el.*, 1982b; du Cros *et al.*, 1982). Associations between γ-gliadin 43.5 and strong gluten and between γ-gliadin band 40 and weak gluten have also been reported in Italian bread wheats and these bands were believed to be coded by a single locus or two tightly linked loci on chromosome 1B (Pogna *et al.*, 1982).

2.7.3.2 HMW subunits of glutenin

Payne *et al.* (1979) reported for the first time that individual HMW subunit of glutenin is associated with bread-making quality by analysing segregating progeny from crosses between high and low quality wheat parents. They found that the quality, as measured by the SDS-sedimentation test, of the progeny containing HMW glutenin band 1 (145 kd) was better than those lacking this band. Similarly, Burnouf and Bouriquet (1980) showed that HMW glutenin bands 3 (122 kd) and 5 (108 kd) were present in cultivars possessing good dough strength as measured by the Alveograph. Subsequently, Payne *et al.* (1981b) compared the relationship between SDS-sedimentation volume and various allelic HMW subunits of glutenin controlled by chromosomes 1A (band 1 and null), 1B (bands 13+16, 6+8, 7+9, 14+15 and 7) and 1D (5+10, 2+12 and 3+12) in the segregating progeny of several crosses. Moonen *et al.* (1982,1983) also compared the relative effects of band 2* controlled by chromosome 1A and its null counter part on sedimentation and bread loaf volumes. The relative importance of the different bands controlled by the *Glu-1* loci on flour quality has been summarised (Payne *et al.*, 1984d) in the following order: *Glu-D1*(5+10 > 2+12 = 3+12 > 4+12) > *Glu-A1* (1 = 2* > null) > *Glu-B1* ([17+18 = 13+16 = 7+8] > 7+9 > 7 ≥ 6+8). Moonen and Zeven (1985) analysed isogenic lines and showed that absence of the bands

5+10 (3+10 in their system) was correlated with severely reduced loaf volume. The association between these bands and the various dough parameters measured by the Chopin Alveograph has also been recorded by analysing wheat cultivars of diverse genetic origins and quality (Branlard and Dardevet, 1985b). Bands 1 and 17+18 were positively related to extensibility whereas band 2* was positively related with strength. Bands 5+10 showed a positive correlation whereas bands 2+12 showed a negative correlation with strength and tenacity. Certain band combinations viz. 5+10 and 1 (Payne *et al.*, 1981b) and 5+10 and 2* (Moonen *et al.*, 1983) were found to have cumulative effects on quality parameters.

The comparative analysis of gliadins and HMW subunits of glutenin for their association with dough quality have shown that gliadin composition (particularly bands 2, 4, 14, and 19) was more closely related to dough strength than the HMW subunits of glutenin (Wrigley *et al.*, 1982b). In contrast, Payne *et al.* (1984d) concluded that the HMW subunits of glutenin had greater effects on flour quality that gliadins controlled by either group 1 or group 6 chromosomes, i.e.the ranking was Glu-1 > Gli-1 > Gli-2.

It was not known whether LMW subunits of glutenin, which form a large proportion of seed proteins in bread wheat (about 30%), are also related to bread-making quality. The LMW and HMW subunits of glutenin together may have cumulative effect on flour quality and these aspects have been investigated in present thesis. In durum wheat, however, it has been suggested the gliadin-associated difference in gluten quality (Damidaux *et al.*, 1978) may be due to the difference in the composition of LMW subunits of glutenin as described below.

2.8 Biochemical and molecular basis of quality differences associated with different alleles

In many instances, certain biochemical differences between proteins (viz. relative amount, molecular weight, amino acid composition) coded by tightly linked genes have been used to explain the associated difference in dough quality. For example, Payne *et al.* (1984b) and Autran and Berrier (1984) suggested that quality differences associated with gliadin bands 42 and 45 in durum wheats could be due to linked LMW glutenin bands LMW-1 and LMW-2, respectively, mainly because of the greater amount of these LMW subunits of glutenin and their ability to form aggregates. These observations derived supports form the findings of

Cottenet *et al.* (1983) that gliadin bands 42 and 45 have identical molecular weight and similar amino acid composition, and the observed difference in their overall hydrophobicity was insufficient to account for the difference in gluten quality. Autran and Berrier (1984) suggested that the observed large difference in the amounts of LMW-1 (14% of total proteins in 42 type durum) and LMW-2 proteins (27% of the total proteins in 45 type durum) could explain the difference in gluten characteristics of these wheats. However, since the amino acid sequences of these proteins have not been determined, it cannot be decided whether the effects of the allelic proteins on quality are due to qualitative (structural) or quantitative differences. Amino acid sequence differences might lead to differences in their three-dimensional structures and which in turn may affect their ability to interact with other proteins or flour constituents.

Some progress has recently been made in the amino acid sequencing (Field *et al.*, 1982), cDNA sequencing (Forde *et al.*, 1983) and 2-Nitro-5-thiocyanobenzoic acid cleavage (Moonen *et al.*, 1985) of various HMW subunits of glutenin and the data have shown that cysteine residues are located near both the N- and C-termini in these subunits. These residues may allow the subunits to form long polymers linked by head-to-tail interchain disulphide bonds and thus differences in the location and/or number of these residues would result in differences in the reactivity of these subunits and hence in visco-elastic properties of a dough (Graveland *et al.*, 1985). The large differences in quality associated with HMW subunits of glutenin 2+12 and 5+10, which have the same number of cysteine residues would thus be due to the different positions of these residues in bands 10 and 12 (Moonen *et al.*, 1985). Shewry *et al.* (1984d) obtained N-terminal amino acid sequences of these HMW subunits (2+12, 5+10) but found no differences between them. However, the differences between these subunits could exist at C-terminus and thus complete amino acid sequencing is necessary for verification. Subsequent reports have presented complete amino acid sequences derived from cDNA clones of HMW subunits of glutenin viz. 1Dx 2 (Sugiyama *et al.*, 1985), 1Dx 12 (Thompson *et al.*, 1985) and a silent gene 1Ay (Forde *et al.*, 1985). Further physico-chemical studies will be required, however, to determine how structural differences in these proteins affect their interaction with other proteins and flour constituents, giving the observed differences in dough properties and eventually in pasta- and bread-making quality.

2.9 Functional behaviour of gluten proteins and their interactions with other flour constituents in dough development

There is wide acceptance that gliadins and glutenins (gluten proteins) have a causal relationship with dough visco-elasticity (Dimler, 1965; Wrigley *et al.*, 1982b; Shewry *et al.*, 1984c). The gluten forms a continuous protein network during dough formation which imparts visco-elastic characteristics on the dough and the framework for gas retention during baking. It has been suggested that α-, β-, γ-and ω-gliadins all contribute to gluten viscosity whereas HMW subunits of glutenin confer gluten elasticity and LMW subunits of glutenin contribute to both gluten elasticity and viscosity (Shewry loc cit) . Nevertheless, the chemical basis of these relationships and in particular how these proteins interact to form the protein matrix which in turn confers the visco-elastic property to gluten and dough, are yet not fully understood.

There is some controversy in the literature about whether the subunits of glutenin are held together principally by disulphide bonds or hydrophobic interactions. Based on changes in physico-chemical properties of glutenin after reduction, it was proposed that intermolecular disulphide bonds were the principal factor controlling glutenin structure (Pence and Olcott, 1952; Jones *et al.*, 1961; Nielsen *et al.*, 1962; Bietz and Wall, 1972). Beckwith and Wall (1966) however showed that both intra and interchain disulphide bonds were present in glutenin and an appropriate ratio of them is essential for the cohesive-elastic properties of glutenin. Kaczkowsky and Mieleszko (1980) also suggested that both intra and intermolecular disulphide bonds were present in gluten and they divided them into four types on the basis of their accessibility to 2-mercaptoethanol (2-ME) treatments and showed that all four types of bonds were correlated with dough quality. Moonen *et al.* (1985) also found evidence for both types of disulphide bonds (interchain and intrachain) among the HMW subunits of glutenin but suggested that only the interchain bonds, which are easily accessible to 2-ME, are involved in dough development. In contrast, Kasarda *et al.* (1976a) suggested that all of the disulphide bonds in glutenin were intrapolypeptide in nature and that they were not involved directly in glutenin structure.

In contrast, other researchers, on the basis of their observations that native glutenin could be completely solubilized with solvents having a strong capacity to disrupt hydrophobic bonds, suggested that glutenin aggregates predominantly consisted of subunits held together by specific hydrophobic interactions (Meredith and Wren, 1966; Kobrehel and Bushuk, 1978). Other reports also implicated hydrophobic interactions in glutenin aggregation (Preston, 1984; Popineau, 1985). Kasarda *et al.* (1976a) proposed that non-covalent interactions were the major force in glutenin aggregation and that the intramolecular disulphide bonds (covalent forces) present in glutenins affect only their specific conformation which in turn affected their ability to aggregate.

Khan and Bushuk (1979) found that purified native glutenin contained some proteins which were separated in SDS gels without reduction whereas the majority were released only after reduction of disulphide bonds. They proposed that besides disulphide bonds, strong hydrophobic interactions play a major role in the structure of functional glutenin and this has been supported by the similar findings of Huebner and Wall (1980).

Physico-chemical studies of gliadins after reduction suggested that most of disulphide bonds in gliadins are intrapolypeptide (Pence and Olcott, 1952; Beckwith *et al.*, 1965) except for ω-gliadins which lack cysteine ~~and methionine~~ and thus have no disulphide bonds (Bietz and Wall, 1972). A large body of evidence suggests that gliadins aggregate through non-covalent forces due to their high surface hydrophobicity (Kasarda *et al.*, 1967; Caldwell, 1979; Bietz and Wall, 1980; Preston, 1984; Popineau, 1985).

Based on these information, a number of models have been proposed to explain the interaction and aggregation of various components of flour into bigger and more complex molecules which in turn impart visco-elasticity to wheat flour doughs. These models can be grouped into (a) those which emphasize the importance of covalent forces in glutenin aggregation (Ewart, 1968; Graveland *et al.*, 1985), (b) those stressing the role of non-covalent interactions (Kasarda *et al.*, 1967; 1976a) in gluten structure and (c) mixed models suggesting the involvement of both covalent and non-covalent forces (Dimler, 1965; Ewart, 1979; Khan and Bushuk, 1979; Wall, 1979; Bushuk *et al.*, 1980; Bietz and Wall, 1980). The mixed models also allow for the involvement of some albumin and globulin type polypeptides (based on amino acid compositions) of mainly 30, 40, 64-70 kd sizes in glutenin and gluten

structure. These molecules are tenaciously attached to glutenin even under strongly dissociative conditions. In fact, the Bietz and Wall's model was the most comprehensive as it considered the interactions of all flour proteins (albumins, globulins, gliadins, LMW glutenin, HMW glutenin) with each other in the formation of a complex visco-elastic protein matrix in dough.

Specific carbohydrates (Huebner and Wall, 1979; Bushuk *et al.*, 1980, 1984; McMaster and Bushuk, 1983) and lipids (Hoseney *et al.*, 1970; MacRitchie, 1977; Pomeranz, 1980; Bekes *et al.*, 1983; Bushuk *et al.*, 1984; Zawistowska *et al.*, 1984) also interact with specific proteins of the gluten complex (both covalently and non-covalently) to form large aggregates contributing to gluten rheology and thus bread-making potential of flour. Bushuk *et al.* (1980) recognized the importance of these non-protein components in their model for functional glutenin (glutenin as it exists in gluten and dough) and suggested that glutenin is a complex of covalently and non-covalently bound components of mainly proteins and trace amounts of carbohydrates and lipids. This model however, does not consider the interactions of gliadins with lipids (Hoseney *et al.*, 1970) and with other protein components of flour. All of these models thus are insufficient to account for various types of interactions among the flour constituents during gluten and dough development and hence need to be modified.

In general, these models tend to suggest the involvement of both covalent (disulphide bond) and non-covalent (hydrogen bonds, electrostatic, hydrophobic interactions etc.) forces in gluten and dough formation. Further studies are needed to account for the differences caused in the structure of the protein-lipid-carbohydrate matrix by specific protein components showing different structure and behaviour.

Chapter 3

VARIATION AND GENETIC CONTROL OF LMW SUBUNITS OF GLUTELIN IN WHEAT AND ITS RELATIVES

3.1 INTRODUCTION

The HMW subunits of glutenin and gliadins are controlled by the *Glu-1*, *Gli-1* and *Gli-2* loci in bread wheat and these are easily resolved by 1-D SDS-PAGE and have shown extensive pattern variation among different cultivars (see Chapter 2 for a review). In contrast to these two groups of proteins, the LMW subunits of glutenin have proven much more difficult to analyse in a 1-D electrophoretic system, mainly because of their overlapping mobilities with the gliadins. Using 2-D electrophoresis, however, 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. Payne *et al.* (1984e), using 1-D SDS-PAGE, were able to map the genes controlling some easily recognizable B subunits of glutenin at the *Gli-1* loci. These separation techniques, however, lacked general application for studying the variability in LMW subunits of glutenin and their inheritance because the 2-D system can analyse only one or two samples per gel. Moreover, these subunits cannot be directly distinguished from gliadins in either a 1-D or a 2-D system. Consequently, these methods have allowed only a few LMW subunit differences to be identified in bread and durum wheats (Payne *et al.*, 1984b, 1984e). The recent introduction of a two-step 1-D SDS-PAGE procedure by Singh and Shepherd (1984b, 1985), however, has provided a rapid method for analysing a large number of samples in a single gel in a gliadin-free background.

In this chapter using this two-step method, variation in the LMW subunits of glutenin among many bread wheat cultivars has been documented at band pattern level and the chromosomal control of some of the patterns has been determined by using the substitution lines and a translocation line. The variation and chromosomal control of these subunits have also been studied in tetraploid and diploid wheats and some of the species related to wheat.

3.2 MATERIALS AND METHODS

3.2.1 Genotypes analysed

Genotypes from both wheat and its relatives were included in the survey of LMW glutelin subunit variation. These species are described in this thesis according to the nomenclature used by Morris and Sears (1967) based on Bowden (1959) with the following exceptions: Although all of the 'A' genome diploid species (eg. *T. monococcum*, *T. boeoticum*, *T. thaoudar* and *T. urartu*) were included under *T. monococcum* by Kimber and Feldman (1987), their separate identity has been retained in the present study for ease of description. Similarly, *T. longissimum* and *T. sharonense* are separated. *Agropyron elongatum* has been described as *Elytrigia elongata* following Dvorak (1981).

The seed samples of these genotypes, unless specified otherwise, were obtained from the collection maintained at the Waite Agricultural Research Institute by Dr. K. W. Shepherd.

3.2.1.1 Hexaploid wheats

Two hundred and twenty-two hexaploid wheat cultivars (including some advanced generation breeding lines) from 32 countries were analysed. A list of these genotypes along with their country of origin is attached as Appendix A. The seeds of overseas wheat cultivars were kindly provided by Mr. M. Mackey (Curator, Australian Winter Cereals Collection, Tamworth). The intervarietal group 1 chromosome substitution lines of Cheyenne (Morris *et al.*, 1966), Capelle-Desprez (Law *et al.*, 1978), Thatcher, Kenya Farmer, Hope and Timstein (Sears *et al.*, 1957) in Chinese Spring (CS) were analysed to determine the chromosomal control of some of the LMW subunits of glutenin. Seed samples of the substitution lines involving Capelle-Desprez, Kenya Farmer and Thatcher were kindly provided by Dr. R. A. McIntosh (University of Sydney).

The following intergeneric substitution lines were also tested to determine the chromosomal control of the LMW subunits of glutenin in the recipient wheat cultivars: Rescue–*E. elongatum* substitution (1A) and (1D) (Hurd, 1959) and Kharkov–Dakold rye

substitution 3R (1A), 3R (1B) (Lee *et al.*, 1969). A translocation stock Warigal–Imperial rye 1DL-1RS (Shepherd, unpublished) was also analysed.

3.2.1.2 Tetraploid wheats

The tetraploid wheats analysed were as follows (number analysed shown in parentheses); *T. turgidum* cv. group *durum* (15), cv. group *turgidum* (3), cv. group *carthlicum* (2), cv. group *dicoccum* (3), cv. group *polonicum* (1), cv. group *turanicum* (1), var. *dicoccoides* (3), and *T. timopheevi* var. *timopheevi* (2). The genetic control of the LMW subunits of glutenin in durum wheat cultivars was determined by analysing the Chinese Spring 1D substitutions for chromosomes 1A and 1B in durum wheat cultivar Langdon and the Edmore and Kharkof-5 chromosome 1B substitutions in Langdon (Joppa and Williams, 1983; Josephides *et al.*, 1987).

3.2.1.3 Diploid wheats and related species

The following 10 diploid *Triticum* species were represented by one or more accessions (as indicated in parentheses) and were screened for LMW glutenin subunit patterns; *T. monococcum* (1), *T. boeoticum* (1), *T. thaoudar* (1), *T. urartu* (1), *T. speltoides* (1), *T. bicorne* (1), *T. longissimum* (2), *T. sharonense* (1), *T. tauschii* (3), *T. umbellulatum* (1). Genetic stocks involving chromosomes of *T. umbellulatum* (2n=14, UU) and *T. longissimum* (2n=14, S^lS^l) in bread wheat were also examined to determine the genetic control of LMW subunits of glutenin in these diploid species; these were CS–*T. umbellulatum* amphiploid (Kimber, 1967) and substitution lines 1U (1A), 1U (1B) and 1U (1D) (Shepherd, 1973) and also CS–*T. longissimum* substitution $1S^l$ (1B) (Netzle and Zeller, 1984).

One accession of *E. elongata* (2n=14) was analysed for its LMW glutenin subunit banding patterns. Seeds of CS–*E. elongata* amphiploid stock and the substitution lines 1E (1A), 1E (1B), 1ES (1B), 1E (1D) and 1ES (1D) (Dvorak and Sosulski, 1974; Dvorak, 1980) were analysed to determine the genetic control of LMW subunits of glutenin in diploid *E. elongata* . The Rescue–*E. elongata* substitutions for 1A and 1D (Hurd, 1959) involving the chromosomes of decaploid *E. elongata* (2n=70), were also screened for genetic analysis.

Seeds from five cultivars of rye (*Secale cereale*) namely, Imperial, King-II, South Australian, Petkus and Dakold and two accessions (R-15, R-42) of wild rye (*S. montanum*) were also analysed to determine the composition of the LMW subunits of glutelin. Seeds of CS–*S. montanum* R–15 amphiploid and four addition lines having chromosome $1R^{ms}S$, $2R^{m}$ and $6R^{m}$ added to Chinese Spring wheat (Miller, unpublished) were analysed to determine the chromosomal control of LMW subunits of glutenin in *S. montanum* R–15.

3.2.2 Protein extraction

The endosperm halves from single kernels were placed in a folded glassine sheet [Lilley, Powder paper (glassine) 8.75 × 10.5 cm] and crushed with a hammer. The crushed material was transferred to 1.5 ml Eppendorf tubes for extracting total unreduced protein following the methods of Lawrence and Shepherd (1980) as described below. In a later modification, crushed material was treated with ethanol to extract prolamins only and this allowed better resolution of LMW subunits of glutenin.

3.2.2.1 Extraction of unreduced total endosperm proteins

Total unreduced protein extracts were obtained by treating the crushed endosperm with 200 µl of freshly prepared SDS-Tris-HCl extracting buffer (pH 6.8), containing 0.125M tris (hydroxymethyl) aminomethane (Tris), 8.0% (w/v) SDS, 30% (v/v) glycerol and 0.005% (w/v) bromophenol blue made to pH 6.8 with HCl, with an equal volume of distilled water. The samples were incubated overnight (16 h) in an oven at 40°C. Samples were centrifuged in a Beckman Microfuge-11 at 10,000 rpm for 2 min and 40-70 µl of clear supernatant was loaded into individual slots of the gel for electrophoresis.

3.2.2.2 Extraction of unreduced prolamins from endosperm

The unreduced prolamins were extracted by treating crushed endosperm in about 800 µl of 70% aqueous ethanol (pH. 5.9) at 50°C for at least 5 hours. The top 70% portion of supernatant was collected after 5 minutes of centrifugation at 10,000 rpm in a Beckman Microfuge-11 and was dried by allowing the ethanol to evaporate at 60°C. The residue was

dissolved in 80 µl of Tris-HCl buffer containing 4% SDS and 40-70 µl of prolamin extract of each sample was loaded into individual slot of the gel for electrophoresis.

When reduced total protein or reduced prolamin proteins were required, 1.5% 2-merceptoethanol (2-ME, v/v) was included in the extraction buffer prior to incubation.

3.2.3 Two-step one-dimensional SDS-PAGE

The two step one-dimensional SDS-PAGE procedure of Singh and Shepherd (1988a) was modified to achieve clearer resolution of LMW subunits of glutenin. Both steps of this procedure are based on the discontinuous-buffer system of SDS-PAGE of Laemmli (1970) as described by Lawrence and Shepherd (1980).

In the first step, protein extracts from 16 individual seeds were electrophoresed by 1-D SDS-PAGE in thin vertical slab gels having a separating and a stacking gel. The separating gel (140mm long × 80mm wide × 1.2 mm thick) contained 10.0% (w/v) acrylamide and 0.08% (w/v) bisacrylamide (Bis), 0.1% (w/v) SDS, and 0.375M Tris adjusted to pH 8.8 with HCl and the stacking gel (140 × 20 × 1.2 mm) contained 3% (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. The protein samples were loaded into wells at the cathodal end and were electrophoresed at a constant current of 50 mA/gel for about 45 minutes until the marker dye front had migrated about 5 cm in the separating gel. Thereafter, the top 0.8-1.0 cm portion across the separating gel was cut, transferred into 75–100 ml of an equilibration solution and incubated for 2–3 h at approximately 60°C in the presence of 2% 2–ME to achieve protein reduction. The equilibration solution consisted of 10.3% (w/v) glycerol, 0.07 M Tris and 2.4% (w/v) SDS adjusted to pH 6.8 with HCl. A small quantity of bromophenol blue was added to this solution for tracking the migration of proteins present in the gel strip during electrophoresis.

In the second step, this gel strip containing the reduced proteins, was subjected to electrophoresis in another vertical slab gel at a constant current of 25 mA/gel for about 6 h. The strip was first rinsed with distilled water and then loaded onto a flat stacking gel (20 ×

150 × 1.5 mm) containing 3% acrylamide. The separating gel (160 × 150 × 1.5 mm) used in this step was a gradient gel (7.5–15.0% acrylamide) prepared by using a twin cylinder (4.5cm internal diameter) apparatus and a proportioning pump. The acrylamide gel solutions from the two cylinders (with 7.5% and 15.0% acrylamide) were mixed at a constant mixing input and the resulting solution was pumped into the vertical slab gel apparatus at a rate of 2 ml/min.

3.2.4 Staining and destaining

Gels were stained overnight in a staining 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). A two-step destaining procedure was employed for two-step gels, first in distilled water (Caldwell and Kasarda, 1978) for 24 h and then in a solution of water: methanol:glacial acetic acid (111:48:8 ratio) (Lawrence and Shepherd, 1980) for about 12 h. Gels were frozen overnight to sharpen the bands for photography.

3.3 RESULTS

The data obtained on the characterization of endosperm proteins by two-step 1-D SDS-PAGE and variation and genetic control of LMW subunits of glutelin in wheat and its relatives will be described in the following order: hexaploid wheat, tetraploid wheat and diploid wheat and related species.

3.3.1 Hexaploid wheat

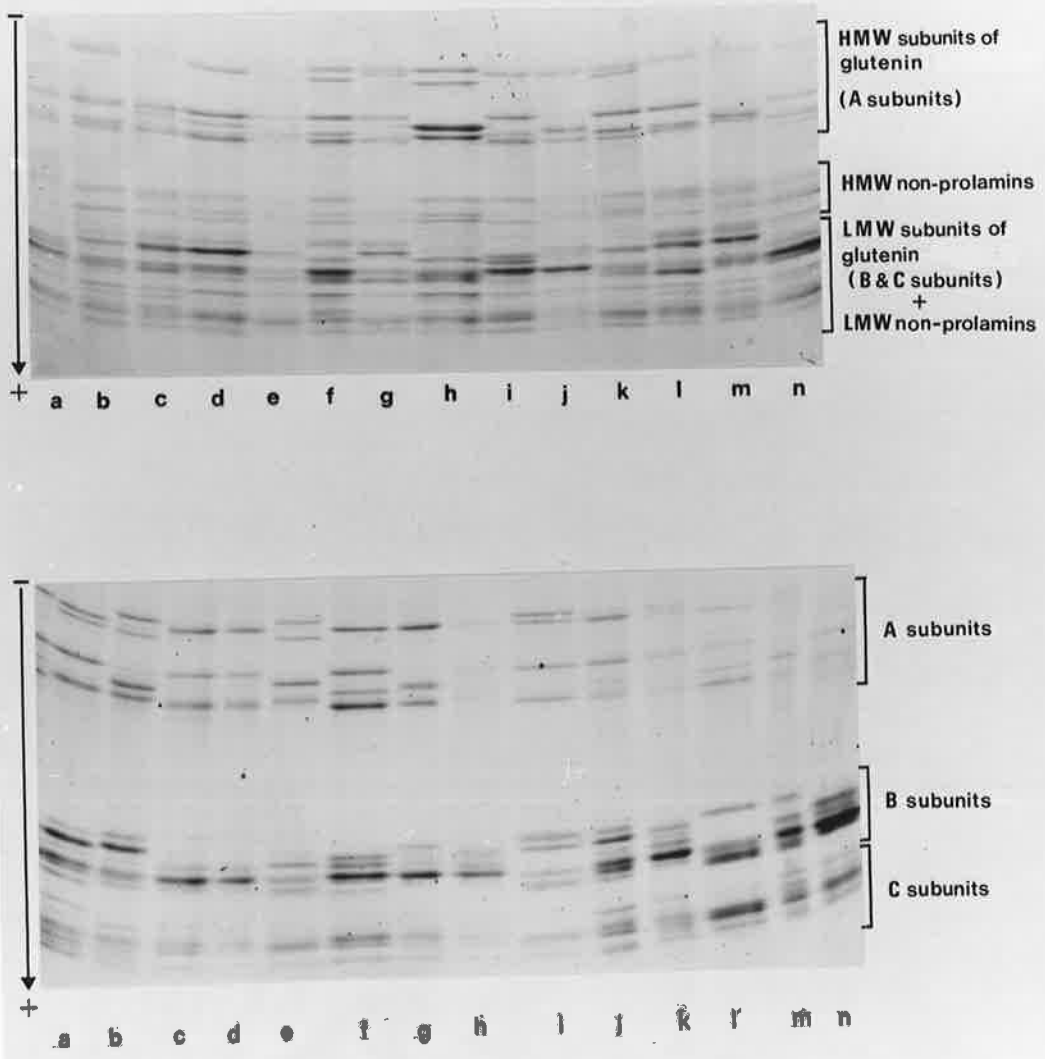
3.3.1.1 Characterization of endosperm proteins by Two-step 1-D SDS-PAGE

When the proteins extracted in Tris-HCl buffer (pH 6.8) containing SDS, were separated by two-step SDS-PAGE, they were resolved into three groups of bands with different electrophoretic mobility (slow, medium and fast) in the second step of separation (Fig. 3.1). On the basis of mobility and genetic evidence, the slow moving bands correspond to HMW subunits (A subunits) of glutenin while the majority of the fast moving bands corresponded to LMW subunits (B and C subunits) of glutenin. All the bands with medium mobility and some of the fastest moving bands corresponded to non-prolamins (see below) and these were arbitrarily divided into HMW and LMW groups, respectively. Some of the these non-prolamin bands overlapped the B and C subunits (Fig. 3.1). When the second step gels containing these non-prolamins were scored soon after staining with Coomassie blue R-250, they had pink-purple colouration while the A, B and C subunits were blue. This differential colouration of bands, also observed by Payne *et al.* (1986) in a two-dimensional system, was helpful in resolving the identity of these bands. For example, where overlap occurred, the band showing blue colouration was considered to be the LMW glutenin band.

In a later modification of the electrophoresis procedure, when the proteins were extracted with 70% aqueous ethanol at 60°C, the bands of medium mobility and some of the fastest moving bands, were absent (Fig. 3.2) indicating that they were ethanol insoluble, hence non-prolamins by definition. The absence of these non-prolamin bands allowed the resolution of all glutenin subunits into three clearly defined groups, namely A, B and C (Fig. 3.2) and this facilitated the analysis of variation in B and C subunits. The main emphasis is

Figure 3.1. Two-step 1-D SDS-PAGE patterns of endosperm proteins extracted in Tris-HCl buffer (pH 6.8) containing SDS from hexaploid wheat cultivars; (a) Chinese Spring, (b) Tincurrin, (c) Chhoti Lerma, (d) Giza-150, (e) Lovrin-13, (f) Nanbukomugi, (g) Orca, (h) Insignia, (i) Chinese Spring, (j) Gabo, (k) Kozara, (l) Norin-61, (m) Tanori-71 and (n) Vicam-71. The arrow indicates the direction of protein migration.

Figure 3.2. Two-step 1-D SDS-PAGE patterns of endosperm proteins extracted in 70% aqueous ethanol from hexaploid wheat cultivars; (a) Arzu, (b) BT–2296, (c) Bencubbin, (d) Bungulla, (e) Halberd, (f) Chinese Spring, (g) Gabo, (h) Cheyenne, (i) Orca, (j) Tincurrin, (k) Timgalen, (l) Cebecco–97, (m) Chanab–70 and (n) Una. It should be noted that HMW- and LMW-non-prolamin bands are absent.


HMW subunits of glutenin
(A subunits)
HMW non-prolamins
LMW subunits of glutenin
(B & C subunits)
+
LMW non-prolamins
+
a b c d e f g h i j k l m n
A subunits
B subunits
C subunits
+
a b c d e f g h i j k l m n

given to the B subunits in the first part of this chapter since they showed the greatest amount of variation. Variation in the C subunit is described subsequently.

3.3.1.2 Inter-varietal variation in LMW subunits and its genetic control

A collection of 222 bread wheat cultivars (including some advanced generation breeding lines) from 32 countries was examined in detail for variation in LMW glutenin subunit patterns, initially by using proteins extracted from these cultivars in Tris-HCl buffer containing SDS (for the first two rounds of screening) and subsequently once by using 70% aqueous ethanol extracts. Several control cultivars viz. Chinese Spring, Gabo, Halberd, Cheyenne, Norin-61 and Orca, were used routinely to determine the relative mobilities of the subunits in the test samples. Initially four seeds from each cultivar were screened to test the homogeneity of the cultivar for LMW glutenin composition. Subsequently, two more seeds from each homogeneous cultivar and several other seeds from heterogeneous cultivars were tested. Since LMW glutenin bands exhibit a narrow range of mobility in SDS-PAGE, the banding patterns of each of these cultivars were determined systematically by comparing them several times against the standards. The cultivars showing similar banding patterns in the preliminary examination were then re-run in a single gel to establish their identity. The results showed an extensive amount of variation in B subunit patterns among the cultivars and a total of 28 clearly scorable B subunits with different mobility, could be identified (Figs. 3.1 and 3.2). Each cultivar showed from five to nine different B subunits.

Close examination of the banding patterns of these cultivars revealed that some LMW bands, or band combinations occurred as alternatives to each other. Based on their mutual exclusiveness and their inheritance patterns (Chapter 4), these subunit bands were assigned to three groups (Fig. 3.3). The chromosomal control of these patterns was determined by analysing the intervarietal and intergeneric substitution lines and translocation lines and the results obtained clearly indicated that patterns within each group are controlled by genes on the same chromosomes.

Six patterns were assigned to group 1 (Fig. 3.3). Patterns '**a**' and '**b**' corresponded to Chinese Spring (CS) and Gabo bands, respectively and were controlled by genes on chromosome arm 1AS (see Chapter 4 for details). Additional evidence that pattern '**b**' is controlled by chromosome 1A came from the analysis of the Chinese Spring–Kenya Farmer

Figure 3.3. Diagram showing the three groups of B subunit bands or band combinations identified by two-step SDS-PAGE analysis of 222 bread wheat cultivars. The grouping is based on the mutual exclusiveness of these bands or band combinations among the cultivars. This diagram also incorporates the information obtained on these subunits from the analyses of substitution lines, translocation line and test-cross progeny. Patterns '**a**' and '**b**' in each group are from Chinese Spring and Gabo, respectively.

Symbols used;

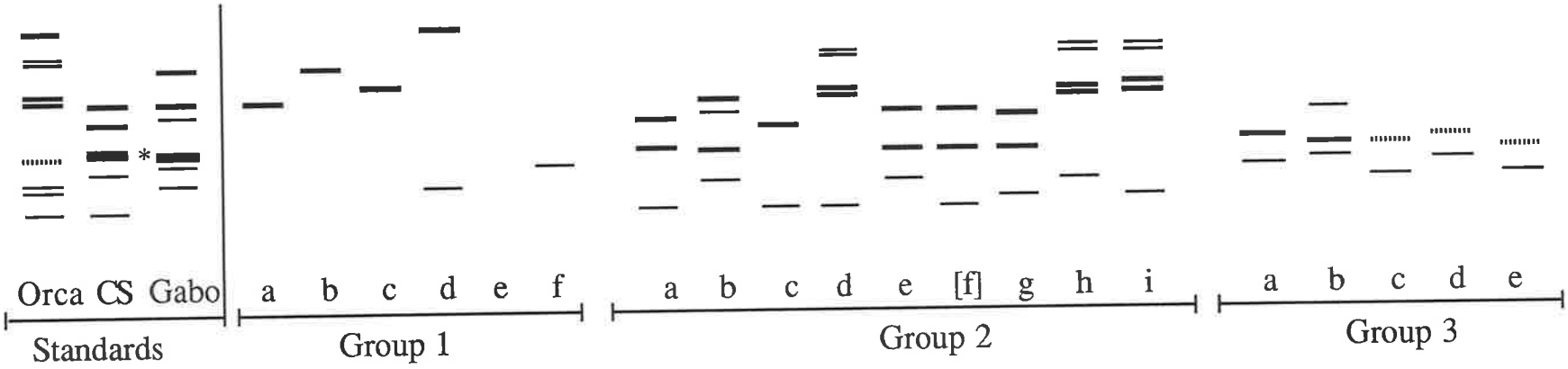
a, b, c.....denote the band patterns.

Group 1, 2 and 3 represents band patterns controlled by chromosomes 1A, 1B and 1D, respectively

[f] = direct evidence for the chromosomal location of pattern '**f**' has not been obtained

The thickness of bars in the patterns indicates the approximate thickness of the bands. Faintly stained bands are shown by broken lines.

* denotes that this thick band represents two bands of same mobility, one controlled by 1BS and other by 1DS in Chinese Spring and Gabo. These two bands have been included in group 2 and group 3 patterns of these as well as other cultivars having the denoted thick band.


Orca CS Gabo
*
a b c d e f
a b c d e [f] g h i
a b c d e
Standards
Group 1
Group 2
Group 3

1A substitution line (Fig. 3.5, slot b) which carried chromosome 1A from cultivar Kenya Farmer. This line exhibited pattern '**b**' (see band marked by ➤) instead of pattern '**a**' of Chinese Spring. Similarly in CS–Cheyenne 1A (Fig. 3.4, slot b) and CS–Capelle-Desprez 1A (Fig. 3.5, slot g) substitution lines, patterns '**c**' from Cheyenne and '**d**' from Capelle-Desprez replaced '**a**' in Chinese Spring (see bands marked by ➤ in the respective slots). The inheritance test showed further that pattern '**d**' in cultivar Orca was controlled by genes on chromosome arm 1AS (see Chapter 4 for details). Many cultivars did not reveal any of these patterns controlled by chromosome 1A and thus they were classified as null phenotypes (pattern '**e**'). For example, substitution lines containing chromosome 1A from cultivars Hope (Fig. 3.6A, slot c), Thatcher (Fig. 3.4, slot g) and Timstein (Fig. 3.6 B, slot b) in Chinese Spring background did not show any extra band while lacking pattern '**a**' from Chinese Spring. Cultivar Kharkov also carried this null phenotype since the reported substitution line Kharkov–Dakold rye 3R (1A) (Fig. 3.7B, slot c) had the same band composition as the Kharkov parent (Fig. 3.7B, slot b) (the identity of Dakold rye 3R is described on page 44). A similar analysis of a Rescue–*E. elongata* (1A) substitution line (Fig. 3.18B, slot b) revealed that pattern '**f**' in Rescue (band marked by ➤ in Rescue, slot a) was controlled by genes on chromosome 1A.

Nine different patterns have been assigned to group 2 and each pattern had two or more B subunit bands (Fig. 3.3). Patterns '**a**' and '**b**' were controlled by chromosome arm 1BS in Chinese Spring and Gabo, respectively (see Chapter 4 for details). The banding patterns of Chinese Spring substitution lines carrying chromosome 1B from Hope (Fig. 3.6A, slot d), Kenya Farmer (Fig. 3.5, slot c) or Timstein (Fig. 3.6B, slot c) also showed that pattern '**b**' in these cultivars was controlled by genes on chromosome 1B (see bands marked by ▲ in the respective slots). Patterns '**c**' and '**d**' were present in cultivars Insignia and Orca, respectively, and were also controlled by genes on chromosome arm 1BS as shown in Chapter 4. Cultivar Cheyenne exhibited pattern '**e**' which replaced the pattern '**a**' of Chinese Spring in the CS–Cheyenne 1B substitution line (bands shown as ➤, Fig. 3.4, slot c) thus indicating that pattern '**e**' was controlled by chromosome 1B. Similar analysis of Kharkov–Dakold rye 3R (1B) (Fig. 3.7B, slot d) and Chinese Spring–Thatcher 1B (Fig. 3.4, slot h) substitution lines showed that genes controlling pattern '**g**' in Kharkov (bands marked by →) and pattern '**h**' in Thatcher (bands shown as ➤) were located on chromosome 1B. The

Figure 3.4. Two-step 1-D SDS-PAGE patterns of protein extracted in 70% aqueous ethanol from bread wheat cultivars and intervarietal substitution lines; (a) Cheyenne, (b) Chinese Spring (Cheyenne 1A), (c) Chinese Spring (Cheyenne 1B) (d*) Chinese Spring (Cheyenne 1D), (e) Chinese Spring, (f) Thatcher, (g) Chinese Spring (Thatcher 1A), (h) Chinese Spring (Thatcher 1B), (i*) Chinese Spring (Thatcher 1D) and (j) Thatcher.

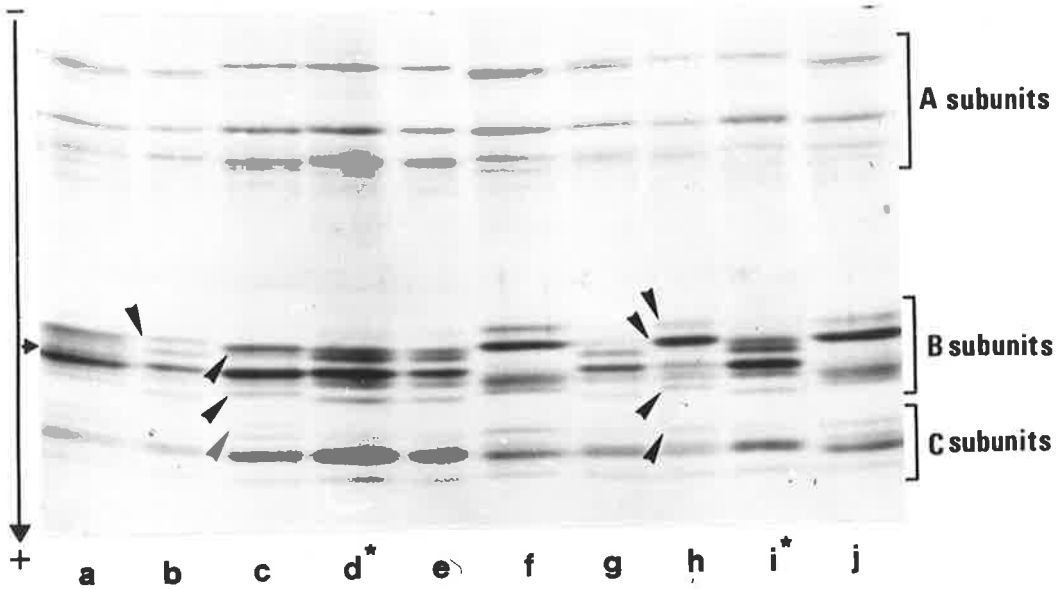
Figure 3.5. Two-step 1-D SDS-PAGE patterns of protein extracted in 70% aqueous ethanol from bread wheat cultivars and intervarietal substitution lines; (a) Kenya Farmer, (b) Chinese Spring (Kenya Farmer 1A), (c) Chinese Spring (Kenya Farmer 1B) (d*) Chinese Spring (Kenya Farmer 1D), (e) Chinese Spring, (f) Capelle -Desprez, (g) Chinese Spring (Capelle -Desprez 1A), (h*) Chinese Spring (Capelle -Desprez 1B), (i) Chinese Spring (Capelle -Desprez 1D) and (j) Chinese Spring.

Symbols used in above figures;

* refers to the genetic stocks showing unexpected protein phenotypes,

➤ = B and C subunits substituted for B and C subunits of Chinese Spring controlled by chromosome 1A, 1B and 1D,

→ = a unique B subunit in Cheyenne possibly controlled by 1D.


A subunits
B subunits
C subunits
+
a
b
c
d*
e
f
g
h
i*
j

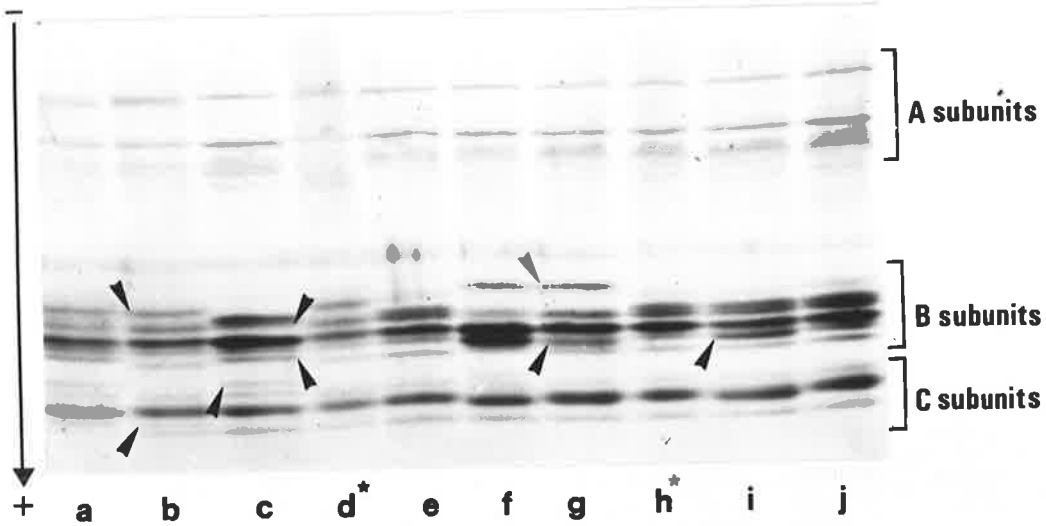

A subunits
B subunits
C subunits
+
a
b
c
d*
e
f
g
h*
i
j

Figure 3.6 A, B. Two-step 1-D SDS-PAGE patterns of protein extracted in 70% aqueous ethanol from bread wheat cultivars and intervarietal substitution lines.

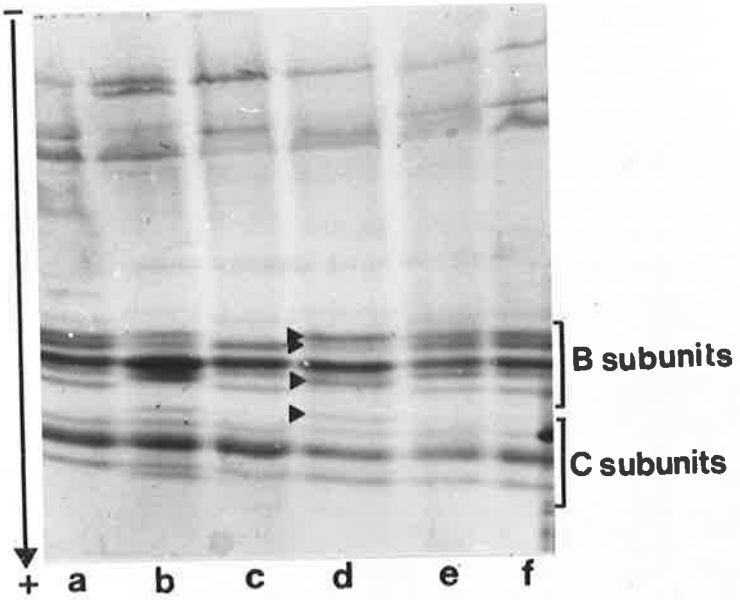
A. (a) Chinese Spring, (b) Hope, (c) Chinese Spring (Hope 1A), (d) Chinese Spring (Hope 1B) and (e) Chinese Spring (Hope 1D).

B. (a) Timstein, (b) Chinese Spring (Timstein 1A), (c) Chinese Spring (Timstein 1B) and (d) Chinese Spring (Timstein 1D)

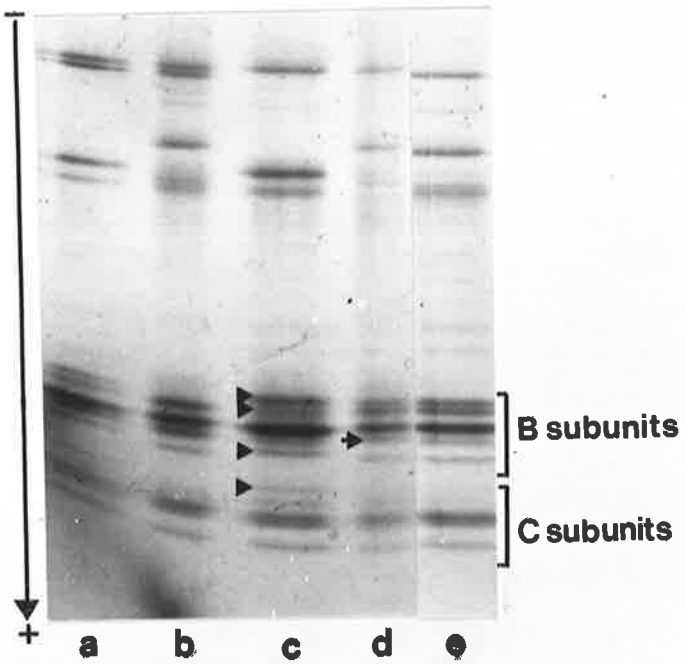
Symbols used;

▲ = LMW subunits in pattern '**b**' (group 2) controlled by chromosome 1B in Hope and Timstein

→ = LMW subunit of pattern '**b**' (group 3) controlled by chromosome 1D in Timstein



A



B

inheritance test revealed that genes controlling pattern '**i**' in Norin–61 were located on 1BS (see Chapter 4). A direct evidence for control of pattern '**f**' by this chromosome has not been obtained yet.

The five different patterns allocated to group 3 (Fig. 3.3) were associated with chromosome 1D. Patterns '**a**' and '**b**', corresponding to the bands in Chinese Spring and Gabo, respectively, were controlled by genes on the short arms of chromosome 1D (see Chapter 4). Pattern '**a**' was also present in cultivars Rescue and Warigal and was found to be absent in Rescue–*E. elongata* (1D) substitution line (bands marked by ➤, Fig. 3.18B, slot c) and in the Warigal 1DL-1RS translocation stock (bands marked by →, Fig. 3.7A, slot c) thus indicating its genetic control by chromosome 1D in these cultivars as well. Timstein possessed pattern '**b**' which replaced pattern '**a**' in the Chinese Spring–Timstein 1D substitution line (Fig. 3.6B, slot d) thus confirmed its genetic control by chromosome 1D. Similarly, by analysing the CS–Capelle-Desprez 1D substitution line (Fig. 3.5, slot i) and by inheritance analysis of Insignia (see Chapter 4), the genetic control of pattern '**c**' in cultivar Capelle Desprez (band indicated by ➤) and Insignia was determined. Patterns '**d**' and '**e**' both were found in cultivar Norin–61 and the inheritance test revealed that they are controlled by genes on chromosome 1D (see Chapter 4). The data obtained on the chromosomal control of the different patterns in these cultivars from analysis of substitution and translocation lines are summarised in Table 3.1.

Attempts were also made concurrently to analyse the C subunit patterns of these 222 cultivars and although some variation could be observed in SDS extracts, it was difficult to study them precisely because they overlapped with the non-prolamin bands. After the ethanol extraction procedure was introduced, the variation in the C subunit bands could be analysed more clearly but these subunits were still poorly resolved in comparison with the B subunits and the slow moving C subunits were usually faintly stained. Thus, the variation in C subunits was analysed in only 125 of the 222 bread wheat cultivars (representing all B subunit patterns) using the ethanol extract. Variation was considerable, most of it occurring in slow moving C subunits and little of it among the fast moving C subunits. Each cultivar exhibited 3-6 C subunits. The genetic control of some of the C subunit variation was determined by analysing the banding patterns of the same genetic stocks used for the analysis of B subunits. These results have been combined with those obtained for the B subunits (Fig. 3.8).

Figure 3.7 A, B. Two-step 1-D SDS-PAGE patterns of proteins extracted in 70% aqueous ethanol from wheat–rye substitution and translocation lines and parental controls.

A. (a) Imperial rye, (b) Warigal and (c) Warigal 1DL-1RS.

B. (a) Dakold rye, (b) Kharkov wheat, (c) Kharkov–Dakold rye 3R (1A) and (d) Kharkov–Dakold rye 3R (1B).

Symbols used;

→ = LMW subunits controlled by chromosome 1B in Kharkov and chromosome 1D in Warigal.

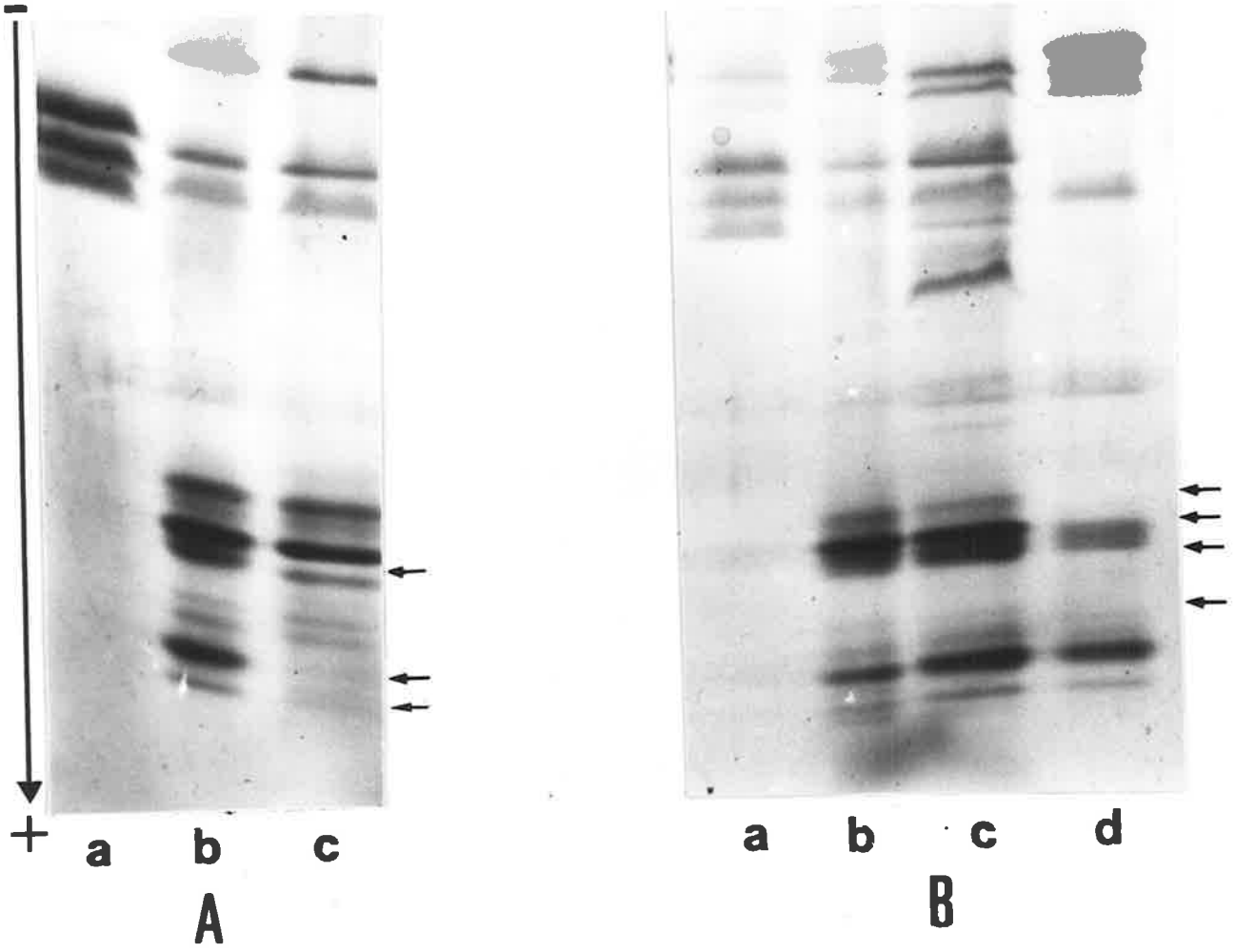

-
+
a
b
c
A
a
b
c
d
B

Table 3.1 Chromosomal control of LMW subunits of glutenin as inferred from the analysis of intervarietal and intergeneric substitution lines and a translocation line.

Genetic Stocks	*LMW glutenin subunit pattern added or deleted
Intervarietal substitutions#	**Pattern added**
Chromosome 1A	
Capelle-Desprez	d
Cheyenne	c
Hope	e
Kenya Farmer	b
Thatcher	e
Timstein	e
Chromosome 1B	
Cheyenne	e
Hope	b
Kenya Farmer	b
Thatcher	h
Timstein	b
Chromosome 1D	
Capelle-Desprez	c
Timstein	b
Intergeneric substitutions	**Pattern deleted**
Kharkov-Dakold rye 3R (1A)	None
Kharkov-Dakold rye 3R (1B)	g
Rescue-*Elytrigia elongata* (1A)	f
Rescue-*Elytrigia elongata* (1D)	a
Translocation line	**Pattern deleted**
Warigal 1DL-1RS	a

* Chinese Spring has pattern '**a**' for all the chromosomes 1A, 1B and 1D.

these intervarietal substitution lines are present in a Chinese Spring background.

The gliadin and HMW glutenin subunit patterns were also analysed to confirm tthe authenticity of these genetic stocks and the results were generally as expected. Exceptions included seeds designated CS–Kenya Farmer 1D and CS–Kenya Farmer 1A substitution lines

Figure 3.8. Diagram showing the three groups of B and C subunit or subunit combinations identified by two-step SDS-PAGE analysis of 222 bread wheat cultivars. The grouping is based on the mutual exclusiveness of these bands or band combinations among the cultivars. This diagram also incorporates the information obtained on the LMW subunits from analyses of substitution lines, translocation lines and the test-cross progeny. Patterns '**a**' and '**b**' in each group are from Chinese Spring and Gabo, respectively.

Symbols used;

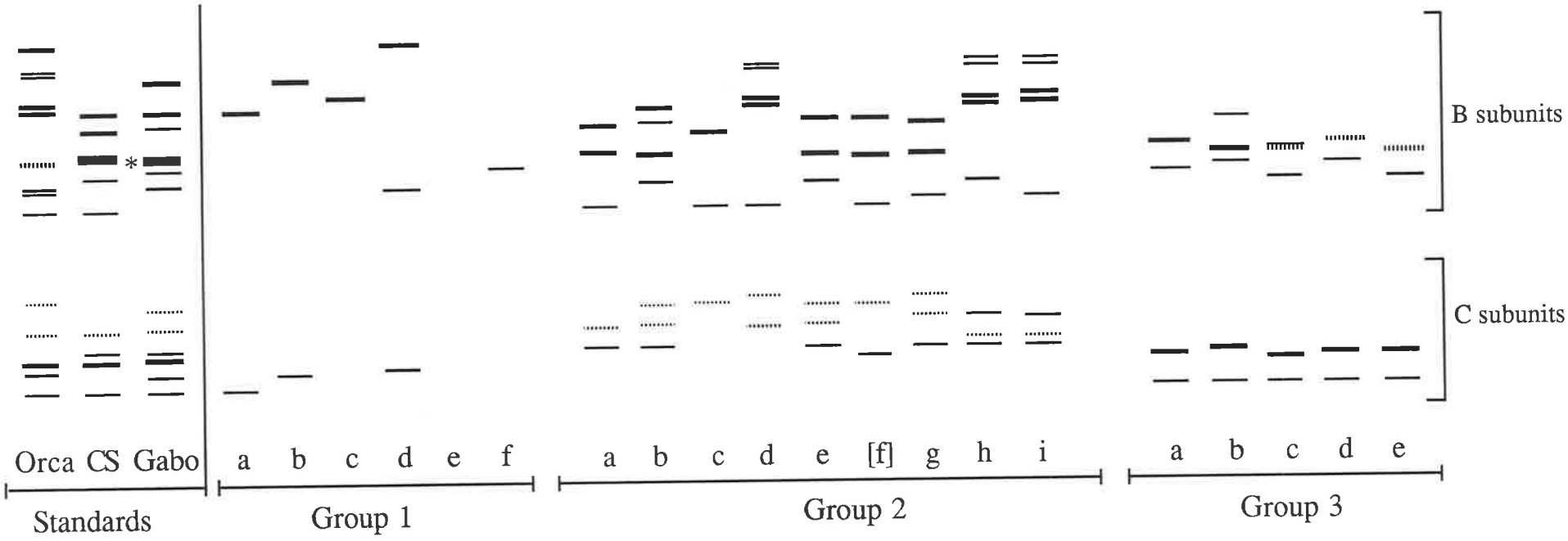
a, b, c.....denote the band patterns.

Group 1, 2 and 3 represents band patterns controlled by chromosomes 1A, 1B and 1D, respectively

[f] = direct evidence for the chromosomal location of pattern '**f**' has not been obtained

The thickness of bars in the patterns indicates the approximate thickness of the bands. Faintly stained bands are shown by broken lines.

* denotes that this thick band represents two bands of same mobility, one controlled by 1BS and other by 1DS in Chinese Spring and Gabo. These two bands have been included in group 2 and group 3 patterns of these cultivars as well as other cultivars having the denoted thick band.


B subunits
C subunits
*
Orca CS Gabo
a b c d e f
a b c d e [f] g h i
a b c d e
Standards
Group 1
Group 2
Group 3

which showed the same differences from Chinese Spring with respect to LMW subunits of glutenin (Fig. 3.5, slot d) and gliadins (marked by→, Fig. 3.9, slot f). Since the 1A substitution protein bands were as expected, it was concluded that 1D substitution was incorrectly labelled. In the absence of an authentic 1D substitution, it was still possible to determine the bands controlled by this chromosome from the pattern of Kenya Farmer. The bands which were not controlled by 1A and 1B were assumed to be controlled by 1D and formed pattern '**a**' in group 3.

Similarly, the CS–Thatcher 1D substitution line was not correct because it showed the same protein phenotype as Chinese Spring even though it should have differed by displaying HMW subunits 5+10 instead of 2+12 (Fig. 3.4, slots e, f) and the lack of an ω-gliadin band (marked by →) present in Chinese Spring (Fig. 3.9, slots j, n). Thus, the LMW glutenin bands in Thatcher, which were not controlled by chromosomes 1A or 1B, were assigned to chromosome 1D (pattern '**e**' in group 3).

The CS–Capelle-Desprez 1B substitution showed the presence of gliadins controlled by chromosome 1B in Chinese Spring (marked by →, Fig. 3.9, slot b) rather than the absence expected in an authentic 1B substitution line (see gap shown as ➤, Fig. 3.9, slot d). Thus, it was inferred that the LMW glutenin bands in Capelle-Desprez (pattern '**g**' of group 2, Fig. 3.8), which were not controlled by 1A or 1D, were controlled by chromosome 1B.

Chinese Spring–Cheyenne 1A substitution line (Fig. 3.4, slot b) possessed the HMW glutenin bands controlled by chromosome 1D in Cheyenne (5+10) rather than those controlled by this chromosome in Chinese Spring (2+12). However, since this line also had the gliadin (Fig. 3.9, slot r) and HMW glutenin band 2* controlled by chromosome 1A of Cheyenne (Fig. 3.4, slot b), it was considered that this substitution line had retained some Cheyenne 1D genes through insufficient backcrossing. Besides HMW subunits of glutenin, Cheyenne also differed from Chinese Spring with respect to gliadins and possibly LMW glutenin bands controlled by chromosome 1D. Cheyenne (Fig. 3.9, slot s) did not carry any detectable ω-gliadin band controlled by this chromosome but the seed designated as CS–Cheyenne 1D substitution line did not show these differences from Chinese Spring (Fig. 3.9, slot p; Fig. 3.4, slot d) suggesting that this stock is not a 1D substitution line. There were three LMW glutenin bands in Cheyenne (Fig. 3.4, slot a) (one of which possessed an unusual mobility as indicated by →), which could not be assigned to either chromosome 1A or 1B and thus, it is

suspected that these are controlled by genes on chromosome 1D. If so, this would constitute another pattern, **'f'** controlled by chromosome 1D in Cheyenne (not shown in group 3).

Further information was obtained from the substitution of chromosome 3R of Dakold rye for chromosomes 1A and 1B of Kharkov wheat. Both lines 3R (1A) and 3R (1B) showed the expected loss of chromosome 1A- and 1B-controlled gliadins (1A band marked by → and 1B bands by ➤, Fig. 3.10A and 3.11B), HMW subunits (1A band indicated by Δ and 1B band by → in Kharkov, Fig. 3.10B, slot b) and LMW subunits of glutenin (Fig. 3.7, B) in Kharkov. These stocks also exhibited the HMW secalin band Sec-3 (Fig. 3.10B) which was similar in mobility to a band controlled by chromosome arm 1RL in Imperial rye (Lawrence and Shepherd, 1981a), so the Dakold rye chromosome in these substitution lines probably contained part or all of chromosome arm 1RL. The 3R addition line (Fig. 3.10B, slot e) also showed Sec-3 bands while the 1R addition line exhibited both Sec-3 (Fig. 3.10B, slot f) and Sec-1 (Fig. 3.10A, slot f) controlled by the long and short arms of 1R, respectively. These data thus indicated that chromosome 3R in addition line was a 3R/1RL translocation but the chromosome 1R in the addition line was normal.

3.3.1.3 Intra-varietal variation

Thirteen of the cultivars were found to consist of two biotypes with respect to LMW glutenin subunit composition (Table 3.2). Biotypes of the cultivars Bayonet, BT–2288, Condor, Gamenya and Norin-61 were grown and shown to have similar or identical plant morphology, so the different patterns represented true biotypes within cultivars and were not the result of seed mixing or chance outcrossing. The other putative biotypes were not compared for morphology but since most exhibited very similar banding patterns for all of the other seed proteins analysed, it could be assumed that they were truly biotypes. A low proportion of seeds of Potam–70 and Huelquen, however, showed other banding patterns for LMW subunits of glutenin, besides those in Table 3.2 and since these seeds also contained different HMW subunits of glutenin, gliadins and triplet bands, they were likely to be seed contaminants. Seed contamination was also found in cultivars C–273 and Bajio–66 (given in Appendix table) which differed at all storage protein loci. Single seeds with null phenotype for group 2 LMW glutenin subunits were also found in two lines, viz. Bajio-66 and (MH3 * Warimba) MH11, but at a very low frequency, so they were not considered as biotypes.

Figure 3.9. One-dimensional SDS-PAGE patterns of endosperm proteins extracted in 70% aqueous ethanol from bread wheats and bread wheat intervarietal substitution lines; (a) Chinese Spring (CS)–Capelle-Desprez (1D), (b*) CS–Capelle-Desprez (1B), (c) CS–Capelle-Desprez (1A), (d) Capelle-Desprez, (e) CS, (f*) CS–Kenya Farmer (1D), (g) CS–Kenya Farmer (1B), (h) CS–Kenya Farmer (1A), (i) Kenya Farmer, (j) CS, (k*) CS–Thatcher (1D), (l) CS–Thatcher (1B), (m) CS–Thatcher (1A), (n) Thatcher, (o) CS, (p*) CS–Cheyenne (1D), (q) CS–Cheyenne (1B), (r) CS–Cheyenne (1A), (s) Cheyenne and (t) CS.

Symbols used in Figure 3.9;

*refers to the genetic stocks showing unexpected protein phenotypes,

➤ = expected gliadin bands absent from the concerned genetic stocks,

→ = unexpected gliadin bands present in the concerned genetic stocks.

Figure 3.10 A, B. One-dimensional SDS-PAGE patterns of unreduced (**A**) and reduced (**B**) endosperm proteins extracted in 70% aqueous ethanol from (a) Dakold rye, (b) Kharkov wheat, (c) Kharkov–Dakold rye 3R (1A) substitution, (d) Kharkov–Dakold rye 3R (1B) substitution, (e) Kharkov+Dakold rye 3R addition and (f) Kharkov+Dakold rye 1R addition line.

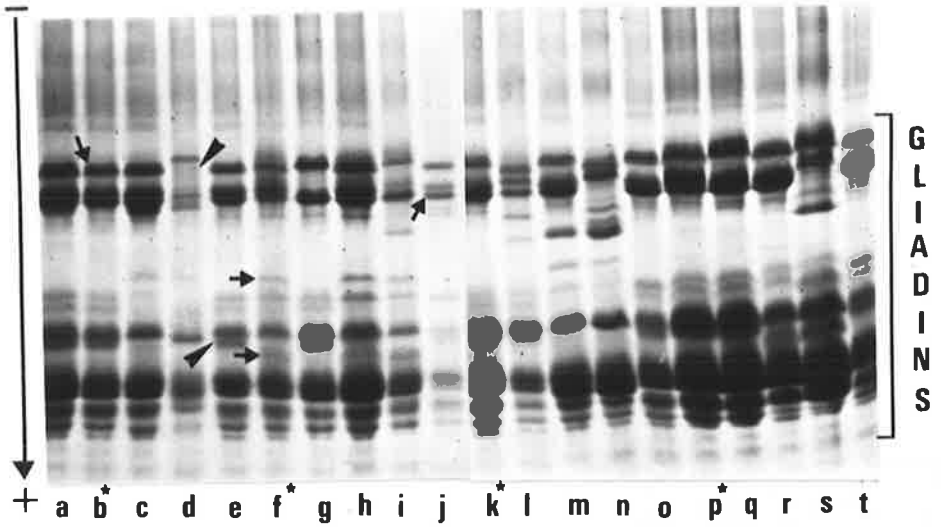
Symbols used in Figure 3.10;

→ = Gli-A1 band in Kharkov,

➤ = Gli-B1 band in Kharkov,

Δ = Glu-A1 band in Kharkov,

→ = Glu-B1 band in Kharkov.


GLIADINS
a b c d e f g h i j k l m n o p q r s t

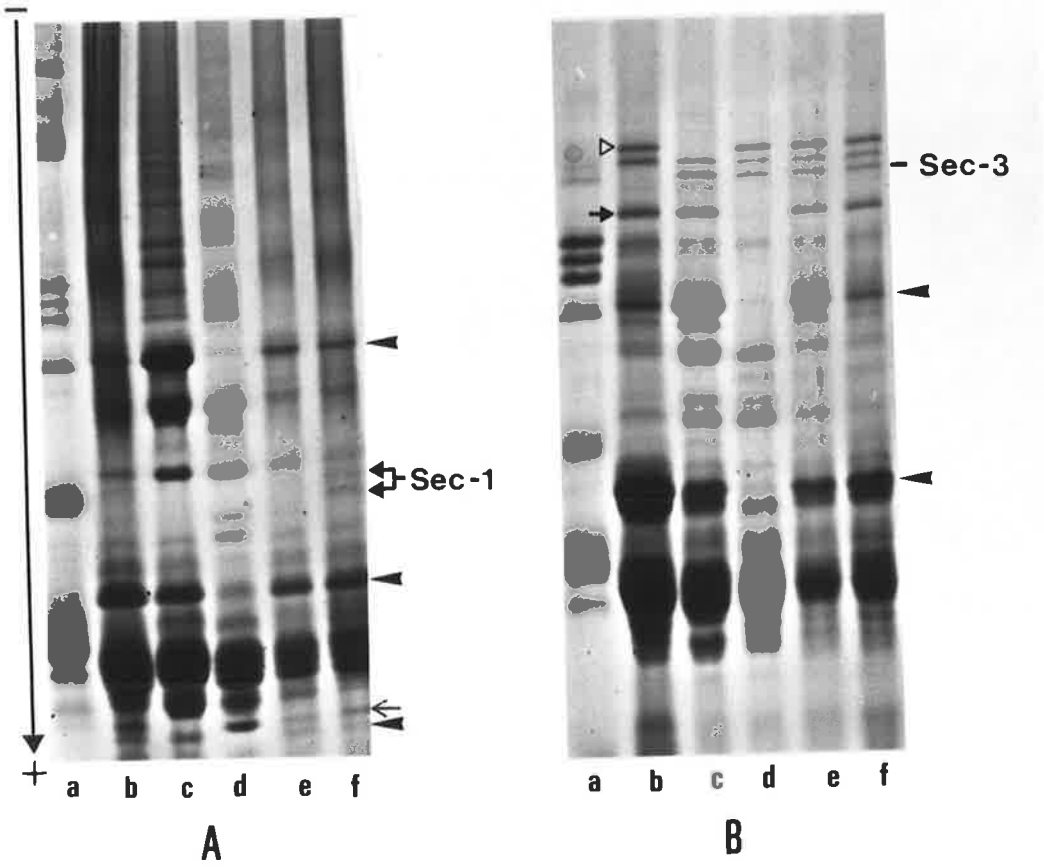

Sec-1
Sec-3
a b c d e f
a b c d e f
A
B

Table 3.2 Bread wheat cultivars heterogeneous for low-molecular-weight subunit (B and C subunits) composition.

Cultivar	Biotype	Seeds tested	LMW subunit pattern 1A	1B	1D
Bayonet	A	4	c	b	b
	B	4	e	b	b
BT–2288	A	9	f	i	a
	B	8	e	i	a
C–306	A	3	c	b	a
	B	6	c	i	a
Condor	A1	19	c	b	b
	A2	27	b	b	b
Gamenya	A	13	b	b	b
	B	16	c	b	b
Huelquen	A	5	c	g	c
	B	4	b	g	c
Norin–61	A	14	e	i	d
	B	17	d	i	e
Opal	A	6	e	g	a
	B	8	e	b	a
Potam–70	A	6	c	g	a
	B	11	c	i	a
Okukomugi	A	3	d	d	e
	B	4	e	d	e
Timstein	A	2	c	b	b
	B	4	e	b	b
Toquifen* S*	A	2	e	b	c
	B	2	e	h	c
8156 (White Grain)	A	9	c	g	b
	B	2	b	g	b

Lawrence (1986) described sixteen Australian bread wheat cultivars which contained mixtures of two or more biotypes of HMW subunits of glutenin. In the present study, these biotypes were also examined for their LMW glutenin subunit patterns and only the HMW glutenin biotypes of cultivar Bayonet showed variation for LMW subunits of glutenin as well.

One of the two HMW glutenin biotypes in cultivar Condor (biotype A) was found to be heterogeneous for LMW subunits of glutenin and these variants were designated A1 and A2.

3.2.2 Tetraploid wheats

Thirty genotypes belonging to six cultivated groups and two wild groups of tetraploid wheats were fractionated by two-step electrophoresis. The results are described in two sections; the first considers the most widely grown cultivated group of tetraploid wheats (*T. turgidum* cv. group *durum*) and the second section deals with the remaining groups.

3.2.2.1 Durum wheat

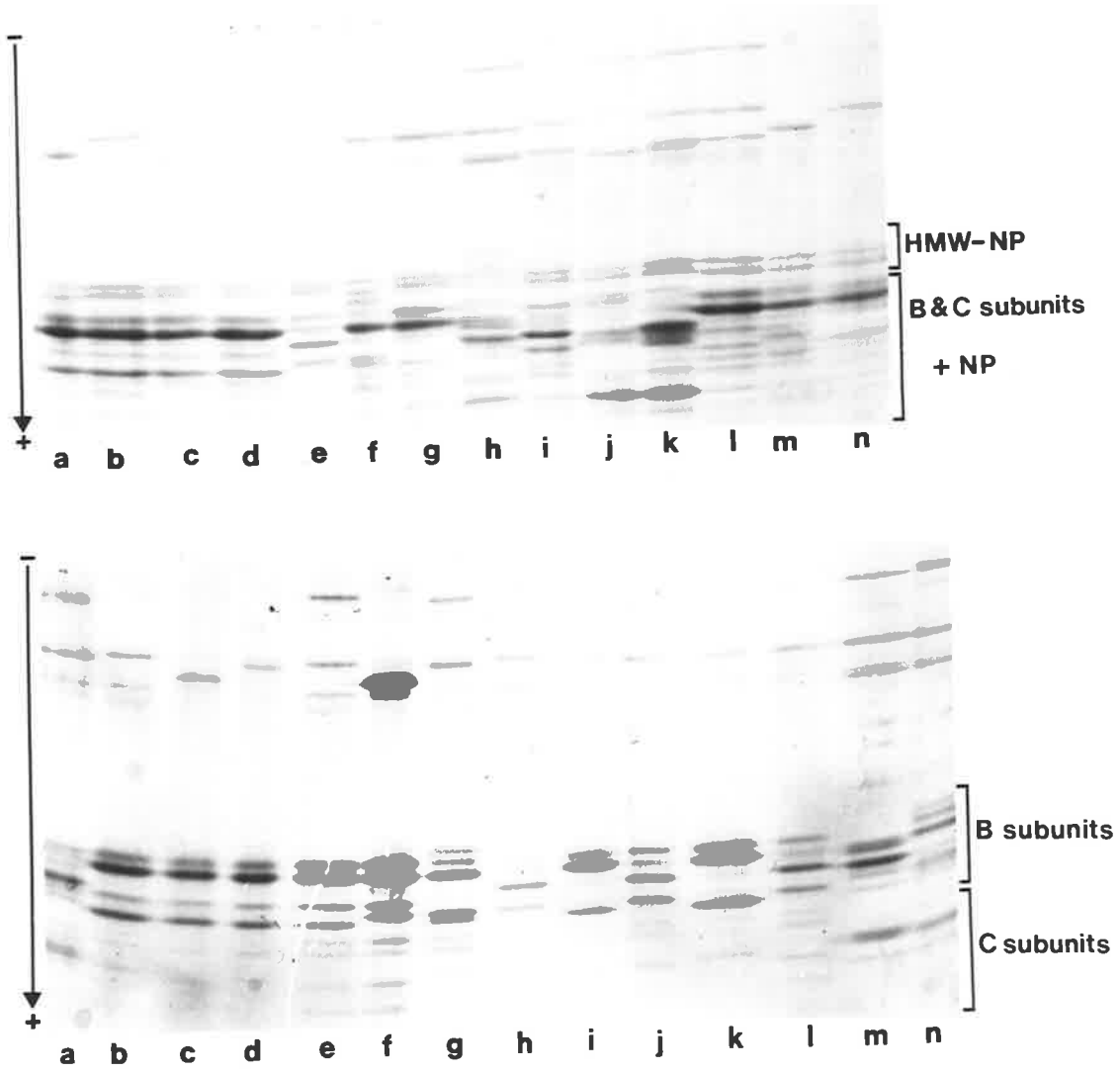
Two-step separation of SDS extracts from durum wheat also revealed much overlap between the LMW subunits of glutenin and non-prolamins (Fig. 3.11). When this overlap was removed by using ethanol extraction, the proteins were resolved into three clearly defined zones, A, B and C subunits of glutenin (Fig. 3.12) as found in hexaploid wheat.

Analysis of the B and C subunit patterns of 11 durum wheat cultivars showed that as in hexaploid wheat, there was much variation in the banding patterns of B subunits in these cultivars and each cultivar had from 7 to 9 LMW glutenin bands (Fig. 3.11). The C subunits were, however, quite faint, particularly the faster moving ones.

The genetic control of the B and C bands in some cultivars was determined by analysing the intervarietal and interspecific substitution lines available in durum wheat cultivar Langdon. Langdon had seven bands (Fig. 3.13A, slots a, f and j). Analysis of the Langdon-CS 1D (1A) (Fig. 3.13A, slot i), and Langdon-CS 1D (1B) (Fig.3.13A, slot h) substitution lines indicated that the slowest of these bands was controlled by chromosome 1A and the next five bands by chromosome 1B. The genetic control of the fastest band could not be determined due to its overlap with a C subunit controlled by chromosome 1D in Chinese Spring. A similar analysis of Langdon (Kharkof-5 1B) (Fig. 3.13A, slot e) and Langdon (Edmore 1B) (Fig. 3.13A, slot b) substitution stocks revealed two more band combinations controlled by chromosome 1B. The Kharkof–5 band pattern corresponded to pattern '**d**' controlled by chromosome 1B in bread wheat. Cultivar Gionp–1954 had another band combination which

Figure 3.11. Two-step SDS-PAGE patterns of endosperm proteins, extracted in Tris-HCl buffer (pH 6.8) containing SDS, from durum wheat cultivars (*Triticum turgidum* cv. group *durum*) and genetic stocks; (a) Dural, (b) Durati, (c) Langdon (Edmore 1B), (d) Edmore, (e) Langdon, (f) Kharkof-5, (g) Langdon (Kharkof-5 1B), (h) Chinese Spring (bread wheat), (i) Langdon, (j) Langdon-Chinese Spring 1D (1B), (k) Langdon-Chinese Spring 1D (1A), (l) ALP–153, (m) Gionp–1954 and (n) Duramba A.

Figure 3.12. Two-step SDS-PAGE patterns of endosperm proteins, extracted in 70% aqueous ethanol, from durum wheat cultivars (*Triticum turgidum* cv. group *durum*) and bread wheat cutlivars used as standards; (a) Kenya Farmer (bread wheat), (b) Edmore, (c) Dural, (d) Durati, (e) ALP-153, (f) Gionp–1954, (g) Kharkof-5, (h) Langdon, (i) Duramba A, (j) Duramba B, (k) Duramba C, (l) Duramba D and bread wheat cultivars, (m) Chinese Spring and (n) Orca.


HMW-NP
B&C subunits
+ NP
a b c d e f g h i j k l m n
B subunits
C subunits
a b c d e f g h i j k l m n

differed from Langdon 1B pattern for one band and was probably controlled by chromosome 1B but direct evidence for its genetic control has not yet been obtained.

The chromosome 1A controlled band in Langdon corresponded in mobility to the band '**b**' controlled by chromosome 1A in bread wheat. There were two bands in Kharkof–5 which were not added in Langdon (Kharkof-5 1B) substitution line (Fig. 3.13A, slot e) and it is assumed that they were controlled by chromosome 1A since they corresponded to the bands in pattern '**d**' controlled by chromosome 1A in bread wheat. Some of the durum wheat cultivars analysed lacked the bands controlled by chromosome 1A and were believed to carry null phenotype similar to many bread wheat cultivars.

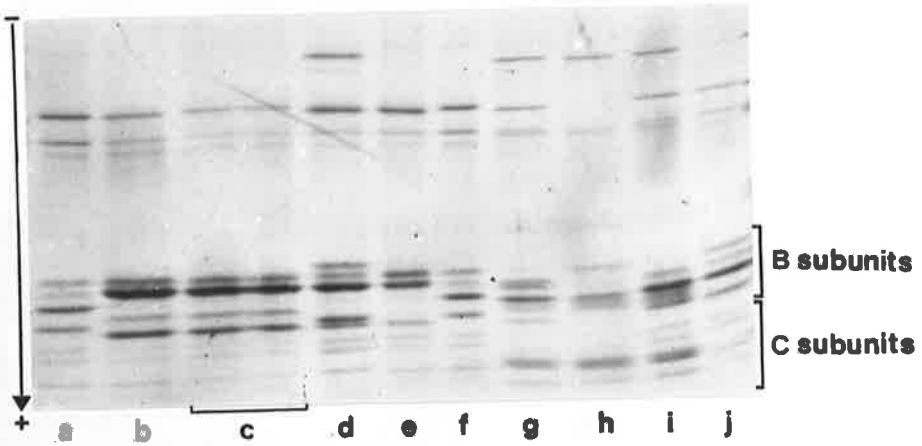
Thus, three and four different LMW glutenin band patterns controlled by chromosome 1A and 1B, respectively were recognised among these durum wheats (Fig. 3.13B). Three of the patterns controlled by chromosome 1B were different from those detected in hexaploid wheats and have been designated with '**j**' to '**l**' (Table 3.3).

Table 3.3 Classification of eleven durum wheat genotypes with respect to their LMW glutenin subunit composition

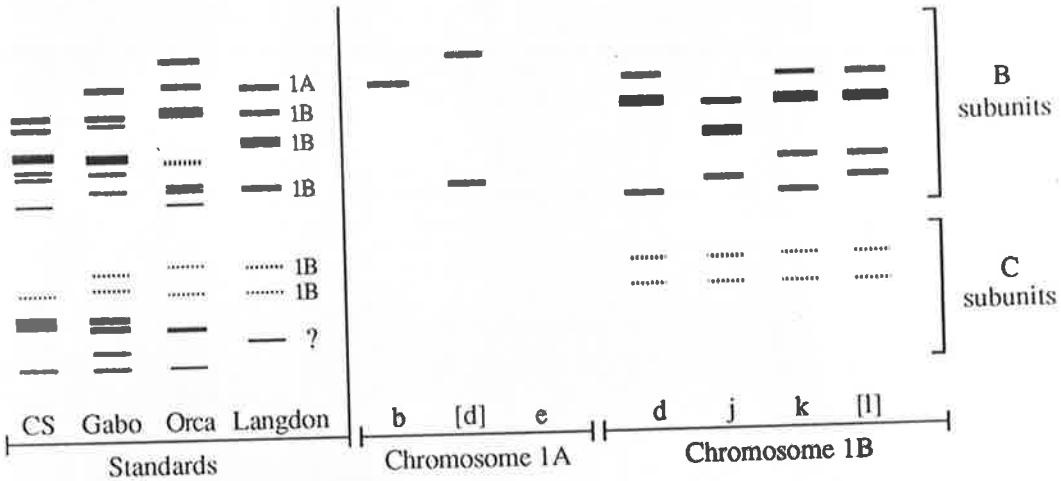
Durum wheat	LMW glutenin subunit patterns	
	1A	1B
ALP–153	e	k
Dural	e	k
Durati	e	k
Duramba–A	d	d
Duramba–B	b	j
Duramba–C	d	d
Duramba–D	b	j
Edmore	e	k
Gionp–1954	e	l
Kharkof–5	d	d
Langdon	b	j

Figure 3.13A. Two-step SDS-PAGE patterns of endosperm proteins, extracted in 70% aqueous ethanol, from durum wheat cultivars (*Triticum turgidum* cv. group *durum*) and genetic stocks; (a) Langdon, (b) Langdon (Edmore 1B), (c) Edmore, (d) Kharkof-5, (e) Langdon (Kharkof-5 1B), (f) Langdon, (g) Chinese Spring (bread wheat), (h) Langdon-CS 1D (1B), (i) Langdon-CS 1D (1A) and (j) Langdon.

Figure 3.13B. Diagram showing the two-step SDS-PAGE patterns of LMW subunits (B and C subunits) of glutenin controlled by chromosomes 1A and 1B in durum wheat cultivars. The LMW subunits of Chinese Spring (CS), Gabo and Orca bread wheat cultivars and Langdon durum wheat cultivar are included for comparison. The chromosomal control of LMW glutenin subunit patterns in durum wheats has been determined by using various substitution lines in Langdon. Patterns '**j**', '**k**' and '**l**' are additional patterns to those controlled by chromosome 1B in bread wheats. [d] and [l] = indicate that direct evidence for the chromosomal control of these patterns has not been obtained yet.


B subunits
C subunits
+
a
b
c
d
e
f
g
h
i
j

A


1A
1B
1B
1B
1B
1B
?
B subunits
C subunits
CS
Gabo
Orca
Langdon
b
[d]
e
d
j
k
[l]
Standards
Chromosome 1A
Chromosome 1B

B

3.2.2.2 Non-durum tetraploid wheats

The two-step gel patterns of SDS-extracted endosperm proteins from 13 accessions belonging to five cv. groups (*carthlicum*, *dicoccum*, *turgidum*, *polonicum*, *turanicum*) and a wild group (var. *dicoccoides*) of *Triticum turgidum* and two accessions of *T. timopheevi* var. *timopheevi* are shown in Fig. 3.14. Chinese Spring was included as a bread wheat control. On the basis of the staining behaviour described in section 3.1, the faint bands in the C subunit region of the gel photograph were considered as non-prolamins whereas the dark bands were classified as the C subunits of glutenin. There was no overlap with the non-prolamins in the B subunit region and thus all the bands labelled as B subunits were glutenin bands. The number of LMW subunits of glutenin ranged from 5 to 8 in the accessions of *T. turgidum* whereas 9 to 11 bands were recorded in the accessions of *T. timopheevi*. B and C subunits were approximately equal in number (to each other) in both groups of tetraploid wheats.

The LMW glutenin banding pattern of the *polonicum* sample (Fig. 3.14, slot m) was identical to one of the accessions of the *dicoccum* (Fig. 3.14, slot d) and *carthlicum*, (Fig. 3.14, slot a). Similarly, the *turanicum* sample (Fig. 3.14, slot n) gave the same LMW glutenin pattern as one of the accessions of wild var. *dicoccoides* (Fig. 3.14, slot j). Banding patterns of the remaining two accessions of var. *dicoccoides* (Fig. 3.14, slots k, l) showed the presence of only two B subunits which were also present in the other accession of this group (Fig. 3.14, slot j). In general, banding patterns of *dicoccoides*, *turanicum* and *timopheevi* were different from the other tetraploid wheats analysed. All the genotypes seemed to have bands with the mobility of 1B-controlled bands in bread wheat but some of the *dicoccum*, *turgidum* and *dicoccoides* samples may have lacked bands with the mobility of 1A controlled bands. These differences in the banding patterns suggest duplication and divergence of genes controlling LMW subunits of glutenin in these tetraploid wheats.

The B subunit banding patterns of the non-durum wheats showed some resemblance with the durum and bread wheats. For example, *carthlicum*, *polonicum* and one accession of *diccocum* (Fig. 3.14, slot d) exhibited pattern '**b**' (1B controlled) while another accession of *diccocum* (Fig. 3.14, slot e) and both accessions of *turgidum* showed pattern '**d**' (1B controlled).

3.3.3 Diploid wheats and relatives of wheat

3.3.3.1 Diploid Triticum *species: variation*

One to three accessions of several diploid *Triticum* species were analysed to investigate the number of B and C subunits present and their LMW glutenin banding patterns were compared with those of hexaploid wheat cultivars Chinese Spring and Sonalika (Fig. 3.15A, B, C). The gel portions containing B and C subunits have been labelled in the figure and only the dark bands on the basis of its staining property, were regarded as the subunits of glutenin.

The diploid wheats with the 'AA' genome, viz. *T. monococcum* (Fig. 3.15A, slot b), *T. boeoticum* (Fig. 3.15A, slot c), *T. urartu* (Fig. 3.15A, slot d) and *T. thaoudar* (Fig. 3.15A, slot e) contained 2-4 LMW subunits of glutenin and showed much greater staining intensity than bands of similar mobility in bread and durum wheats. LMW subunits of *T. boeoticum* (Fig. 3.15A, slot c) were very similar in mobility to the bands in pattern '**d**' in bread and durum wheats controlled by chromosome 1A. In addition, two novel banding patterns were found between *T. monococcum* (Fig. 3.15A, slot b) and *T. thaoudar* (Fig. 3.15A, slot e). *T. urartu* (Fig. 3.15A, slot d) possesed a band very similar to chromosome 1A band in pattern '**a**' of bread wheat and moreover its banding pattern resembled more closely to that of *T. monococcum* than the other species. Any '**a**' or '**d**' patterns in other species were not detected because of the necessarily small number of sample.

Analysis of the diploid species thought to be related to the B genome donor of polyploid wheat revealed 5-6 LMW subunits of glutenin in *T. speltoides* (Fig. 3.15B, slot c), *T. longissimum* (Fig. 3.15B, slot a) and *T. sharonense* (Fig. 3.15B, slot b) and 2 subunits in *T. bicorne* (Fig. 3.15B, slot d). While the LMW glutenin bands in these species were within the mobility range of the LMW bands of hexaploid and tetraploid wheats, they did not appear to be identical with band patterns controlled by chromosome 1B in the polyploid wheats.

Since the D genome of hexaploid wheat is known to have come from *T. tauschii* (Pathak, 1940; Kihara, 1944; McFadden and Sears, 1946), three accessions of this species were examined for any similarilities between their LMW glutenin composition and that of hexaploid wheats. Chinese Spring (Fig. 3.15C, slot b) and Sonalika (Fig. 3.15C, slot a) were the hexaploid wheat checks. All three accessions had two B and three C subunits and

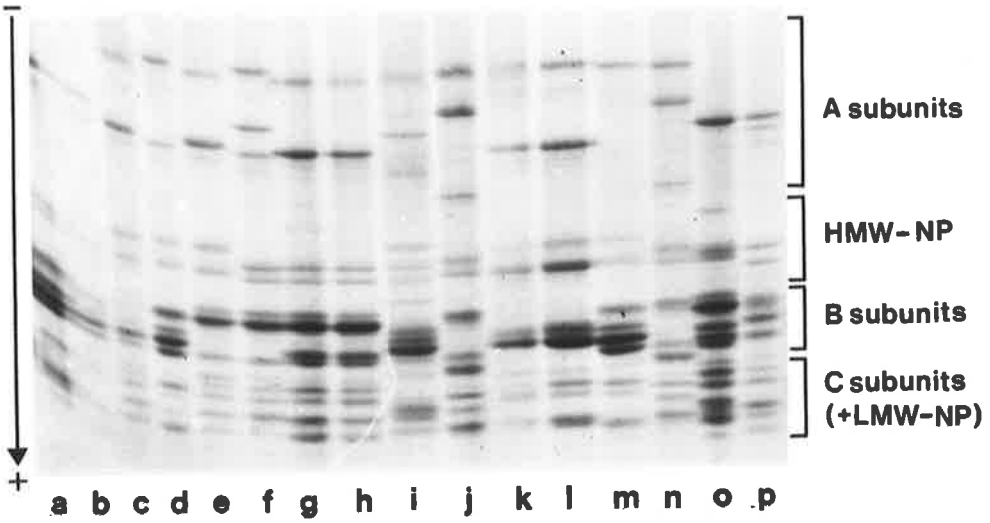
Figure 3.14. Two-step SDS-PAGE patterns of SDS-extracted seed proteins from tetraploid wheats and bread wheat Chinese Spring. *Triticum turgidum* (a, b) cv. group *carthlicum*, (c, d, e) cv. group *dicoccum*, (f, g, h) cv. group *turgidum*, (j, k, l) var. *dicoccoides*, (m) cv. group *polonicum*, (n) cv. group *turanicum*, (o, p) *T. timopheevi* var. *timopheevi* and (i) *Triticum aestivum* cv. Chinese Spring.

Figure 3.15 A, B, C. Two-step SDS-PAGE patterns of SDS-extracted seed proteins from diploid *Triticum* species related to bread wheat. The bracketed portion in gel photographs **A**, **B** and **C** represents the B and C subunits of glutelin and some non-prolamins, particularly the faintly stained bands.

A. (b) *T. monococcum*, (c) *T. boeoticum* , (d) *T. urartu*, (e) *T. thaoudar* and (a) *T. aestivum* cv. Chinese Spring.

B. (a) *T. longissimum*, (b) *T. sharonense*, (c) *T. speltoides*, (d) *T. bicorne* and (e, f) *T. aestivum* cv. Chinese Spring.

C. *T. tauschii* (c) var. *meyeri*, (d, e) var. *strangulata* and *T. aestivum* (a) cv. Sonalika and (b) cv. Chinese Spring.


A subunits
HMW-NP
B subunits
C subunits
(+LMW-NP)
a b c d e f g h i j k l m n o p

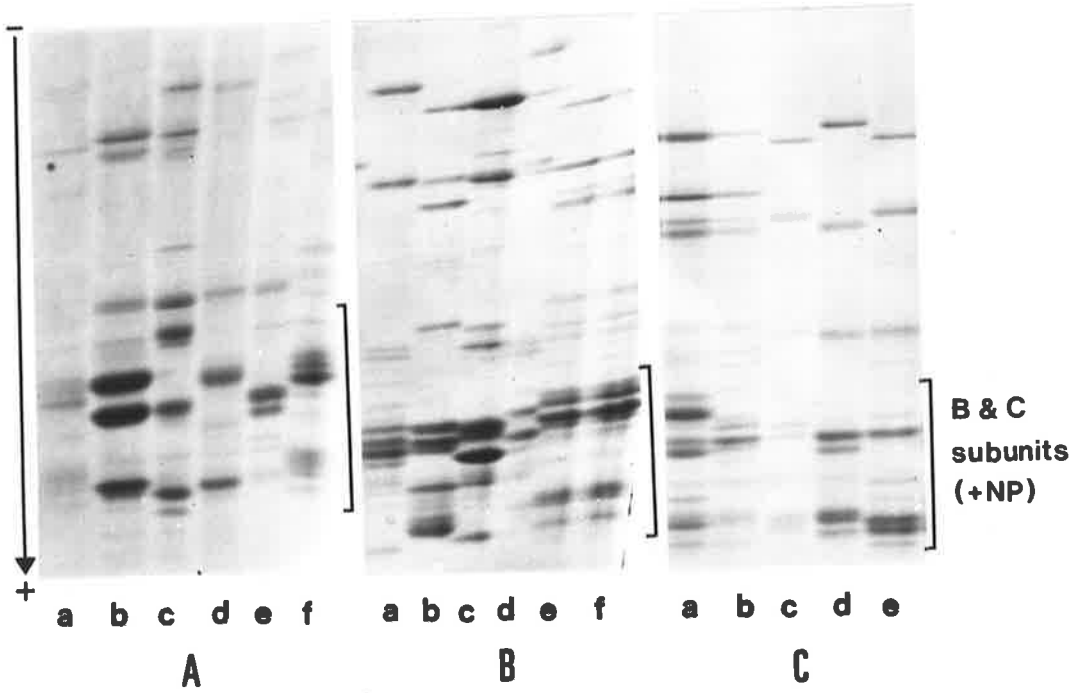

B & C
subunits
(+NP)
a b c d e f
a b c d e f
a b c d e
A
B
C

Figure 3.16. Fractionation by Two-step SDS-PAGE (**A**) and One-dimensional SDS-PAGE (**B**) of 70% ethanol-extracted endosperm proteins from (a) *Triticum longissimum* (2n =14, S^lS^l), (b) Chinese Spring and (c) Chinese Spring–*T. longissimum* $1S^l$ (1B) substitution line.

Figure 3.17. Two-step SDS-PAGE patterns of 70% ethanol-extracted seed proteins from (a) *Triticum umbellulatum* (2n =14, UU), (b) Chinese Spring, (c) Chinese Spring–*T. umbellulatum* amphiploid (2n =56), (d) Chinese Spring–*T. umbellulatum* substitution line 1U (1D), (e) Chinese Spring–*T. umbellulatum* substitution line 1U (1B) and (f) Chinese Spring–*T. umbellulatum* substitution line 1U (1A).

Symbols used:

S1-S2 = A subunits controlled by chromosome $1S^l$ in *T. longissimum*

S3-S6 = B subunits controlled by chromosome $1S^l$ in *T. longissimum*

S7-S10 = Monomeric prolamins controlled by chromosome $1S^l$ in *T. longissimum*

U1-U3 = A subunits controlled by chromosome 1U in *T. umbellulatum*

U4-U5 = B subunits controlled by chromosome 1U in *T. umbellulatum*

their banding patterns differed from each other. Var. *meyeri* of this species (Fig. 3.15C, slot c) did not have any of the chromosome 1D patterns from bread wheat but var. *strangulata* (Fig. 3.15C, slots d, e) had patterns similar to the '**b**' and '**c**' controlled by chromosome 1D of bread wheats. In order to compare the other bread wheat patterns with those in *T. tauschii*, a much larger sample of accessions of this species needs to be examined.

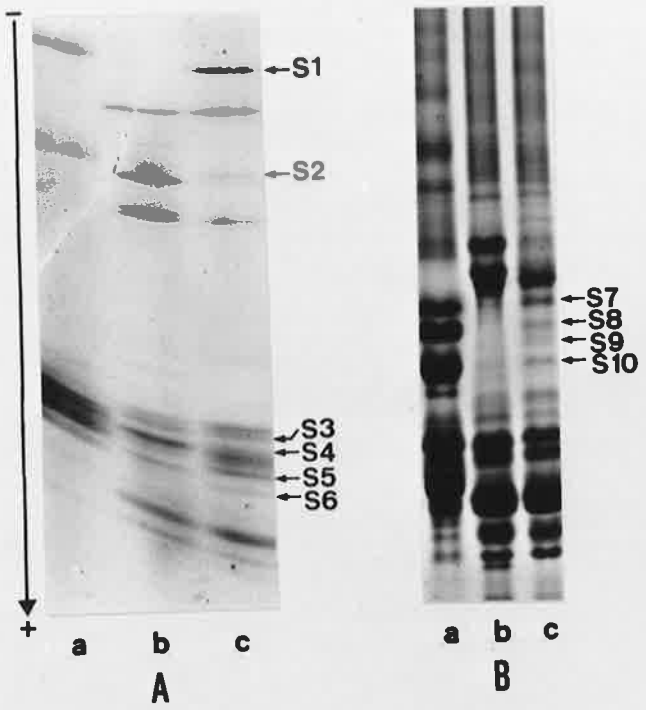
3.3.3.2 Diploid Triticum *species: chromosomal control*

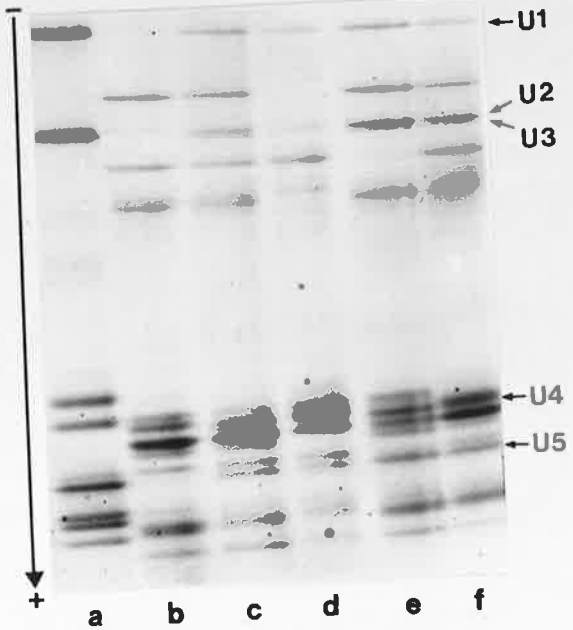
T. longissimum

The chromosomal control of LMW glutenin bands in *T. longissimum* was determined by comparing the banding patterns of the Chinese Spring-*T. longissimum* $1S^l$ (1B) substitution line and Chinese Spring (Fig. 3.16A). The seeds of *T. longissimum* and the Chinese Spring-*T. longissimum* amphiploid used to produce this substitution line were not available for analysis. Another accession of this species was available and it showed at least seven B subunits and probably lacked the C subunits (Fig. 3.16A, slot a). The substitution line (Fig. 3.16A, slot c) possessed three extra LMW glutelin bands (S4, S5, S6) while the chromosome 1B-controlled LMW subunits of Chinese Spring were absent, as expected. There was another B subunit band in this stock which overlapped one of the 1B-controlled LMW subunits of Chinese Spring (S3). This indicated that four LMW subunits of glutelin are controlled by chromosome $1S^l$ in *T. longissimum*. This stock also showed the presence of two extra HMW subunits of glutelin (S1 and S2, Fig. 3.16A, slot c) and four extra gliadin bands (S7, S8, S9, S10, Fig. 3.16B, slot c). The genes controlling these subunits have not been located to a chromosome arm. Since these protein groups are controlled by group 1 chromosomes in bread wheat, the results provide a biochemical basis for the homoeologous relationship between chromosome $1S^l$ of *T. longissimum* and group 1 chromosomes (particularly chromosome 1B) of hexaploid wheat.

T. umbellulatum

The two-step banding patterns of an accession of *T. umbellulatum*, Chinese Spring and the amphiploid between *T. umbellulatum* and Chinese Spring are given in Fig. 3.17. The banding pattern of an accession of *T. umbellulatum* showed the presence of seven LMW


S1
S2
S3
S4
S5
S6
S7
S8
S9
S10
a b c
A
a b c
B


U1
U2
U3
U4
U5
a b c d e f

subunits and two HMW subunits of glutelin (Fig. 3.17). Although this accession is not the one used to produce the amphiploid (Shepherd, personal communication), it had four bands with the same mobility to the bands U1, U2, U3 and U5 in the amphiploid (Fig. 3.17, slot c). Besides these four bands, the amphiploid revealed a glutelin subunit (U4) additional to those present in Chinese Spring. Bands U4 and U5 were LMW subunits of glutelin while the others were HMW subunits of glutelin. Analysis of the banding patterns of the substitution lines 1U (1D) (Fig. 3.17, slot d), 1U (1B) (Fig. 3.17, slot e), and 1U (1A) (Fig. 3.17, slot f) showed that these bands were added while the LMW and HMW glutelin bands controlled by chromosomes 1A, 1B and 1D in Chinese Spring were deleted, respectively in these stocks. Thus, chromosome 1U was shown to control two LMW subunits (U4, U5) and three HMW subunits (U1, U2, U3) of glutelin (Fig. 3.17).

3.3.3.3 Elytrigia *and* Secale *species: variation and chromosomal control*

Elytrigia elongata

The two-step method was applied to endosperm proteins from an accession of *E. elongata* (2n=14), the amphiploid involving *E. elongata* (2n=14) and Chinese Spring wheat and various substitution lines involving replacement of group 1 wheat chromosomes (Fig. 3.18A) to investigate the genetic control of LMW subunits of glutelin in *E. elongata.* Four LMW subunits of glutenin were identified in *E. elongata* (Fig. 3.18A, slot a) but the amphiploid (Fig. 3.18A, slot c) did not exhibit any extra LMW glutelin bands over those present in Chinese Spring. Unexpectedly, it lacked two (B3, B4) of the four labelled LMW glutenin bands known to be controlled by chromosome arm 1BS in Chinese Spring. This stock, however, possessed a HMW glutelin band (E1) additional to those present in the Chinese Spring. To investigate the band composition of this stock further, one-dimensional SDS-PAGE patterns of unreduced proteins were analysed and it showed a lack of gliadin bands (B5, B6) controlled by the *Gli-B1* locus in Chinese Spring but this contained three extra gliadin bands E4, E5 and E6 (Fig. 3.19A, slot c). Four other seeds from this stock were analysed, three of which exhibited gliadin bands B5 and B6 (Fig. 3.19B) while one lacked them (not shown). Since the frequency of seeds lacking gliadins and LMW subunits of Chinese Spring was high, the parental amphiploid seed used to multiply this stock was

Figure 3.18 A, B. Fractionation by two-step SDS-PAGE of 70% ethanol-extracted seed proteins. **A.** (a) *Elytrigia elongata* (2n =14, EE), (b) Chinese Spring, (c) Chinese Spring–*E. elongata* amphiploid, (d) Chinese Spring–*E. elongata* substitution line 1E (1A), (e) Chinese Spring–*E. elongata* substitution line 1E (1B), (f) Chinese Spring–*E. elongata* substitution line 1ES (1B), (g) Chinese Spring–*E. elongata* substitution line 1E (1D) and (h) Chinese Spring–*E. elongata* substitution line 1ES (1D). **B.** (a) Rescue, (b) Rescue–*E. elongata* substitution line (1A) and (c) Chinese Spring–*E. elongata* substitution line (1D).

Figure 3.19 A, B. One-dimensional SDS-PAGE patterns of 70% ethanol-extracted seed proteins. **A.** (a) *Elytrigia elongata* (2n =14, EE), (b) Chinese Spring, (c) Chinese Spring–*E. elongata* amphiploid, (d) Chinese Spring–*E. elongata* substitution line 1E (1A), (e) Chinese Spring–*E. elongata* substitution line 1E (1B), (f) Chinese Spring–*E. elongata* substitution line 1ES (1B), (g) Chinese Spring–*E. elongata* substitution line 1E (1D) and (h) Chinese Spring–*E. elongata* substitution line 1ES (1D). **B.** (a, b, c) Chinese Spring–*E. elongata* amphiploid.

Symbols used ;

E1 = A subunit controlled by chromosome arm 1EL in diploid *E. elongata*

E2-E3 = B and C subunits controlled by chromosome arm 1ES in diploid *E. elongata*

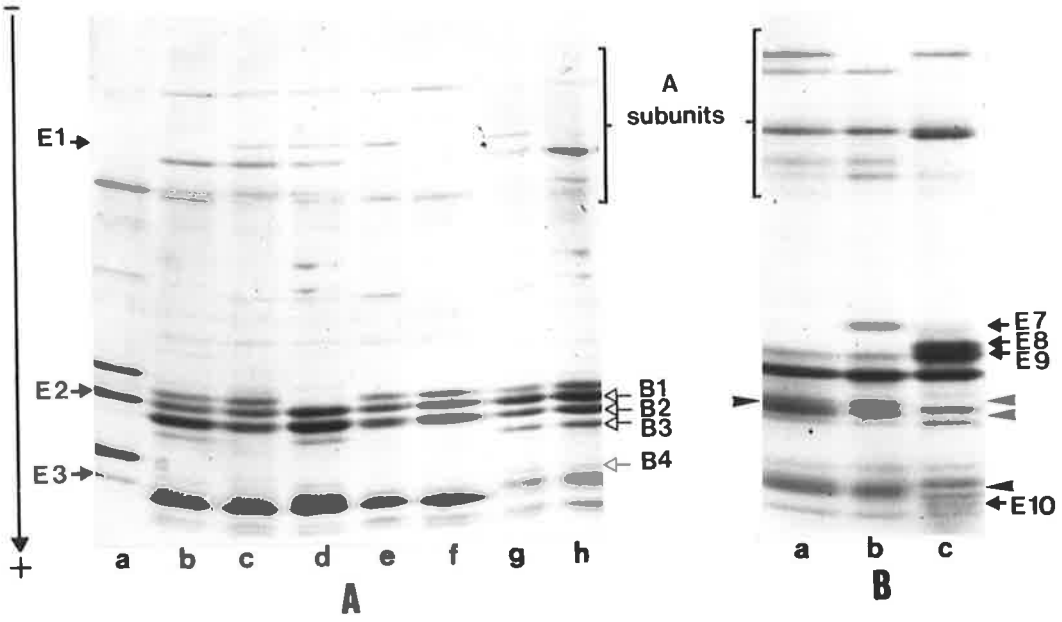
E4-E6 = Monomeric prolamins controlled by chromosome arm 1ES in diploid *E. elongata*

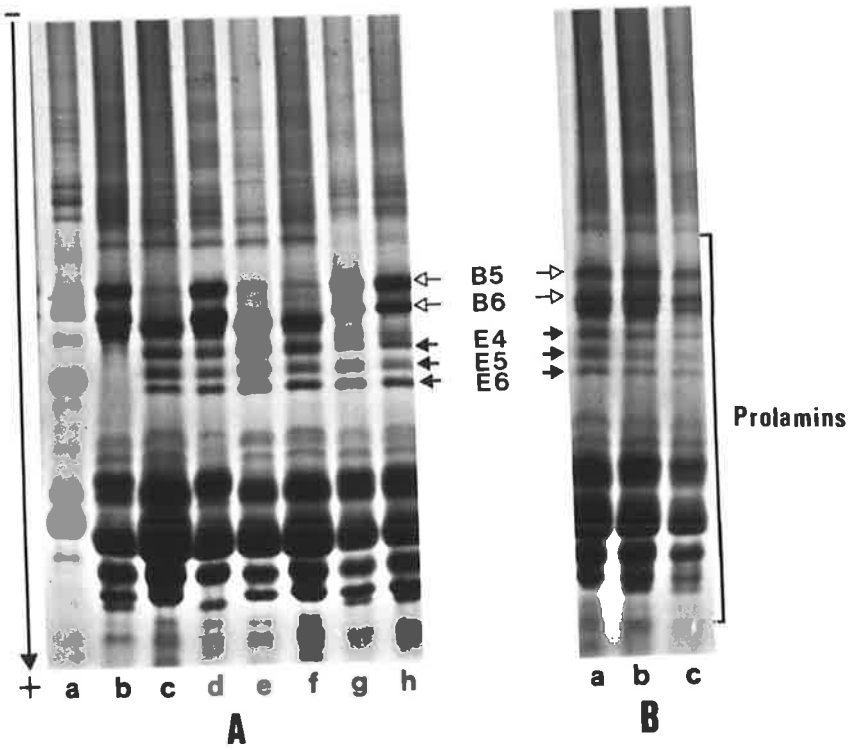
E7 = B subunit controlled by chromosome 1E' in decaploid *E. elongata*

E8-E10 = B and C subunits controlled by chromosome 1E" in decaploid *E. elongata*

B1-B4 = B and C subunits controlled by chromosome arm 1BS in Chinese Spring

B5-B6 = Gliadins controlled by chromosome arm 1BS in Chinese Spring


E1
E2
E3
A subunits
B1
B2
B3
B4
E7
E8
E9
E10
a b c d e f g h
A
a b c
B


B5
B6
E4
E5
E6
Prolamins
a b c d e f g h
A
a b c
B

probably hemizygous for chromosome arm 1BS. Consequently, the presence of a band (E2) with similar mobility to a 1BS-controlled LMW subunit (B1) in these seeds indicated that band E2 came from *E. elongata,* but this could not be identified in the normal amphiploid because of the overlap.

Since bands E2 and E3 were present in both the substitution lines 1E (1B) (Fig. 3.18A, slot e) and ditelocentric 1ES (1B) (Fig. 3.18A, slot f) which lacked the 1B-controlled LMW subunits of Chinese Spring, they were controlled by genes on chromosome arm 1ES. Additional evidence for the genetic control of these two bands by 1ES and for their presence came from the observations that substitution stocks 1E (1A) (Fig. 3.18A, slot d), 1E (1D) (Fig. 3.18A, slot g) and 1ES (1D) (Fig. 3.18A, slot h) showed increased staining intensity in the B1and B2 bands of Chinese Spring which were similar in mobility.

This study also showed that HMW glutenin subunit (E1) was controlled by genes on chromosome arm 1EL as this band was not present in 1ES (1B) and 1ES (1D) substitution lines (Fig. 3.18A). On the other hand, lack of chromosome arm 1EL did not have any effect on the presence of gliadin bands E4, E5 and E6 in these stocks (Fig. 3.19A, slots f, h) indicating that these were governed by genes on chromosome arm 1ES. These results conformed with those obtained by Lawrence and Shepherd (1981a) for HMW glutenin subunits.and supported the homoeologous relationship between chromosomes 1A, 1B, 1D and 1E (Dvorak, 1980; Lawrence and Shepherd, 1981a).

To determine the chromosomal control of the LMW glutelin bands in decaploid *E. elongata* (2n= 70), the banding patterns of two lines thought to have a chromosome from it substituted for Rescue chromosomes 1A and 1D were also analysed (Fig. 3.18B). Seeds from the amphiploid between wheat and the parental *E. elongata* were not available. The purported substitution line Rescue– *E. elongata* (1A) (Fig. 3.18B, slot b) showed one extra band (E7) while the Rescue– *E. elongata* (1D) line (Fig. 3.18B, slot c) had three other bands (E8, E9, E10) of which E8 and E10 occupied a distinct position whereas E9 overlapped the slowest moving LMW glutenin subunit of Rescue and hence increased its staining intensity. Since both lines showed the presence of different LMW glutelin bands from *E. elongata*, it seemed that decaploid *E. elongata* carried two different chromosomes (arbitrarily designated 1E' and 1E") controlling LMW subunits E7 and E8, E9, E10, respectively, which were homoeologous to group 1 chromosomes of hexaploid wheat. Since chromosomes 1E' and

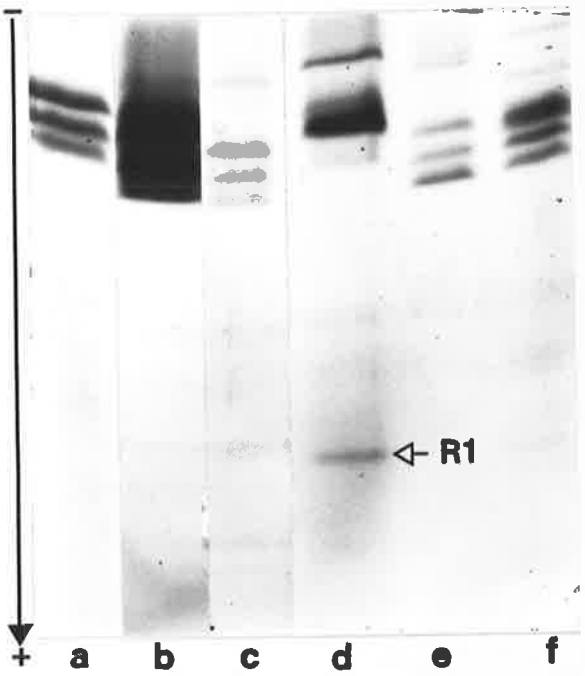
1E" of decaploid and 1E of diploid *E. elongata* all are homoeologous to group 1 chromosomes of hexaploid wheats, the chromosomes 1E' and 1E" would be homoeologous to chromosome 1E. This would support that decaploid *E. elongata* had at least two genomes that were related to the E genome of diploid *E. elongata* (Dvorak, 1981).

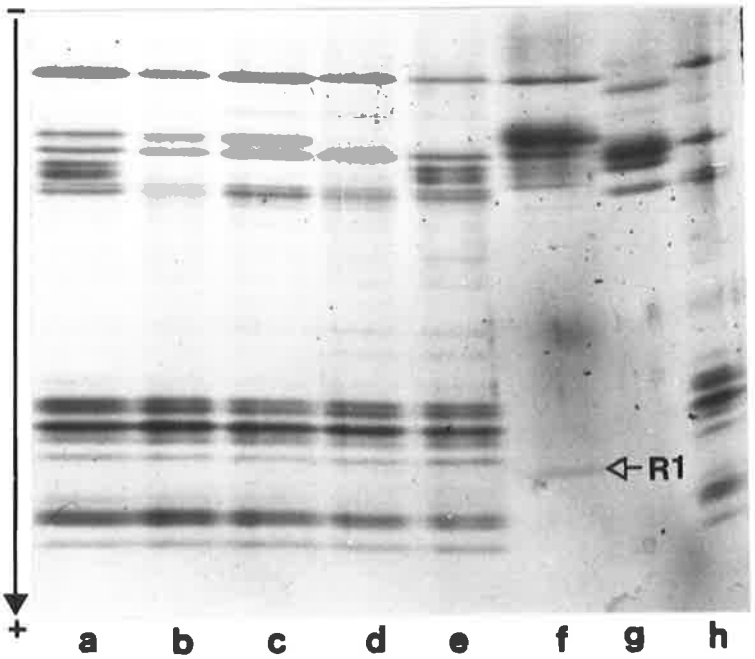
Secale species

Two-step banding patterns of five cereal rye (*S. cereale*) cultivars Imperial, King II, Dakold, Petkus, South Australian rye and two wild rye (*S. montanum*) genotypes R-15 (Fig. 3.20) and R-42 (Fig. 3.21, slot g) were analysed for the presence of LMW glutelin subunits.

The two-step gels provided no evidence that any of the rye cultivars produced LMW glutelin bands. Similarly, *S. montanum* R–42 did not have any LMW bands. However, *S. montanum* R-15 carried one band R1 (Fig. 3.20, slot d).

The Chinese Spring–*S. montanum* R-15 amphiploid (Fig. 3.21, slot a) stock did not demonstrate the band R1 nor was it present in any of the addition lines involving chromosomes $1R^m$ (Fig. 3.21, slot b), $1R^mS$ (Fig. 3.21, slot c), $2R^m$ (Fig. 3.21, slot d) or $6R^m$ (Fig. 3.21, slot e). The genes controlling band R1 might be suppressed in Chinese Spring wheat background, or the R1 band might not be synthesized in sufficient amount, or the *S. montanum* genotype involved in producing the amphiploid and addition lines did not carry this band.


R1
a
b
c
d
e
f


R1
a
b
c
d
e
f
g
h

3.4 DISCUSSION

3.4.1 Solubility of native glutelins in aqueous ethanol

Although glutenin in its native state has been defined as being insoluble in aqueous ethanol (Osborne, 1907), the fraction known as LMW glutenin is readily soluble in 70% ethanol at room or even lower (4°C) temperatures (Nielsen *et al.*, 1968; Bietz and Wall, 1973; Byers *et al.*, 1983; Shewry *et al.*, 1983b) and this fraction consists of B and C subunits (Payne and Corfield, 1979; Graveland *et al.*, 1982). In contrast, native HMW glutenins (consisting of A, B and C subunits) are readily soluble only at higher (≥50°C) temperatures and they are either insoluble (Byers *et al.*, 1983) or sparingly soluble (Bietz and Wall, 1973; Singh and Shepherd, 1985) in ethanol at room or low temperatures. Byers *et al.* (1983) also found that native prolamins when extracted at 60°C contained significant amounts of the HMW subunits (A subunits). The quantitative differences in the solubility of native HMW glutenins at these temperatures can be attributed to the molecular weights of the glutenins (Graveland *et al.*, 1985). Since both covalent (inter-chain disulphide bonds) and non-covalent forces (hydrophobic bonds etc.) are involved in the stabilization of glutenin polymers (Dimler, 1965; Khan and Bushuk, 1979), it is likely that at higher temperatures, these forces between the polymers may be disrupted (Schofield *et al.*, 1983) thus causing reduction in the sizes of HMW glutenins and hence the increased solubility. These data clearly indicate that HMW glutenins, like gliadins, are soluble in ethanol in their native state and hence can be included in the prolamins as suggested by Shewry *et al.* (1986b).

3.4.2 Variation of LMW subunits of glutenin in bread wheat

Comparison of the two-step banding patterns of endosperm proteins extracted in two different solvents, viz. 70% ethanol and Tris-HCl buffer with 4% SDS showed that ethanol extraction provided much improved resolution of LMW subunits of glutenin because non-prolamins, which overlap the B subunits to some extent and the C subunits to a large extent, were not extracted. Hence, the analysis of the genetic variation in LMW subunits of glutenin described here has been modified from that previously carried out on SDS-extracted seed proteins from

these wheats (Gupta and Shepherd, 1988). The composition of B and C subunits were found to be the same in both the extraction procedures.

The band patterns of the 222 bread wheat cultivars are listed in the Appendix table. Some difficulties were encountered in the analysis of LMW subunit patterns of these cultivars. The bands controlled by chromosome 1B (group 2, Fig. 3.8) represented a wide range of mobilities and some of them overlapped with those controlled by 1A and 1D (group 1 and 3, Fig.3.8). For example, the four slowest moving bands in patterns '**d**', '**h**' and '**i**' (chromosome 1B) often separated as two distinctly different thick bands and the faster one had similar mobility to a band in patterns '**a**' and '**c**' controlled by chromosome 1A. Similarly, the slowest band in pattern '**b**' controlled by chromosome 1B often coincided with that of pattern '**a**' (chromosome 1A) but the former was usually paler and thinner which assisted in distinguishing between them. Many cultivars had the thickest B subunit band (marked by * in Fig. 3.8) but this did not show mutual exclusiveness with any other bands. Further studies revealed that this band in cultivars Gabo and Chinese Spring represented two subunits one controlled by 1BS and other by 1DS (Gupta and Shepherd, 1987) and hence, it could not be assigned to a single group. Based on this information, it was assumed for simplicity that this band in other cultivars was also controlled by 1BS+1DS (though this may not have been true for all the cultivars) and hence, one band of similar mobility was included in the respective group 2 and 3 patterns of all the cultivars exhibiting it. This composite band sometimes joined the adjacent faster moving B subunit in group 3 pattern '**b**' and formed an even thicker band. The composite band was, however, missing from most of the cultivars having group 3 patterns '**c**', '**d**' and '**e**' and in some cases only a faint band was present in the background. The cultivars recommended as standards for each pattern are given in Appendix B.

The apparent occurrence of extensive heterogeneity in the size of HMW glutenin subunits and gliadins in bread wheat cultivars has been reported by using the one-dimensional SDS-PAGE system (Lawrence and Shepherd, 1980; Payne *et al.*, 1981a; Galili and Feldman, 1983a). (Since SDS-PAGE separates the proteins based on their size differences (Weber and Osborn, 1969), it is likely that the extent of variation recorded here in the LMW glutenin subunit composition among bread wheat cultivars is probably an underestimate. Separation based on both size and charge differences would reveal the maximum variation in these

proteins, but only one or two samples can be analysed by using such a system e.g. IEF/NEPHGE × SDS, so it cannot be used for direct comparison of banding patterns of several samples in a single gel.

The number of band combinations found in the LMW subunits of glutenin of hexaploid wheats is much lower than the expected number of such combinations on the basis of random association, indicating that genes coding for these bands are closely linked. Such close linkage has been demonstrated by Singh and Shepherd (1988a) who detected a low level of recombination between LMW subunits of glutenin on chromosome 1B. The exact genetic and molecular nature of variation of LMW subunits of glutenin is not yet known, but rare recombination and point mutation can generate new LMW subunit combinations. Moreover, the analysis of amino acid and nucleotide sequences of some HMW glutenin subunits has revealed the presence of repetitive sequences which differ in their size and number (Halford *et al.*, 1987b). If a similar situation pertains with LMW glutenin subunit genes, unequal crossing over between these repeats might produce new subunits and thus would contribute towards the multiplicity and variation of LMW subunits of glutenin. Such changes in the structure of these proteins will be retained and perpetuated probably because storage proteins are able to tolerate random mutations (Wrigley and Shepherd, 1973; Kasarda 1976a) since they are mainly used as an energy source during seed germination and hence their structural requirements may not be very stringent.

Considering the close genetic linkage between the genes coding for LMW subunits of glutenin and gliadins (Payne *et al.*, 1984e; Singh and Shepherd, 1984b, 1988a; Gupta and Shepherd, 1988), it will be expected that classification of bread wheat cultivars on the basis of LMW subunits of glutenin will be comparable to that based on gliadins at the *Gli-1* loci. It was found that the gliadin classification of 98 cultivars (determined by using starch gels at acid pH) by Wrigley *et al.* (1982b) corresponded to the LMW glutenin classification of this study. In the present study, the SDS-PAGE analysis of the gliadin compositon of some bread wheats showing LMW subunit differences revealed that they also contained differences in *Gli-1* alleles. Nevertheless, since gliadins and LMW subunits of glutenin are coded by different genes (Bartels *et al.*, 1986; Singh and Shepherd, 1988a), it is not essential that a change in LMW subunit genes would be accompanied by a similar change in gliadin genes.

Intra-varietal null phenotypes were detected in a few bread wheat cultivars namely, Bajio-66 and (MH3* Warimba) MH11 for chromosome 1B-controlled LMW subunits of glutenin. Since these cultivars also lacked all the gliadin bands at the *Gli-B1* locus, it is suspected that they arose due to deletion of either the whole chromosome arm 1BS or a segment of chromosome arm 1BS carrying these genes. India-115 lacked some C subunits and ω-gliadins controlled by chromosome 1D but the other LMW subunits were present (Gupta and Shepherd, 1988; Singh and Shepherd, 1988a) indicating that this probably represents a deletion of the genes controlling them. In contrast, many bread wheat varieties having chromosome 1A-associated null LMW phenotypes were found to have gliadins bands controlled by the *Gli-A1* locus e.g. Kite (Gupta *et al.*, 1989), Insignia (Gupta and Shepherd, 1988), Hope (Singh and Shepherd, 1988a) and since these loci are tightly linked with each other, it is expected that the genes coding for both LMW subunits and gliadins would be present. Hence, the gene on chromosome 1A associated with null LMW phenotype was more likely to be inactive than to be deleted. The possibility of a very small deletion involving only the LMW subunit gene cannot, however, be ruled out and in that case, the allocation of a null phenotype to an allele would be misleading. To determine, whether the null phenotype was due to a very small deletion or an inactive allele, comparison of nucleotide sequences will be required and the simple inheritance tests will not be sensitive enough to distinguish between them. Other studies have also reported the occurrence of null phenotypes for group 1 chromosome-controlled gliadins in bread and durum wheats (Wrigley and Shepherd, 1974; Damania *et al.*, 1983; Payne *et al.*, 1984c; Lafiandra *et al.*, 1987), but the exact cause is not known except that Payne *et al.* (1984c) showed that the null phenotype at *Gli-B1* locus was due to a terminal deletion on chromosome arm 1BS.

3.4.3 Variation of LMW subunits in tetraploid and diploid wheats and wheat relatives and the evolution of wheat

The survey of tetraploid wheats (30 genotypes) revealed that there was considerable variation in the LMW glutenin subunit composition among different cultivated and wild groups. Although only 11 durum wheats were analysed in the present study, the number of patterns controlled by chromosome 1B was more than obtained by other researchers among over 100

durum wheats by a 1-D method or 2-D method (Payne *et al.*, 1984b; Autran and Berrier, 1984; du Cros, 1987). They detected only two patterns (LMW–1 and LMW–2) controlled by this chromosome. Moreover, Payne *et al.* (1984b) reported that Duramba A and Durati both carried the same LMW–2 pattern but it is evident in the present study that they had two different patterns, '**d**' and '**k**', respectively. It is clear that the two-step separation method gives better resolution of the LMW glutenin bands than the methods used by the earlier workers.

Allelic variation has also been reported in HMW glutenin subunit patterns in cv. group *durum* (Vallega, 1986; Vallega and Mello-Sampayo, 1987; Vallega, 1988), cv. group *dicoccum* (Vallega and Waines, 1987) and wild var. *dicoccoides* (Levy and Feldman, 1988; Levy *et al.*, 1988) and some of the alleles including the null allele at the*Glu-A1* locus in bread wheat were also present in these tetraploid wheats. Furthermore, allelic variation at the *Glu-B1* locus was much greater that that at the *Glu-A1* locus. Similarly, the highest number of alleles have been reported at *Glu-B1* and *Gli-B1* loci coding for HMW glutenin subunits (Payne and Lawrence, 1983) and gliadins (Sozinov and Poperelya, 1980; Galili and Feldman, 1983a), respectively, in bread wheat cultivars. Examination of the LMW subunits of glutelin also revealed greater allelic variation at the *Glu-B3* locus than the *Glu-D3* and *Glu-A3* loci in both tetraploid and hexaploid wheats and thus all of these studies have provided supportive evidence to the hypothesis that the B genome in polyploid wheat is polyphyletic (Sarkar and Stebbins, 1956; Zohary and Feldman, 1962; Athwal and Kimber, 1972, Dennis *et al.*, 1980).

The alternative hypothesis of a monophyletic origin of the B genome in polyploid wheats (Riley, 1965), however, cannot be rejected if the B genome has undergone preferential changes during the evolution of polyploid wheats. As a result, genes on chromosome 1B coding for these storage proteins might have duplicated and diverged more extensively than the genes on chromosomes 1A and 1D and this would explain the observed difference in the extent of LMW glutenin subunit pattern variation on these chromosomes. Several lines of evidence have been cited to support the hypothesis that the B genome of polyploid wheats is monophyletic and possibly derived from *T. searsii* (see review by Kerby and Kuspira, 1987). Moreover, recently Vallega (1988) has provided additional biochemical evidence for *T. searsii* as being the B genome donor. He has shown that HMW glutenin subunits in

tetraploid and hexaploid wheats were quite distinct from those in all the putative donors of this genome except *T. searsii*. In the present study, the LMW glutenin subunit patterns from suggested donors of B genome viz. *T. speltoides*, *T. bicorne*, *T. sharonense*, *T. longissimum* also carried different banding patterns from those of the B genome in tetraploid and hexaploid wheats. Since only one or two accessions of these species were analysed, however, it cannot be generalised that they may not be related with the B genome in wheats and furthermore, *T. searsii* was not analysed in the study.

The LMW glutenin patterns of the putative A genome donors of polyploid wheats revealed that *T. monococcum* and *T. urartu* possessed very similar patterns. Other protein markers viz. albumins and gliadins (Konarev *et al.*, 1979) and HMW glutenin subunits (Galili *et al.*, 1988) of these two species were also very similar and thus supports that *T. urartu* and *T. monococcum* are the same species (Giorgi and Bozzini, 1969). Waines and Payne (1987), however, showed that HMW glutenin subunits of these two species were quite distinct from each other and considered *T. urartu* as a separate species, but since prolamins including HMW glutenin subunits are highly variable, distinction based solely on any one of these would be questionable. Konarev *et al.* (1979) and Galili *et al.* (1988) found that the band patterns of *T. urartu* resembled the bread wheat patterns more closely than those of *T. monococcum*. A similar observation was made with LMW glutelin subunits in the present study. These data thus support the cytological and biochemical evidence that *T. urartu* rather than *T. monococcum* is the source of the A genome in polyploid wheat (Chapman *et al.*, 1976; Nishikawa, 1983).

T. tauschii was proposed as the donor of the D genome to hexaploid wheat (Pathak, 1940) and this proposal has been substantiated by others on the basis of several lines of evidence (Kihara, 1944; McFadden and Sears, 1946; Riley and Chapman, 1960; Fernandez de Caleya *et al.*, 1976; Lawrence and Shepherd, 1980; Shewry *et al*, 1984c). The LMW glutenin band patterns of three accessions of this species revealed that the banding patterns of var. *strangulata* conformed very closely with patterns '**b**' and '**c**' in bread wheats. Lawrence and Shepherd (1980) also detected HMW glutenin banding patterns in var. *strangulata* which were commonly present in bread wheats. Jaaska (1980, 1984), on the basis of a series of isozyme studies, concluded that var. *strangulata* is the most likely donor of D genome to

hexaploid wheat. The present finding is thus consistent with these results and provides supportive evidence for *T. tauschii* var. *strangulata* as the D genome donor.

3.4.4 Evolution of genes controlling LMW subunits of glutelin

Since there were 5-8 LMW glutenin bands in *T. dicoccoides* and 5 in *T. tauschii*, bread wheats should have 10-13 bands. Infact, 7-14 bands were found in bread wheat cultivars, much as expected. Although a large proportion of bread wheat cultivars carried either a null phenotype or 1-2 bands encoded by chromosome 1A and others possessed only 1 or 2 bands, the A genome diploid species carried 2 to 4 bands. This would indicate that the activity of genes on this chromosome synthesizing LMW subunits of glutenin has been greatly reduced in hexaploid wheats. Galili *et al.* (1988) analysed 155 accessions of A genome for HMW glutenin subunits and showed that the *Glu-A1* locus in tetraploid and hexaploid wheats coded for many fewer HMW glutenin subunits than the *Glu-A1* locus in the diploid A genome wheats. A similar comparison between polyploid and the diploid wheat relatives containing B and D genomes did not show any significant differences in the bands controlled by the *Glu-B1* and *Glu-D1* loci. Thus, they suggested that a massive non-random diploidization and silencing of genes occurred on chromosome 1A (Galili and Feldman, 1983b, c). The findings from this study also indicate a similar phenomenon for LMW subunits of glutelin, but it is valid only within the limited sample of A genome species analysed.

Genetic diploidization can be brought about by deletion, spontaneous mutation or suppresion of genes at both intra- and inter-genomic levels (Galili and Feldman, 1983c; 1984a, 1985; Galili *et al.*, 1986). Although it has been shown that the HMW glutenin null phenotype controlled by the *Glu-A1* locus is due to insertion of a chain terminating codon in the gene (Thompson *et al.*, 1983), more recent data suggest that deletion in the –300 upstream region of the controlling element is the cause of this null phenotype (Halford *et al.*, 1987a). The cause for the null LMW subunit phenotype for chromosome 1A is not determined yet. It is also not known why the non-random process of diploidization has occurred so extensively on chromosome 1A. It was noted, however, that the A genome related LMW subunits of glutelin are produced in much greater amount (as inferred from their staining intensity) in the relevant diploid wheats than in durum and bread wheats. It is possible that the capacity of the

genes at this locus to produce a large amount of proteins may have caused overproduction of the proteins in the polyploid, so to balance the protein level and the efficiency, these redundant genes might have undergone rapid silencing resulting in the reduced number or intensity of the bands. Such a repression mechanism (dosage compensation or genomic interaction) has been implicated as an explanation for the reduction in number of HMW glutenin subunits in polyploid wheat (Galili and Feldman, 1984a; Galili *et al.*, 1986). An indication that altitude might be related to the occurrence of a HMW glutenin band at the *Glu-A1* locus in *T. turgidum* var. *dicoccoides* in Israel, has recently been reported (Levy and Feldman, 1988). It should be noted that null phenotypes for HMW as well as LMW subunits of glutelin controlled by chromosome 1A were detected in both hexaploid and tetraploid wheats including wild var. *dicoccoides* and this could indicate that the non-random diploidization might have happened at some stage in var. *dicoccoides* and was perpetuated in cultivated wheats.

The findings on the chromosomal location of genes controlling LMW subunits of glutelin in wheats and species related to wheat revealed one obvious similarity between them that they all carried genes for LMW subunits of glutelin on chromosomes belonging to group 1 chromosomes of Triticeae. In other words, chromosomes 1A, 1B, 1D in hexaploid wheats (AABBDD), chromosomes 1A and 1B in tetraploid wheats (AABB), chromosome $1S^l$ in *T. longissimum* (S^lS^l), chromosome 1U in *T. umbellulatum* (UU), chromosome 1E in diploid *Elytrigia elongata* (EE) and two chromosomes (arbitrarily called 1E', 1E") in decaploid *Elytrigia elongata* all carry genes for LMW subunits of glutelin. Gliadins and HMW glutenin subunits are also controlled by chromosome $1S^l$ (this study), 1U (Shepherd, 1973; Lawrence and Shepherd, 1981a), 1E (Lawrence and Shepherd, 1981a; this study), 1E' and 1E" (Koebner and Shepherd, unpublished data). These results thus support the homoeologous relationships between group 1 chromosomes of wheat and $1S^l$ (Netzle and Zeller, 1984), 1U (Athwal and Kimber, 1972) and 1E, 1E' and 1E" (Dvorak, 1980; 1981) and also suggested that all these chromosomes are derived from a common ancestral chromosome.

Evidence from amino acid and nucleotide sequences of HMW glutenin subunits (Shewry *et al.*, 1984c; Halford *et al.*, 1987b) and gliadins (Bietz *et al.*, 1977; Autran *et al.*, 1979; Shewry *et al.*, 1983b; Bartels *et al.*, 1986) have suggested that the different genes at the *Glu-1* or *Gli-1* loci were derived from one ancestral locus through duplication and divergence.

Limited molecular analyses on LMW subunits of glutenin (Okita *et al.*, 1985; Kasarda *et al.*, 1988) have also given similar indications. Duplication or amplification of the ancestral LMW glutenin gene for B and C subunits should have occurred very early in the evolution of wheat since the diploid wheats and *Elytrigia elongata* all already carried two or more LMW subunits of glutelin. Furthermore, the presence of B and C subunits in almost all the wheats and wheat related species analysed, their different isoelectric points (Jackson *et al.*, 1983) and different sizes suggest that they might be coded by two gene sub-families. The presence of the same C subunits with different B subunits and vice-versa between the patterns controlled by chromosomes 1B and 1D supports the hypothesis that they are coded by two different gene sub-families.

Although rye cultivars did not exhibit any LMW subunits of glutelin, there is evidence that Imperial rye carries a LMW subunit of glutelin which is controlled by chromosome arm 1RS (see Chapter 4 for details). The chromosomal location of the LMW subunit in *Secale montanum* R-15 could not be determined. The data support the homoeologous relationship between group 1 chromosomes of wheat and 1R of Imperial rye (Shepherd, 1973). A homoeologous relationship has also been suggested between chromosome 3R of Dakold rye and group 1 chromosomes of wheat, on the basis of its capacity to compensate for the loss of group 1 chromosomes in Kharkov wheat (Gupta, 1969; Lee *et al.*, 1969; Zeller, 1977), but these authors suggested that this was possibly due to a translocation between 3R and 1R. The present study has shown that the supposed 3R in substitution and addition lines has either the long arm 1RL or a segment of 1RL i.e. 3R represents 3R/1RL in these stocks. Since the data also revealed that chromosome 1R was normal in the addition line, this translocation might have occurred during the backcrosing of the amphiploid for the production of the 3R addition line which was used for isolating the 3R (1A) and 3R(1B) substitution lines. Hence, the substitution of group 1 chromosomes (with genes for LMW subunits of glutenin, gliadins and HMW glutenin subunits) by chromosome 3R is not due to the homoeology between chromosome 3R of Dakold rye and group 1 chromosomes of bread wheat but it is rather due to the presence of 1RL translocation on 3R.

3.4.5 Utility of LMW subunits of glutelin

In addition to providing information about the evolution of the Triticeae, the genes controlling seed proteins in wheat and its relatives are also of practical value. They may serve as useful protein markers for the identification of cultivars/varietal mixtures (Wrigley *et al.*, 1982a), as genetic markers for the identification of chromosomes and chromosome arms which can greatly facilitate the transfer and detection of alien genetic material in bread and durum wheat (Lawrence and Shepherd, 1981a; Koebner and Shepherd, 1986; Blanco *et al.*, 1987). The LMW subunits of glutelin will thus serve as additional protein (genetic) markers for these purposes. More importantly, seed storage proteins in wheat, viz. gliadins and HMW glutenin subunits have been shown to be significantly related to flour qualities of bread and durum wheat (see review by Payne, 1987; Chapter 2 for detail). Recent studies have shown that differences in the LMW glutenin subunit composition are also associated with dough qualities of bread and durum wheats (Autran *et al.*, 1987; Payne *et al.*, 1987a; Gupta and Shepherd, 1987; Gupta *et al.*, 1989). This suggests that the wide polymorphism in LMW subunits of glutelin described may be useful for improving the technological qualities of wheat flours. The LMW subunits of glutelin unique to tetraploid and diploid wheats may be used to widen the range of variation in LMW subunits of glutelin in bread wheats and similarly those unique to non-durum tetraploid wheat and diploid wheats may be used to increase the genetic variability for these subunits in durum wheats and eventually to improve their nutritional and technological qualities. HMW glutenin subunits from *T. speltoides* (Moonen and Zeven, 1985) and HMW glutenin subunits and gliadins from *T. umbellulatam* (Law and Payne, 1983; Rogers *et al.*, 1987) have already been transferred to bread wheats.

Considering the intensity and the number of LMW glutenin bands controlled by group 1 chromosomes, it appears that chromosome 1B contributes most to the amount and variability of LMW subunits and thus this may have an important bearing in determining the pasta- and bread-making quality of wheat flours because differences in the amount of LMW subunits of glutenin have been shown to be related to flour quality differences (Autran *et al.*, 1987). The LMW bands controlled by chromosome 1BS were found to be absent in some of the bread wheat cultivars analysed and they were suspected to carry 1B/1R substitution or translocation.

Studies have shown that 1B/1R substitution and translocation lines are associated with poor dough quality (Dhaliwal *et al.*, 1988), and it is possible that the loss of chromosome 1B-controlled LMW subunits of glutenin could be a contributing factor.

Chapter 4

INHERITANCE OF LMW SUBUNITS OF GLUTENIN IN BREAD WHEAT

4.1 INTRODUCTION

As reviewed in Chapter 2, the LMW subunits (B and C groups) of glutenin in hexaploid wheats are coded by genes at the *Glu-3* loci which are tightly linked with the *Gli-1* loci coding for ω- and γ-gliadins on the short arms of group 1 chromosomes. The present studies on the LMW subunits of glutenin have shown that these subunits exhibit extensive pattern variation in hexaploid wheats and that the patterns are controlled by genes on the short arms of group 1 chromosomes (see Chapter 3). The inheritance of the individual LMW subunits of glutenin in bread wheat cultivars, however, has not been studied in detail nor have the allelic relationships of the genes controlling these been elucidated. These aspects have been investigated in the present study using test-cross seeds.

To facilitate these studies, there was a need to find a third parent for use in test-crosses which did not contribute any bands overlapping those expected to segregate in the testcross progeny. A genetic stock referred to as a "Triple Translocation" was produced for this purpose and in the process three double translocation stocks were also isolated.

These stocks have also proved useful for determining the chromosomal location of the genes controlling the LMW subunits of glutenin in cultivars Chinese Spring and Gabo and they have drawn attention to another group of LMW subunits in bread wheat which are not controlled by group 1 chromosomes.

The data obtained from these various studies are presented in this chapter.

4.2 MATERIALS AND METHODS

4.2.1 Plant materials

The seeds of Chinese Spring (CS), CS nullisomic-tetrasomic lines 6A-6D, 6B-6D, 6D-6A, 6D-6B, CS ditelocentric lines 6AL, 6BL, 6DL, 1AL, 1BL and 1DL (Sears, 1954, 1966; Sears and Sears, 1978), CS-Imperial rye amphiploid (Driscoll and Sears, 1971), Imperial rye, 1AL-1RS translocation line [isolated from the progeny of a cross between CS-Hope 1A and CS-Imperial rye 1R̂ (1A) substitution lines, Singh and Shepherd, 1988a], CS 1DL-1RS translocation and CS 1R (1B) substitution lines (Shepherd, 1973), Gabo and Gabo-Imperial rye single translocations, 1BL-1RS, 1DL-1RS and the double translocation 1BL-1RS/1DL-1RS (Shepherd, unpublished) used in this study were kindly provided by Dr. K. W. Shepherd.

The bread wheat parents used for test-crosses included Chinese Spring, Gabo, Orca, Insignia and biotypes Norin-61A and Norin-61B, differing in LMW glutenin subunit patterns.

4.2.2 Crossing procedures

F_1 seeds were produced from crosses between the single translocation 1AL-1RS and double translocation Gabo 1BL-1RS/1DL-1RS and then planted to provide F_2 seeds for isolating the three double and one triple translocation stocks.

Test-cross analysis was used to examine the inheritance of LMW glutenin subunits and the allelic relationship of the genes controlling them among bread wheat cultivars. Five cultivars with contrasting LMW glutenin subunit pattern were chosen as parents and intercrossed to give three different F_1 hybrids. In another F_1 hybrid, Norin–61B was crossed to the "Triple translocation stock" which lacked all the major LMW glutenin subunits. These F_1 hybrids were then crossed as the female parent to the Triple translocation stock so that all of the bands expected to segregate in the F_1 gametes could be scored without ambiguity in the test-cross seeds. The following test-cross combinations were produced;

(Chinese Spring × Orca) × Triple translocation stock

(Norin–61B × Triple translocation stock) × Triple translocation stock

(Chinese Spring × Norin–61A) × Triple translocation stock

(Gabo × Insignia) × Triple translocation stock.

4.2.3 Protein extraction and electrophoresis

The procedures followed for the extraction of endosperm proteins, their separation by one-dimensional and two-step electrophoresis and for staining and destaining of gels were the same as described in Chapter 3. The gel dimensions for the 1-D SDS-PAGE used to separate unreduced proteins were 120 × 100 × 1.5mm and electrophoresis was carried out at a constant current of either 40 or 50 mA per gel for about 2 hours.

A two-dimensional SDS-PAGE was also employed in this study and it was based on the method of Wang and Richards (1974) as modified by Singh and Shepherd (1985) except in the present work slab gels were used for both dimensions. In the first dimension, unreduced seed protein extracts were run for about 1.5 hour in normal SDS-PAGE (1.2 mm thick 10% acrylamide gel) and the whole slot was then cut out and transferred into an equilibration solution containing 2% 2-ME for protein reduction (see Chapter 3 for details on equilibration). Thereafter, the slot was loaded horizontally onto another 10% acrylamide gel with slightly greater thickness (1.5mm) for the run in the 2nd dimension, in order to re-electrophorese the reduced proteins in a direction at right angles to that of the first dimension. The second run was carried out at a constant current of 40mA/gel for about 2 h.

4.2.4 Cytology

Standard Feulgen squashes were used to analyse the chromosome configurations at metaphase-I of meiosis in pollen mother cells (pmcs) of these translocation stocks. Individual anthers at the required stage of meiosis, were selected and fixed in 3 absolute ethanol : 1 glacial acetic acid for 24 hours at 4°C. The anthers were hydrolysed in 1N HCl at 60°C for 15 minutes and then stained with Feulgen reagent for approximately 45 minutes at room temperature, before being squashed in 45% acetic acid for microscopic examination.

4.2.5 Gene symbols used for seed proteins in bread wheat and rye

Payne *et al.* (1982b) originally assigned the symbol *Gli-1* to the genes controlling LMW subunits of glutenin and ω- and γ-gliadins. Singh and Shepherd (1988a) introduced a new gene symbol *Glu-3* specifically for LMW subunits of glutenin in bread wheat because they observed rare recombination between the genes controlling gliadins and LMW subunits of glutenin and also because LMW subunits of glutenin form disulphide-linked aggregates. The *Glu-3* symbol has now been accepted by the Wheat Gene Symbol Committee (McIntosh, 1988) and it has also been adopted in the present study.

The loci coding for HMW secalins and ω- and γ-secalins in rye have been designated *Sec-3* and *Sec-1*, respectively, by Shewry *et al.* (1984b) whereas the genes coding for triplet bands in wheat were designated *Tri-1*, by Singh and Shepherd (1988a).

The allele symbols for the genes controlling LMW glutenin subunits and ω- and γ-gliadins have been given an italicised, single letter suffix to the gene symbol (eg. *Glu-A3a*, *Gli-A1a*). When proteins coded by these alleles are described, they are given in normal print (eg. Glu-A3a). This system of allelic designation has been preferred over the one given by Singh and Shepherd (1988a) using the initial letter (s) of the cultivars possessing them, because it has more general application.

4.2.6 Genetic analysis

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

$$S_p = \sqrt{[\ p(1-p) \div n\ ]} \quad \text{.....(i)}$$

Where no recombinants were detected between two protein markers, in the test-cross progeny, the upper limit (at the 95% confidence limit) for the recombination fraction (p) was calculated using the method of Hanson (1959).

$$p = [\ 1-(0.05)^{-n}\ ] \quad \text{.....(ii)}$$

where, n = number of euploid progeny analysed

4.3 RESULTS

The data presented in this chapter are arranged under two main headings. The first is related to the production of wheat-rye Triple and double translocation stocks, their characteristics and phenotypes in two-step electrophoresis. The second part deals with the inheritance of LMW subunits of glutenin and gliadins in bread wheats, using this stock.

4.3.1 Production and characteristics of double and Triple 1RS translocation stocks

4.3.1.1 Isolation procedure

Since Imperial rye did not appear to have any LMW bands of glutenin (Chapter 3), it was decided to substitute the short arms of 1R for the short arms of the group 1 chromosomes of wheat thought to be responsible for the synthesis of LMW subunits of glutenin to produce a stock having no LMW subunits for use in genetic analysis. This was achieved by using the crossing and selection scheme outlined in Fig. 4.1. F_1 was heterozygous for rye chromosome arm 1RS, wheat chromosome arm 1AS from Gabo and 1BS and 1DS from Chinese Spring. The unreduced protein extracts from F_2 seeds were screened by SDS-PAGE and the gliadin (Gli-1) and Triplet (Tri-1) bands known to be controlled by the short arms of group 1 chromosomes (Shepherd, 1968; Singh and Shepherd, 1985) were used to select out homozygous double and Triple translocations (Fig. 4.1). The γ-gliadin band Gli-A1 produced by chromosome arm 1AS in Gabo (band shown as Δ, Fig. 4.2, slot b) is similar in mobility to one of the secalin bands, Sec-1, produced by chromosome arm 1RS (~~band marked by Δ, Fig. 4.2, slot f~~) and thus could not be scored in the segregating progeny. However, the gliadins Gli-B1, Gli-D1 produced by chromosome arms 1BS and 1DS, respectively, and Triplet bands Tri-A1 and Tri-D1 produced by 1AS and 1DS, respectively, were easily scored. Progeny seeds lacking Tri-A1 and Gli-B1 bands were selected as the putative double translocation (DTr) 1AL-1RS/1BL-1RS. Similarly, progeny lacking Tri-A1, Tri-D1 and Gli-D1 bands were selected as putative DTr 1AL-1RS/1DL-1RS. The seeds lacking all the Triplet bands (Tri-A1, Tri-D1) and gliadins (Gli-B1, Gli-D1) were selected as a Triple translocation (TTr) 1AL-1RS/1BL-1RS/1DL-1RS. The F_3 seeds from these putative translocations were

Figure 4.1. Procedure used to produce double and triple wheat-rye translocation stocks in mixed backgrounds of Gabo and Chinese Spring. The F_1 triple translocation heterozygote carried chromosome arm 1AS from Gabo and 1BS and 1DS from Chinese Spring. In the F_2 and F_3 generations, only the progeny genotypes which were selected as putative translocations are shown in the figure. Protein markers (in brackets) located on the chromosome arms 1AS (Tri-A1, Gli-A1), 1BS (Gli-B1), 1DS (Tri-D1, Gli-D1) and 1RS (Sec-1) were used for selecting these translocation stocks. Tri-1 = triplet bands, Gli-1 = gliadin bands, Sec-1 = secalin bands.

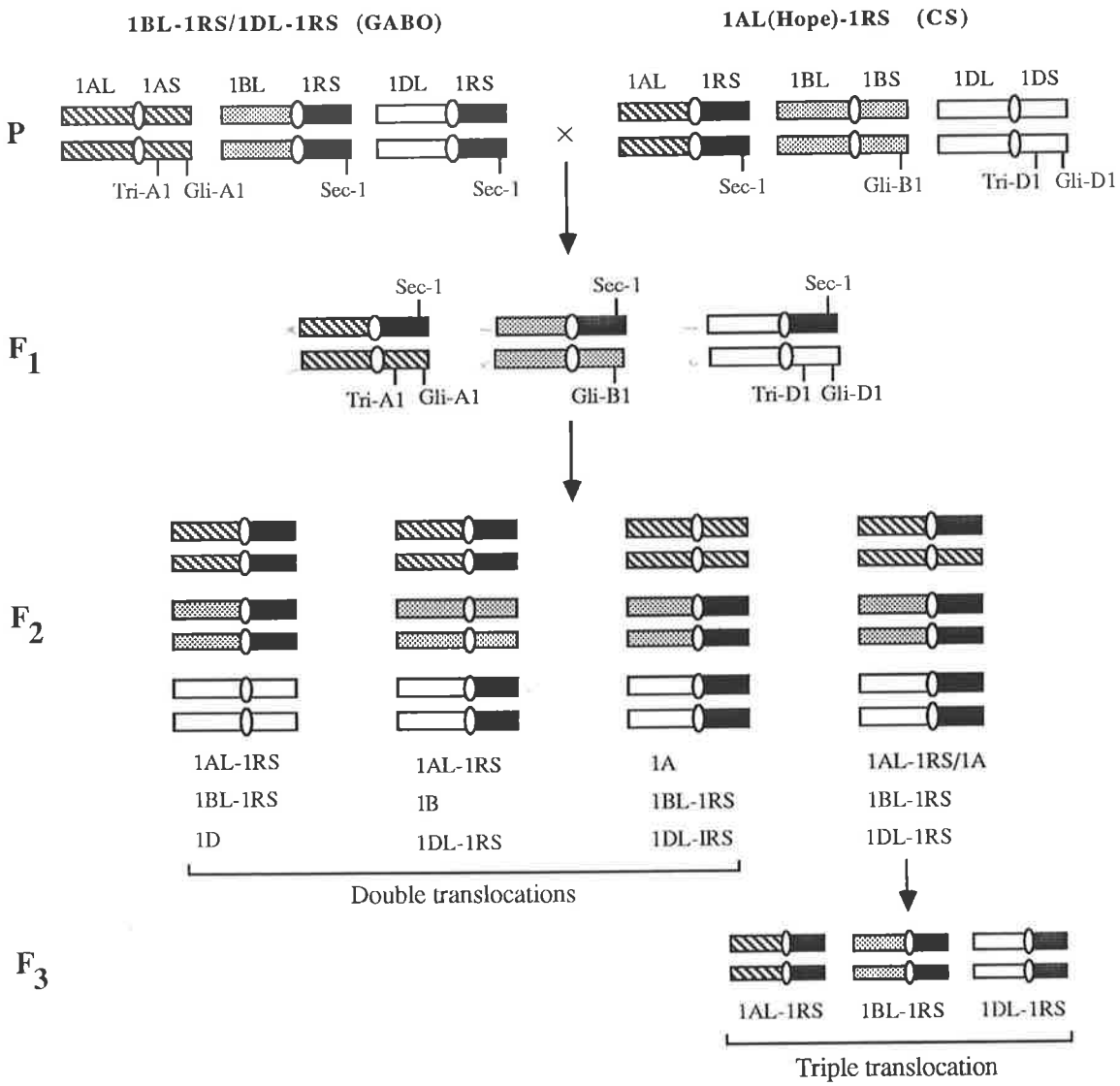

1BL-1RS/1DL-1RS (GABO)
1AL(Hope)-1RS (CS)
P
1AL 1AS 1BL 1RS 1DL 1RS
Tri-A1 Gli-A1 Sec-1 Sec-1
×
1AL 1RS 1BL 1BS 1DL 1DS
Sec-1 Gli-B1 Tri-D1 Gli-D1
F1
Sec-1 Sec-1 Sec-1
Tri-A1 Gli-A1 Gli-B1 Tri-D1 Gli-D1
F2
1AL-1RS 1BL-1RS 1D
1AL-1RS 1B 1DL-1RS
1A 1BL-1RS 1DL-IRS
1AL-1RS/1A 1BL-1RS 1DL-1RS
Double translocations
F3
1AL-1RS 1BL-1RS 1DL-1RS
Triple translocation

Figure 4.2. One-dimensional SDS-PAGE patterns of SDS-extracts of unreduced endosperm proteins from wheat parents and homozygous double (DTr) and triple (TTr) translocation stocks. Parents (a) Chinese Spring, (b) Gabo, and translocations (c) DTr 1BL-1RS/1DL-1RS, (d) DTr 1AL-1RS/1DL-1RS, (e) DTr 1AL-1RS/1BL-1RS and (f) TTr 1AL-1RS/1BL-1RS/1DL-1RS.

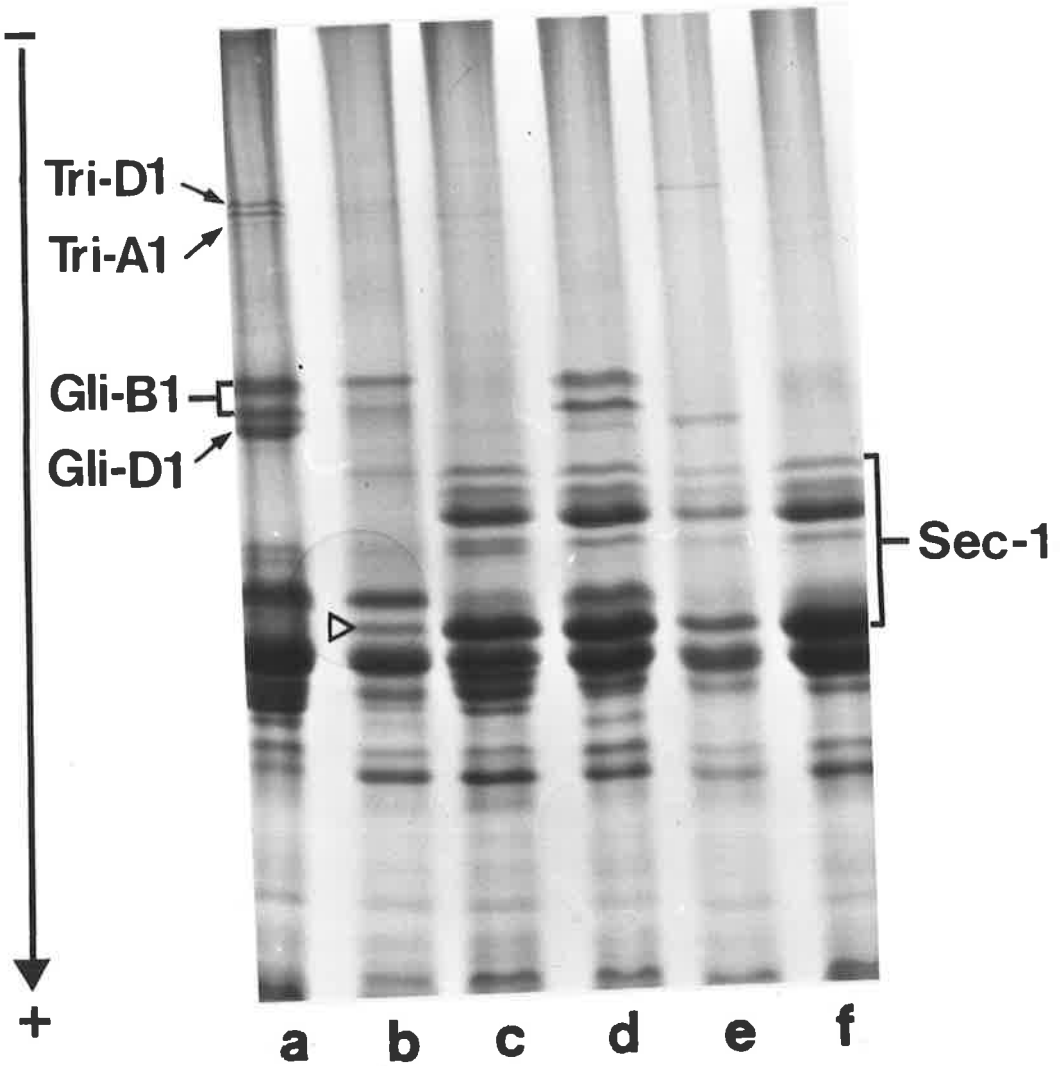
Symbols used in the figure:

Δ = a gliadin band in Gabo controlled by chromosome arm 1AS (Gli-A1) which could not be scored due to its overlap with one of the Sec-1 bands.

Tri-A1, Tri-D1 = triplet bands controlled by chromosome arms 1AS, 1DS, respectively

Gli-B1, Gli-D1 = gliadin bands controlled by 1BS and 1DS, respectively

Sec-1 = secalin bands controlled by chromosome arm 1RS.


−
Tri-D1
Tri-A1
Gli-B1
Gli-D1
Sec-1
+
a
b
c
d
e
f

tested for the same markers to confirm their homozygosity. The homozygous Triple translocation was not obtained in the F_2 generation, but was isolated in the progeny of an F_2 selection which was heterozygous for 1AL-1RS/1A and homozygous for 1BL-1RS and 1DL-1RS. The unreduced protein phenotypes of homozygous double and Triple translocation stocks (F_3 seeds) are shown in Fig. 4.2.

4.3.1.2 Cytology

From 30 to 120 pollen mother cells (pmcs) from homozygous F_3 plants of these translocation stocks were analysed for their chromosomal configurations at metaphase 1 (Fig. 4.3). All the pmcs analysed from these stocks contained 42 chromosomes (Fig. 4.3, a, b and c) and formed multivalents as expected (Fig. 4.3, b and c). The DTr stocks formed 14-17% quadrivalents whereas the TTr stock showed almost equal proportions (25%) of quadrivalents and hexavalents (Table 4.1). These stocks also showed few univalents. Two pmcs from Triple translocation stock contained single trivalents.

Table 4.1 Frequency of multivalents at metaphase 1 in pollen mother cells (pmcs) of wheat-rye translocation lines (F3 plants)

Wheat-rye translocation	No. of pmcs analysed	Frequency of multivalents	
		quadrivalents	hexavalents
1AL-1RS/1BL-1RS	120	20	0
1AL-1RS/1DL-1RS	110	14	0
1BL-1RS/1DL-1RS	30	5	0
1AL-1RS/1BL-1RS/1DL-1RS	50	13	14
1BL-1RS/1DL-1RS (Gabo)	110	14	0

4.3.1.3 Plant morphology

The plant and spike morphology of the translocation lines and the Chinese Spring and Gabo controls are shown in Fig. 4.4. Plants were grown singly in 15 cm pots (diameter) during the spring of 1987 in the glass house for general observations on their plant morphology and fertility. These stocks resembled Chinese Spring more than the Gabo parent in growth behaviour, spike morphology (Fig. 4.4) and maturity (time to spike emergence = 70 to 85 days). Spikes of the double translocation 1AL-1RS/1BL-1RS line (Fig. 4.4, e), however,

Figure 4.3. Chromosome configurations at metaphase I in pollen mother cells (pmcs) of homozygous triple translocation stock (F3 plant). Pmcs showing (a) 21^{II}, (b) $19^{II} + 1^{IV}$ and (c) $18^{II} + 1^{VI}$. Q = Quadrivalent, H = Hexavalent

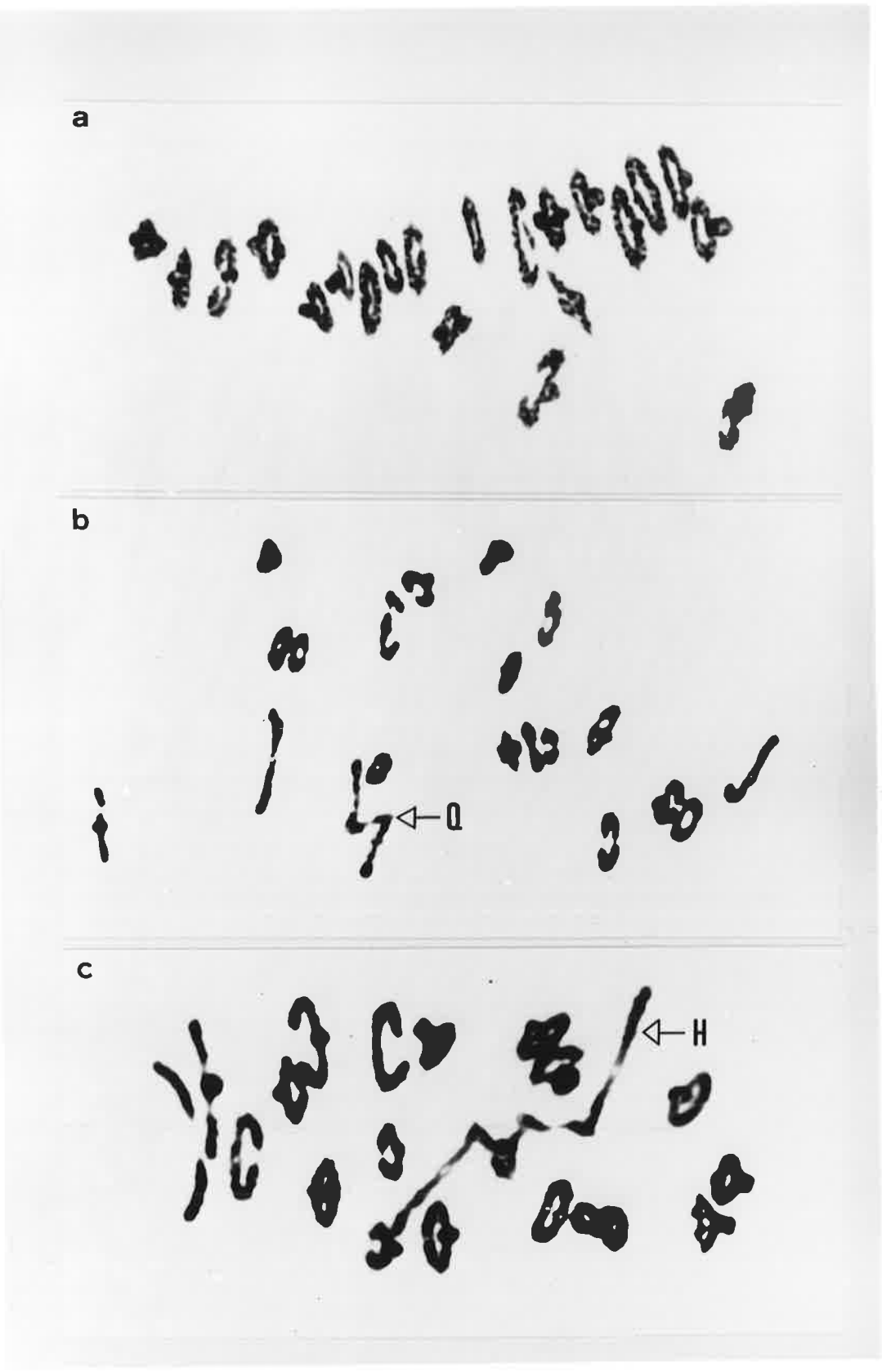
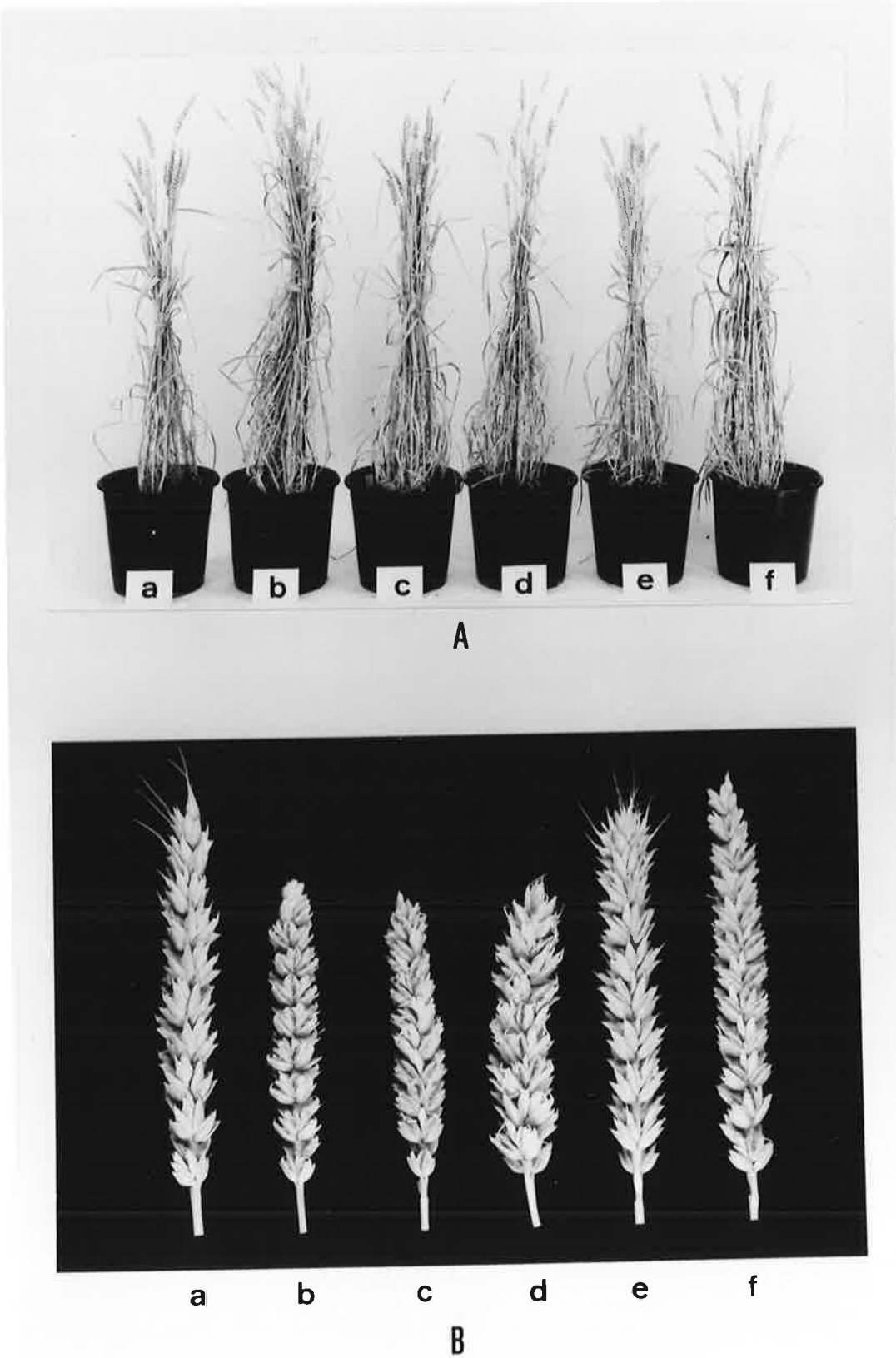

a
b
Q
c
H

Figure 4.4 A, B. Plant (**A**) and spike (**B**) morphology of the parental cultivars (a) Gabo, (b) Chinese Spring, and double (DTr) and triple translocation (TTr) stocks (c) DTr 1BL-1RS/1DL-1RS, (d) DTr 1AL-1RS/1DL-1RS, (e) DTr 1AL-1RS/1BL-1RS and (f) TTr 1AL-1RS/1BL-1RS/1DL-1RS. The photographs show mature plants and spikes.


a
b
c
d
e
f
A
a
b
c
d
e
f
B

were awned and compacted at the apex. In general, these stocks were fertile with vigorous vegetative growth and thus the short arm of 1R showed partial compensation for the loss of the short arms of chromosomes 1A, 1B and 1D, showing homoeologous relationship between group 1 chromosomes of wheat and rye (Shepherd, 1973; Lawrence and Shepherd, 1981a).

Table 4.2 Plant characters of parents and novel translocation stocks

Stocks	No. of plants analysed	Spikes/ plant	Spikelets/ spike	Seeds/ spikelet
Chinese Spring	1	42	14.8	1.6
Gabo	2	15	14.4	2.1
1AL-1RS/1BL-1RS	2	38	15.5	1.8
1AL-1RS/1DL-1RS	2	25	15.3	0.8
1BL-1RS/1DL-1RS	1	35	16.2	1.0
1AL-1RS/1BL-1RS/1DL-1RS	2	25	17.7	1.1

4.3.1.4 LMW glutenin subunit (B and C subunits) composition

The nullisomic-tetrasomic (NT) and ditelocentric stocks of Chinese Spring had been utilized earlier for locating the genes controlling the LMW subunits of glutenin in Chinese Spring (Jackson *et al.*, 1983; Singh and Shepherd, 1985). With these stocks, the chromosomal location of the genes controlling LMW subunits could be determined by comparing the protein phenotype of nullisomic-tetrasomic lines lacking one chromosome pair of a homoeologous group (eg. NT 1A-1B missing chromosome pair 1A) with the euploid having a normal chromosomal complement (ie. with all the chromosomes 1A, 1B and 1D). These comparisons would detect any bands which were uniquely controlled by these chromsomes providing their electrophoretic mobilities were different. Similarly, the chromosome arm location of a LMW band could be determined by using ditelocentric lines. These lines could not be used, however, to determine the chromosome/chromosome arm location of those LMW subunits which were similar in mobility but of different genetic control. This problem was encountered by Singh and Shepherd (1985) and they could determine the chromsomal controls of only five of the LMW subunits of glutenin in Chinese Spring using the two-step separation procedure. Some of these overlapping bands, however, could be separated by

using two-dimensional electrophoretic methods (IEF/NEPHGE × SDS) and Jackson *et al.* (1983) were able to locate the genes controlling nine of the B and C subunits to group 1 chromosomes but they could not assign the genes controlling some of the C subunits.

The wheat-rye Triple and double translocation stocks provide a new approach for locating genes controlling LMW subunits with either unique or overlapping electrophoretic mobility, onto the short arms of group 1 chromosomes of wheat. This is because the Triple translocation stock lacks all of the chromosome arms 1AS, 1BS and 1DS, thought to be responsible for the synthesis of all LMW subunits of glutenin (Jackson *et al.*, 1983), whereas the double translocation stocks carry just one of these arms at a time (eg. 1AL-1RS/1BL-1RS has only 1DS and lacks 1AS and 1BS). Thus comparison of the protein phenotypes of these stocks would give a better indication of the LMW bands controlled by the chromosome arm present in the DTr stocks than obtained with the nullisomic-tetrasomic and ditelocentric stocks. The Triple translocation stock would also give definite evidence on whether all the LMW subunits of glutenin in bread wheat are controlled by the short arms of group 1 chromosomes as suggested by Jackson *et al.* (1983). Since these translocation stocks have been produced in mixed backgrounds of Chinese Spring and Gabo, involving chromosome arm 1AS from Gabo and arms 1BS and 1DS from Chinese Spring, the single translocation stocks lacking 1BS (1BL-1RS) and 1DS (1DL-1RS) in Gabo and 1AS (1AL-1RS) in Chinese Spring were also required to allocate the LMW subunits of glutenin to group 1 chromsomes in these cultivars. The 70% ethanol extracts from the endosperm of these stocks as well as Chinese Spring, Gabo and Imperial rye were fractionated by two-step SDS-PAGE and the results are described for Gabo and Chinese Spring separately below.

Gabo

Gabo carries 12 LMW subunits (six B and six C subunit) of glutenin (Fig. 4.5, slot l). Three of these bands (two B and one C) were absent in the Gabo 1BL-1RS stock (Fig. 4.5, slot a) while Gabo 1DL-1RS (Fig. 4.5A, slot b) lacked two other bands (one B and one C) and showed reduced staining intensity in two C subunits. Analysis of the double translocation Gabo 1BL-1RS/1DL-1RS (Fig. 4.5, slot c) lacking both chromosome arms 1BS and 1DS, indicated that these 7 bands were also lacking in this stock and thus confirmed their genetic control by genes on these chromosome arms. Three other bands (marked by →, Fig. 4.5,

Figure 4.5. Two-step 1-D SDS-PAGE patterns of endosperm proteins extracted in 70% ethanol from (a) Gabo 1BL-1RS, (b) Gabo 1DL-1RS, (c) Gabo 1BL-1RS/1DL-1RS, (d) Chinese Spring(CS)-Hope 1AL-1RS, (e) F_1 between Gabo 1BL-1RS/1DL-1RS and CS-Hope 1AL-1RS and the F_2-derived double (DTr) and triple translocation stocks (TTr), namely (f) DTr 1AL-1RS/1BL-1RS, (g) DTr 1AL-1RS/1DL-1RS, (h) DTr 1BL-1RS/1DL-1RS, (i) Triple translocation 1AL-1RS/1BL-1RS/1DL-1RS and rye cultivar (j) Imperial and wheat cultivars (k) Chinese Spring and (l) Gabo.

Symbols used in the figure;

< band controlled by 1BS in Chinese Spring (suspected to be a D subunit)

» usually separated as a single band in Chinese Spring

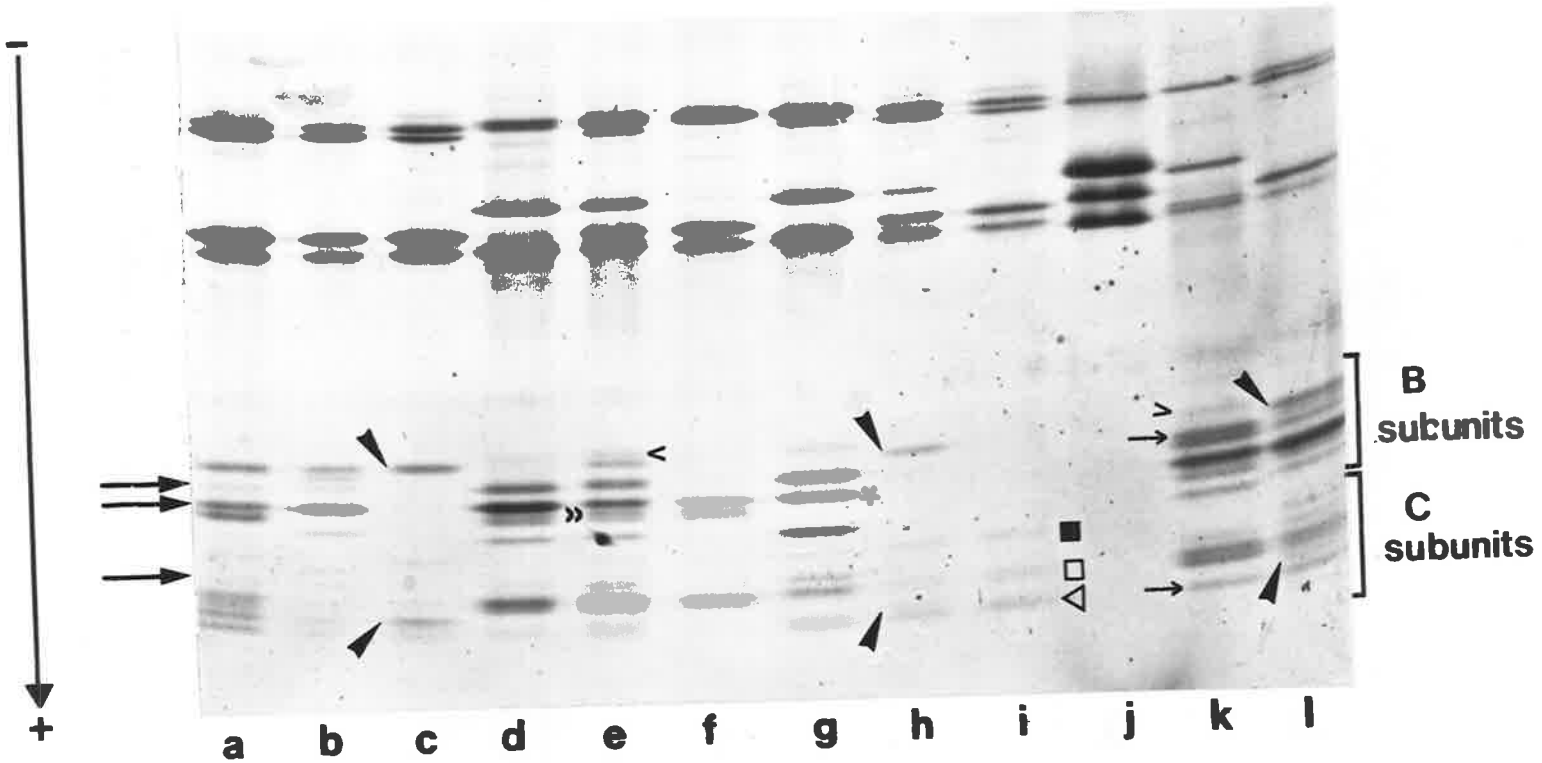
* overlapping bands controlled by 1BS and 1DS in Chinese Spring,

→ = bands controlled by 1AS in Chinese Spring,

⟶ = bands controlled by 1BS and 1DS in Gabo,

➤ = bands controlled by 1AS in Gabo,

Δ, ❑ and ■ = bands controlled by chromosomes other than 1AS, 1BS and 1DS of Chinese Spring and Gabo


−
+
a
b
c
d
e
f
g
h
i
j
k
l
B
subunits
C
subunits

slot a), which were present in both of the single translocation stocks, were also missing and this indicated that each of these bands consisted of at least two bands with the same mobility, one controlled by 1BS and the other by 1DS. Thus, chromosome arms 1BS and 1DS in Gabo control 6 and 7 LMW subunits, respectively.

The presence of five LMW bands (2 dark and 3 faint bands) in the Gabo 1BL-1RS/1DL-1RS line (Fig. 4.5, slot c) indicated that they were not controlled by 1BS and 1DS. Initially, it was thought that they might be controlled by 1AS present in this stock, but three of these were also present in the Triple translocation stock (Fig. 4.5, slot i) indicating that they must have been controlled by other chromosome(s). The two bands which were absent from the TTr stock (marked by ➤ in Fig. 4.5, slot c) were controlled by 1AS of Gabo.

Thus, Gabo carried at least 15 LMW glutenin bands (8 B and 7 C subunits) which were controlled by genes on chromosome arms 1AS, 1BS and 1DS, respectively. Three pairs of these bands coincided in mobility and thus only 12 separate LMW bands (6 B and 6 C subunits) were separated in Gabo (Fig. 4.5, slot l).

Chinese Spring

Chinese Spring exhibited 10 LMW bands (Fig. 4.5, slot k, the band marked by > was considered to be the slowest LMW band) whereas Imperial rye (Fig. 4.5, slot j) did not show any LMW bands. Two of the Chinese Spring subunits (shown by →, Fig. 4.5, slot k) were controlled by chromosome arm 1AS because the slower band of these (B subunit band) was missing while the faster one (C subunit) showed reduced staining intensity in the CS 1AL-1RS stock (Fig. 4.5, slot d). To determine whether the remaining 9 LMW bands (including the one overlapping the C subunit controlled by 1AS) of Chinese Spring were controlled by chromosome arm 1BS and 1DS, the banding patterns of Double and Triple translocation stocks involving these arms were compared. It is important to note that the bands present in these lines were transmitted from the F_1 hybrid (Fig. 4.5, slot e) produced between CS 1AL-1RS and Gabo 1BL-1RS/1DL-1RS. The 9 bands of normal Chinese Spring were seen as 10 bands in CS 1AL-1RS and in the F_1 because one of the bands was resolved into two subunits as marked by » in the F_1 seed (Fig. 4.5, slot e).

Eight of the 10 Chinese Spring bands present in the F_1 (Fig. 4.5, slot e) were absent and one band showed reduced staining intensity (band marked ❑) in the TTr stock 1AL-

1RS/1BL-1RS/1DL-1RS (Fig. 4.5, slot i) and the DTr stock 1BL-1RS/1DL-1RS (Fig. 4.5, slot h). This indicated that these bands were controlled by genes on chromosome arms 1BS and 1DS of Chinese Spring (the fastest moving C subunit of Chinese Spring and another two LMW bands, however, were present in the TTr stock, suggesting that these were controlled by arms other than 1AS, 1BS and 1DS). Six bands (including the one overlapping the band marked by ❑ in TTr stock, Fig. 4.5, slot i) were present in the DTr stock 1AL-1RS/1DL-1RS (Fig. 4.5, slot g) having chromosome arm 1BS and similarly, 4 bands were present in 1AL-1RS/1BL-1RS (Fig. 4.5, slot f), having chromosome 1DS. Two of these bands controlled by 1BS and 1DS had common mobility (marked by *, Fig. 4.5, slots f, g) and hence were regarded as one band and were absent only when both arms 1BS and 1DS were missing simultaneously (see Fig. 4.5, slots h, i). Because of this overlap, the chromosomal control of this compound band could not have been determined by using nullisomic-tetrasomic or ditelocentric lines which lack only one chromosome or chromosome arm at a time.

It should be noted that one C subunit in Chinese Spring (marked by →, Fig. 4.5, slot k) also showed a response to change in the dosage of chromosome arm 1DS (Fig. 3.13A, slots g, h, i; Fig. 3.18A, slots g, h) indicating that this arm is involved with its control and thus in Chinese Spring, it coded for at least 5 LMW subunits. Chromosome arm 1BS coded for 6 LMW subunits but only five of them were considered to be the B and C subunits of glutenin because one band (marked by <, Fig. 4.5, slot k) was not always seen and its sporadic occurrence resembled the D subunits designated by Jackson *et al.*, (1983) which are now regarded as unusual gliadins (Payne *et al.*, 1988). Thus, a total of 12 B and C subunits were controlled by chromosome arms 1AS (2), 1BS (5) and 1DS (5) in Chinese Spring and because 3 pairs of these bands had the same mobility, only 9 LMW subunits could be scored in Chinese Spring (Fig. 4.5, slot k).

4.3.2 Further analysis of the LMW prolamin bands present in the Triple translocation stock

4.3.2.1 Genetic control

The slowest moving of the three LMW prolamin bands in the Triple translocation stock (band marked by ■ Fig. 4.5, slot i) was not present in Chinese Spring or Gabo but was present

only in those stocks which carried rye arm 1RS and it was more intense when 1RS was present in 4 or 6 doses viz. double and Triple translocation stocks (Fig. 4.5, slots c, f, g, h, i) indicating that it was controlled by genes on this arm. Arm 1RS in these stocks was derived from Imperial rye but this band was not clearly visible in Imperial rye probably because it was produced in only small amounts when just 2 doses of 1RS are present. Bands with similar mobility to the remaining two bands were, however, present in both Chinese Spring and Gabo and moreover a faint band remained in the background when bands of similar mobility controlled by the short arms of group 1 chromosomes in these cultivars were absent (Fig. 4.5, slots c, e).

To investigate the genetic control of these bands in Chinese Spring, nullisomic-tetrasomic and ditelocentric lines of group 6 chromosomes were analysed (Fig. 4.6). These particular stocks were chosen for analysis because all the prolamins in wheat apparently are controlled by group 1 and group 6 chromosomes (see Shepherd, 1988 for a review). The two-step banding patterns of these stocks showed that the staining intensity of the fastest LMW band in Chinese Spring (marked by a Δ) was much reduced in the nullisomic-tetrasomic stocks 6D-6A, 6D-6B (Fig. 4.6, slots b, e) as well as in the ditelocentric stock 6DL (Fig. 4.6, slots c, f). These results indicated that this band in Chinese Spring was controlled by a gene(s) located on chromosome arm 6DS, this conclusion was confirmed as this band became darker when chromosome 6D was in tetrasomic condition viz. NT stock 6B-6D (Fig. 4.6, slots a, g). On the basis of similarity in mobility, the LMW band controlled by 6DS in Chinese Spring probably corresponded to the band in TTr stock (marked by Δ, Fig. 4.5, slot i). Although an adjacent band slower in mobility than the 6DS-controlled band (marked by ❑, Fig. 4.6) showed a response in staining intensity with change in dosage of chromosome 6D, this was not so clear probably because in this gel, two other bands (much darker) controlled by 1BS and 1DS were identical in mobility. The mobility of this band corresponded to one of the bands in the TTr stock (marked by ❑, Fig. 4.5, slot i). The removal of the other chromosomes of this homoeologous group viz. 6A (not shown) and 6B (Fig. 4.6, slots a, g) did not show any marked effect on any of the LMW prolamin bands in Chinese Spring.

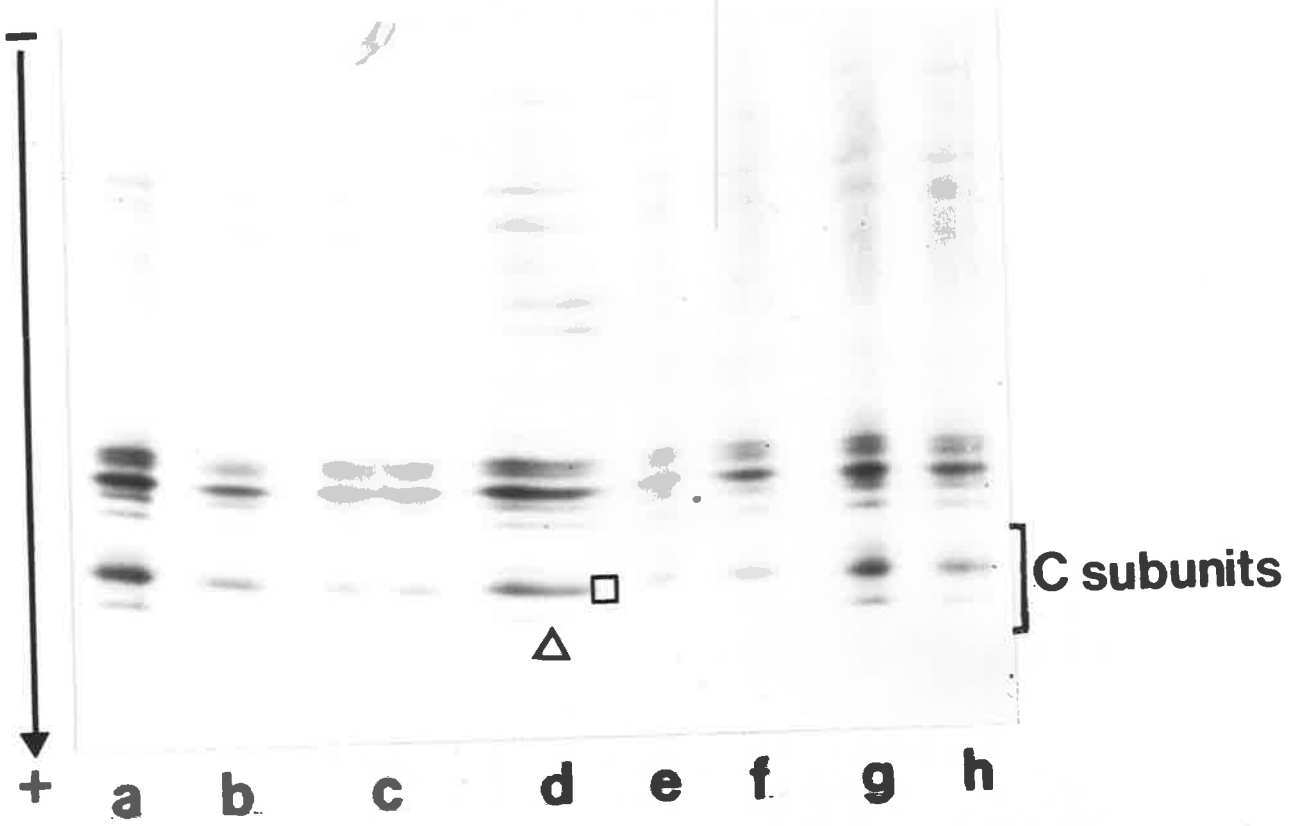
Figure 4.6. Two-step SDS-PAGE patterns of endosperm proteins extracted in 70% ethanol from Chinese Spring (CS) euploid and aneuploid (Nullisomic-tetrasomic = NT, ditelocentric = Dt) stocks, (a) NT 6B-6D, (b) NT 6D-6A, (c) Dt 6DL, (d) CS (e) NT 6D-6B, (f) Dt 6DL, (g) NT 6B-6D and (h) CS.

Symbols used in the figures;

Δ = band controlled by 6DS,

~~■ = band controlled by 1RS~~,

❑ = band possibly controlled by 6DS,


−
+
a
b
c
d
e
f
g
h
C subunits

4.3.2.2 Two-dimensional SDS-PAGE patterns

The discovery that some of the prolamin bands resolved in two-step SDS-PAGE were also controlled by chromosome arms 1RS and 6DS raised some questions requiring further investigation, e.g. whether these proteins were disulphide-linked prolamins (glutelins) or monomeric prolamins (gliadins, secalins) associated in some manner with glutelin molecules. Although the separation of these bands in two-step gels suggested that they were disulphide-linked proteins, the diagonal (unreduced × reduced) electrophoresis procedure (Wang and Richards, 1974) was employed to analyse these bands further. In this procedure, the formation of spots or parallel lines off the diagonal are taken as evidence that proteins occur as the disulphide-linked aggregates and it has been shown that the LMW and HMW subunits of glutenin, which are disulphide-linked aggregates (Beitz and Wall, 1973; Payne and Corfield, 1979; Graveland *et al.*, 1982), are separated as parallel lines (Singh and Shepherd, 1985).

The ethanol-soluble extracts from the Triple translocation stock and Chinese Spring were analysed by diagonal electrophoresis and the Triple translocation stock exhibited three parallel lines. Because of very heavy loading of proteins in the first dimension, some γ-gliadins appeared to have diffused with the parallel lines (Fig. 4.7 A, d). Nevertheless, these parallel lines were not formed as a result of diffusion of the proteins, as they did not extend beyond the origin and they ran only toward one side of the diagonal line. These parallel lines appeared to be similar in electrophoretic mobility to the three prolamin bands detected in this stock in two-step electrophoresis. The parallel line marked by ■ (Fig. 4.7) corresponded to the 1RS-controlled LMW prolamin band whereas the parallel line marked by Δ corresponded to the 6DS-controlled band in Chinese Spring. The remaining band (marked by ❑) was also present in Chinese Spring. In Chinese Spring itself (Fig. 4.7A, a), however, these two bands were much darker because it contained additional overlapping bands (C subunits controlled by group 1 chromosomes) in this region.

Thus, these prolamin bands (including 1RS- and 6DS-controlled bands) are capable of forming disulphide-linked aggregates. Since proteins which are soluble in ethanol and are disulphide-linked, have been regarded as subunits of glutelin (Shewry *et al.*, 1986b), these bands can also be considered as the LMW subunits of glutelin. Hence, it can be concluded that the majority of the LMW subunits of glutenin in bread wheats are controlled by group 1 chromosomes but at least one LMW subunit (C subunit) is also controlled by a group 6

Figure 4.7. SDS-PAGE separation of endosperm proteins extracted in 70% ethanol.

A. Two dimensional (a, d) and one-dimensional (b, c) patterns of (a, b) Chinese Spring and (c, d) Triple translocation stock.

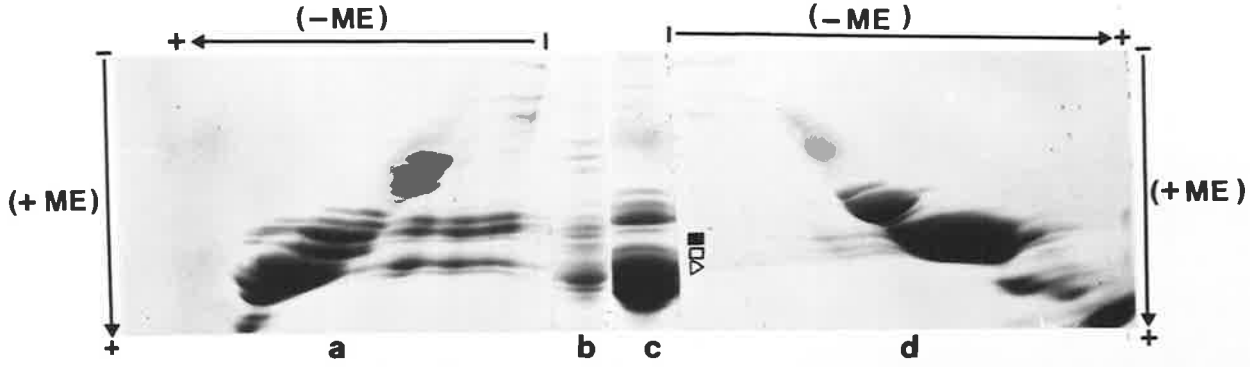
B. Two-dimensional pattern of the Triple translocation stock.

Symbols used in the figures:

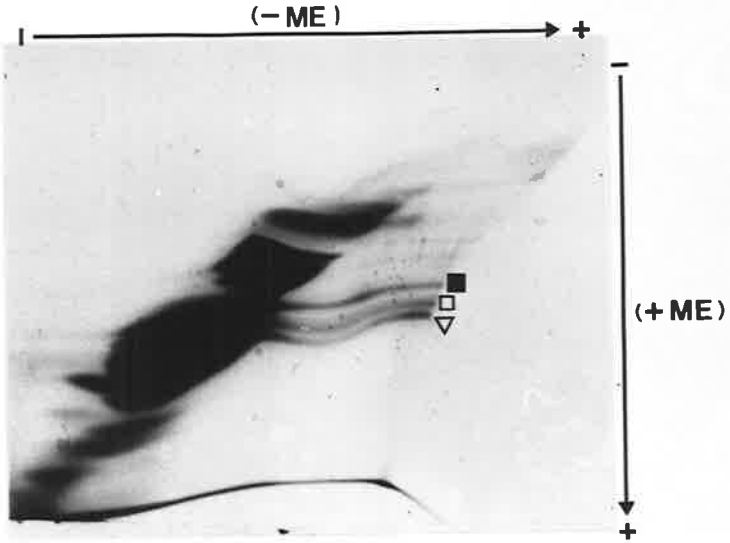
Δ = band controlled by 6DS,

■ = band controlled by 1RS,

❑ = band possibly controlled by 6DS,



A



B

chromosome (6D). Chromosome arm 1RS in Imperial rye also codes for a LMW glutelin band. These data are summarised in Table 4.3 and in the diagram showing the LMW subunits of glutenin in Chinese Spring and Gabo and their chromosomal control (Fig. 4.8).

Table 4.3 Chromosome arm location of the genes controlling LMW subunits of glutelin in bread wheat and cereal rye

Cultivars	Number of LMW subunits of glutenin				
Chromosome arm →	1AS	1BS	1DS	6DS	1RS
Chinese Spring wheat	2	5	5	1	–
Gabo wheat	2	6	7	?	–
Imperial rye	–	–	–	–	1

4.3.3 Inheritance of LMW subunits of glutenin and gliadin bands in bread wheat cultivars

Seeds from four different test-crosses were analysed to determine the inheritance and the allelic relationships of genes controlling individual LMW subunits of glutenin in bread wheats. Four different wheat cultivars, Chinese Spring, Gabo, Orca and Insignia together with two biotypes Norin-61 A and Norin-61B were used in these studies. The inheritance of gliadin differences in these cultivars/biotypes was also studied. It is emphasized that only those LMW bands which were present consistently in the two-step patterns have been considered as LMW glutenin subunits (B and C subunits). The LMW prolamin bands appearing only sporadically were excluded from the analysis since such bands have been assumed to be the D subunits of glutenin. A single band of this nature has been recorded in Chinese Spring (section 4.3.1.4.2) and Insignia (section 4.3.3.3). It has been shown that D subunits of glutenin (Jackson *et al.*, 1983) can become associated occasionally with the glutenin complex through non-covalent forces (Payne *et al.*, 1988) and they have recently been re-classified as gliadins and the loci coding for them have been renamed *Gli-3* instead of *Glu-2* (Payne, loc. cit.). Their occasional appearance with glutenin subunits in the present study may have been due to the variable methods used for extracting the endosperm proteins. For example, incubation at 50-60°C for 5-10 hours in 70% ethanol may not always have

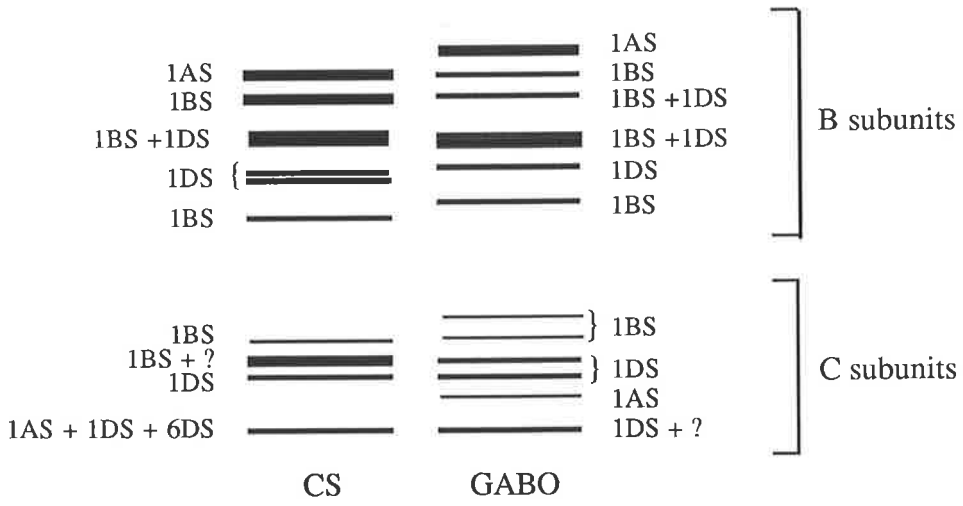


Figure 4.8. Diagrammatic representation of the B and C subunits of Chinese Spring and Gabo. The thickest band in Chinese Spring and Gabo has occasionally been fractionated into two and the slower component is controlled by 1DS and the faster component by 1BS.

disrupted the non-covalent associations between these bands and glutenin complex. The test-cross seeds were analysed only for B and C subunits of glutenin and gliadins and the data are presented as below.

4.3.3.1 Chinese Spring and Orca

The two-step banding patterns of Orca and Chinese Spring exhibited 9 and 8 LMW glutenin bands (Fig. 4.9A, slot e, f), respectively. Certain bands in Orca were sometimes resolved into two (eg. the bands marked by →, Fig. 4.9B, slot f). The Triple translocation stock used as the third parent in the test-crosses lacked almost all of the LMW glutenin subunits (Fig. 4.9, slot g) so that the bands segregating from the F_1 could be scored without much overlap in the test-cross seeds.

As described in Chpater 3, examination of the LMW subunits of glutenin of a large collection of bread wheat cultivars showed that some bands or band combinations were mutually exclusive and that they could be assigned to three groups. The LMW subunit patterns of Chinese Spring were termed **'a'** in all three groups whilst those of Orca were 1d, 2d and 3e (Fig. 4.9E). The band patterns in Chinese Spring are controlled by genes on chromosome arms 1AS, 1BS and 1DS respectively (see section 4.3.2). To facilitate the interpretation of the inheritance data, the bands within each of these groups have been labelled (>, Δ and ▲, respectively, Fig. 4.9) and are presented diagrammatically in Fig. 4.9E. Four photographs (Fig. 4.9A, B, C, D) showing the segregation of these patterns in the test-cross seeds are given because each of these gels showed slightly different resolution of these bands. For example, the 1a band in Chinese Spring (marked by > , Fig. 4.9A, slot f) overlapped one of the 2d bands in Orca (slot e) but these two bands differed in mobility in Fig. 4.9C (see bands indicated by >). Overlapping bands were characterized by greater staining intensity and thickness (Fig. 4.9A, slot b, Fig. 4.9, slots a, d).

Inheritance data from the test-cross seeds showed that the bands within each of these patterns were inherited as a unit indicating that the genes controlling them were tightly linked. Analysis of the joint segregation of these patterns taken in pairs revealed that group 1 patterns segregated independently of the patterns in group 2 ($\chi 2$= 3.7, P > 0.25) and group 3 ($\chi 2$= 6.9, P > 0.05) and similarly group 2 patterns were inherited independently of group 3 patterns ($\chi 2$= 5.66, P > 0.1). The data were consistent with a 1: 1: 1:1 test-cross ratio. In

Figure 4.9. Two-step SDS-PAGE patterns of proteins extracted in 70% ethanol from test-cross [(Chinese Spring × Orca) × Triple translocation stock] and the parental seeds.

A. (a-d, h-j) test-cross seeds, (e) Orca, (f) Chinese Spring and (g) Triple translocation stock.

B. (a-d) test-cross seeds, (e) Chinese Spring and (f) Orca.

C. (a) Chinese Spring, (b) Orca, (c) Triple translocation stock and (d-g) test-cross seeds.

D. (a-d) test-cross seeds, (e) Chinese Spring and (f) Orca.

E. Diagrammatic presentation of the B and C subunits of Chinese Spring and Orca divided into three inheritance groups.

Symbols used in figures;

OR = Orca, CS = Chinese Spring, TTr = Triple translocation stock

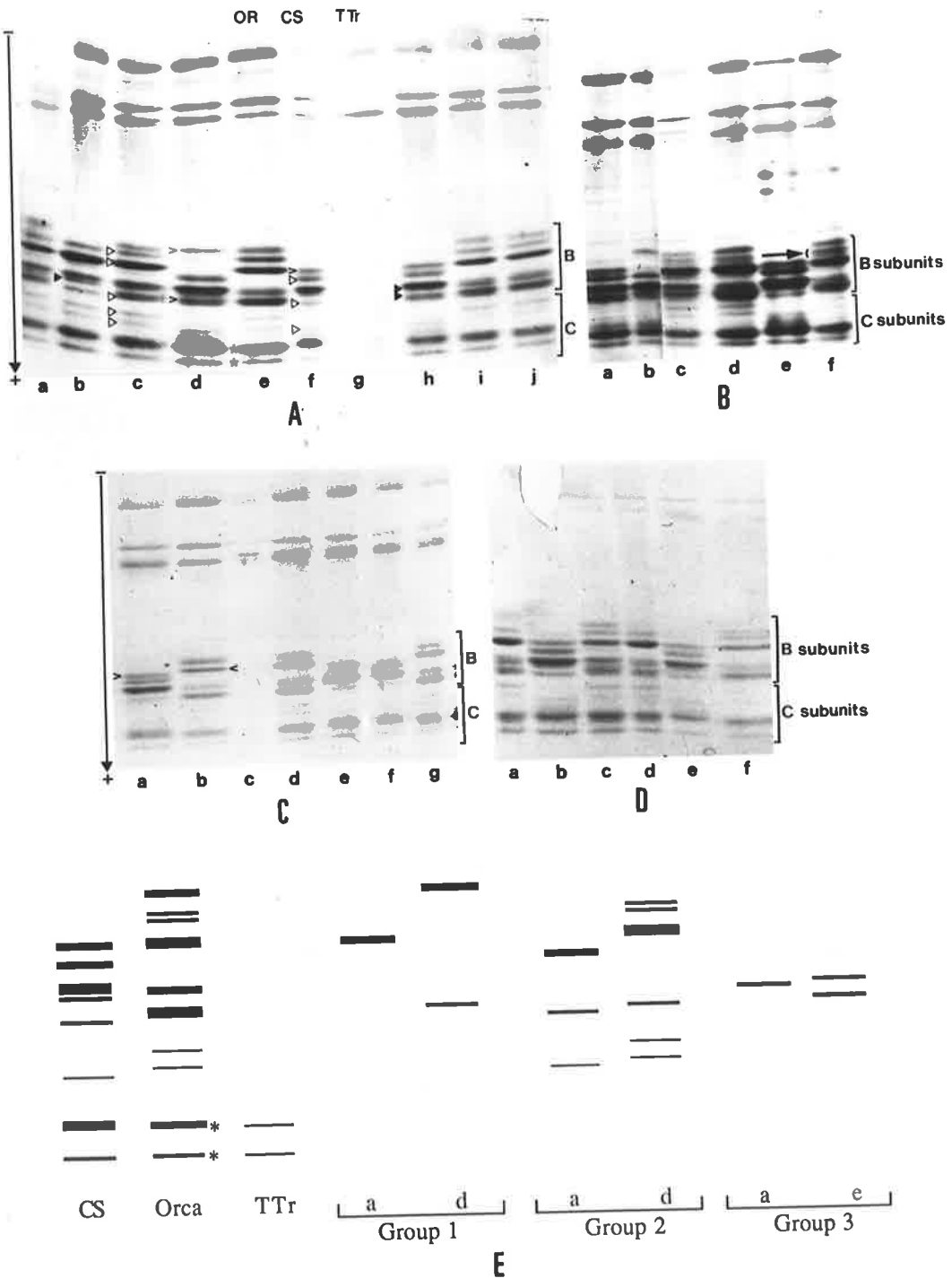
* bands common in both Orca and Chinese Spring and could not be scored in the test-cross seeds.

→ = doublet bands which separated as a single band in Figure 4.7, A, C and D .

> bands controlled by 1AS,

Δ = bands controlled by 1BS,

▲ = bands controlled by1DS.


OR
CS
TTr
B
C
a b c d e f g h i j
A
a b c d e f
B
B subunits
C subunits
C
a b c d e f g
D
a b c d e f
CS
Orca
TTr
a d
Group 1
a d
Group 2
a e
Group 3
E

contrast, the patterns within a group were as inherited alternatives in the test-cross ratio of 1:1 except for one seed which lacked both patterns '**a**' and '**d**' from group 2 (Table 4.4).

All the test-cross seeds possessed either parental pattern 1a or 1d (bands shown by >, Fig. 4. 9A, slots d, h, respectively) and either 3a or 3e (see bands marked ▲ in patterns 3a and 3e in Fig. 4.9A, slots b and h, respectively). The absence of any non-parental band combination suggested that the genes controlling these patterns within a group were either located at the same locus and were allelic to each other or, that the genes were located at two loci which were closely linked in repulsion phase and were inherited as alleles. In the absence of any detectable recombination, the genes controlling patterns 1a and 1d and similarly 3a and 3e in pairs have been considered to be alleles. Since the genes controlling pattern 1a and 3a in Chinese Spring were known to be located on chromosome arms 1AS and 1DS, respectively, patterns 1d and 3e in Orca were concluded to be controlled by genes on the 1AS and 1DS arms. Hence, the genes controlling patterns 1a and 1d have been given the allelic designations *Glu-A3a* and *Glu-A3d* and similarly genes for patterns 3a and 3e have been designated alleles *Glu-D3a* and *Glu-D3e,* respectively. It should be noted that certain C subunits in Orca (marked with ★, Fig. 4.9A, slot e) had the same mobilities as the C subunits in Chinese Spring and thus could not be scored separately in the test-cross seeds. Nevertheless, because these C subunits were controlled by chromosome arm 1DS in Chinese Spring, it was assumed that they were also controlled by 1DS in Orca.

Table 4.4 Segregation of LMW subunits of glutenin among 107 seeds from [(Chinese Spring × Orca) × Triple translocation stock] test crosses

LMW glutenin pattern	Observed frequency		$\chi 2$ value	Probability
	Parental type	Nonparental type*	(1 : 1)	(df=1)
a : d (group 1)	52 : 55	0	0.08	0.7 – 0.8
a : d (group 2)	46 : 60	1	1.84	0.1 – 0.2
a : e (group 3)	45 : 62	0	2.70	0.1 – 0.2

* The nonparental type was excluded from the analysis

Band patterns 2a and 2d (Fig. 4.9E) in these cultivars also segregated as alternatives in the test-cross seeds (the bands in patterns 'd' and 'a' are marked by Δ in Fig. 4.9A, slot c and

slot f, respectively) consistent with a 1:1 ratio except that one seed contained neither parental pattern (Table 4.4). Examination of the gliadin patterns by 1-D electrophoresis and HMW glutenin subunit pattern by the two-step method revealed that this seed also lacked those HMW subunits of glutenin and gliadins from Chinese Spring and Orca (figure not shown) controlled by the long and short arm of chromosome 1B. This indicated that the test-cross seed lacked chromosome 1B and this could have been due to deficiency of this chromosome in the gamete transmitted from the F_1 parent. It was concluded from this evidence that pattern 2d in Orca was controlled by genes on chromosome 1B. Furthermore, the genetic control of pattern 2a by 1BS in Chinese Spring indicated that pattern 2d was also controlled by genes on 1BS and hence the genes encoding them have been designated alleles *Glu-B3a* and *Glu-B3d,* respectively.

The unreduced protein phenotypes of the parents and test-cross seeds were determined by 1-D SDS-PAGE and this revealed that Orca and Chinese Spring also differed for some gliadins (Fig. 4.10A, slots f and g) which (particularly ω-gliadins) are clearly seen in Fig. 4.10B (slots a and b). Since the Triple translocation stock (Fig. 4.10A, slot k and Fig. 4.8B, slot c) revealed no gliadin bands overlapping the parental gliadins, the latter could also be scored in the test-cross seeds without difficulty. The segregation patterns of the gliadin differences in the same test-cross seeds were compared with those obtained for the LMW subunits of glutenin and it was found that all the test-cross seeds possessing LMW glutenin patterns Glu-A3a, Glu-B3a and Glu-D3a also exhibited the same gliadin bands [shown as > (Fig. 4.10A, slot g), Δ (Fig. 4.10A, slot r) and → (Fig. 4.10A, slot m), respectively]. Because of their co-inheritance, these gliadins have been designated Gli-A1a, Gli-B1a and Gli-D1a respectively by using the same letter symbols given for the LMW glutenin band patterns ie. 'a' in Chinese Spring and the gene symbol *Gli-1* for gliadins introduced by Payne *et al.*, (1982b). In the same way, the LMW glutenin patterns Glu-A3d, Glu-B3d and Glu-D3e in Orca always segregated with the gliadin band patterns termed Gli-A1d (Fig. 4.10A, slot d), Gli-B1d (Fig. 4.10A, slot q) and Gli-D1e (Fig. 4.10A, slot l), respectively. These results indicate that there is a tight linkage between the genes controlling these gliadins and LMW glutenin subunit patterns on chromosome arms 1AS, 1BS and 1DS in these cultivars. The absence of any recombinants amongst 107 test-cross seeds sets an upper limit of 2.7% for the recombination fraction between the *Glu-3* and *Gli-1* loci (at the 95% confidence limit,

Figure 4.10 A, B. One-dimensional SDS-PAGE patterns of unreduced proteins extracted in 70% ethanol from the test-cross [(Chinese Spring × Orca) × Triple translocation stock] and the parental seeds.

A. (a-e, l-r) test-cross seeds, (f) Orca, (g) Chinese Spring (k) Triple translocation stock and Chinese Spring ditelocentric lines (h) Dt 1AL, (i) Dt 1BL and (j) Dt 1DL. Gliadin bands Gli-A1, Gli-B1 and Gli-D1 are shown by >, Δ and →, respectively.

B. Test-cross parents (a) Orca, (b) Chinese Spring and (c) Triple translocation stock. Note that gliadin bands Gli-A1d and Gli-D1e are clearly separated in Orca.

Orca
CS
TTr
Gli-A1d
Gli-A1a
Gli-D1e
Gli-D1a
Gli-B1d
Gli-B1a
-
+
a b c d e f g h i j k l m n o p q r
A
Gli-D1e
Gli-A1d
a b c
B

Hanson, 1959). Moreover, the gliadin patterns Gli-A1a, Gli-A1d; Gli-B1a, Gli-B1d and Gli-D1a, Gli-D1e were inherited as alternatives within a pair and did not show any recombinant phenotypes indicating that they were controlled by tightly linked or allelic genes. Furthermore the inheritance of the different gliadin bands as a unit within each of these patterns indicated them to be controlled by closely linked genes.

4.3.3.2 Biotypes of cultivar Norin-61

4.3.3.2.1 Norin-61B

Since all of the 222 bread wheat cultivars analysed carried some LMW subunits of glutenin with overlapping mobilities in the two-step gels, it was not possible to study the inheritance of all the LMW glutenin bands from any two cultivars by producing an F_1 hybrid between them. Thus, a test-cross [(Norin-61B × Triple translocation stock) × Triple translocation stock] was made so that the inheritance of all the LMW subunits of glutenin in Norin-61B could be studied among test-cross seeds without overlap of bands. It should be noted that LMW subunits controlled by genes on the short arms of group 1 would be inherited as blocks because recombination was not expected to occur due to lack of pairing between these wheat chromosome arms and homoeologous rye arm 1RS (Koebner and Shepherd, 1986; Singh and Shepherd, 1988b).

The ethanol soluble protein extracts from 122 test-cross seeds were analysed by two-step SDS-PAGE in order to determine the segregation patterns of LMW subunits of glutenin (Fig. 4.11A). The segregation data revealed that the 10 LMW subunits of glutenin in Norin-61B were inherited in three groups [marked by >, Δ and ➤, respectively (Fig. 4.11A) and shown diagrammatically in Fig.4.11B]. It should be noted that when the C subunits of Norin-61B (Fig. 4.11B) from group 3 were missing in the test-cross seeds, a faint band in the backgrounds was still present (indicated by *, Fig. 4.11A, slot d). Up to four C subunits were found to be present in the test-cross seeds which lacked the bands in these three groups. These bands were presumably group 6-controlled C subunits from the Triple translocation stock and Norin-61B.

The joint segregation ratio of LMW subunits of glutenin in group 1 and group 3 and similarly of bands in group 2 and group 3 indicated that these segregated independently of

Figure 4.11. Two-step SDS-PAGE patterns of proteins extracted in 70% ethanol from test-cross [(Norin-61B × Triple translocation stock) × Triple translocation stock] and the parental seeds.

A. (a-g) test-cross seeds, (h) Orca, (f) Norin-61B and (i) Triple translocation stock.

B. Diagram showing the B and C subunits of Norin-61B divided into three inheritance groups.

C. One-dimensional SDS-PAGE patterns of test-cross seeds (a-f, i-t), (g) Norin-61B and (h) Triple translocation stock.

Symbols used in figures;

NR= Norin-61, TTr = Triple translocation stock,

> bands in group 1,

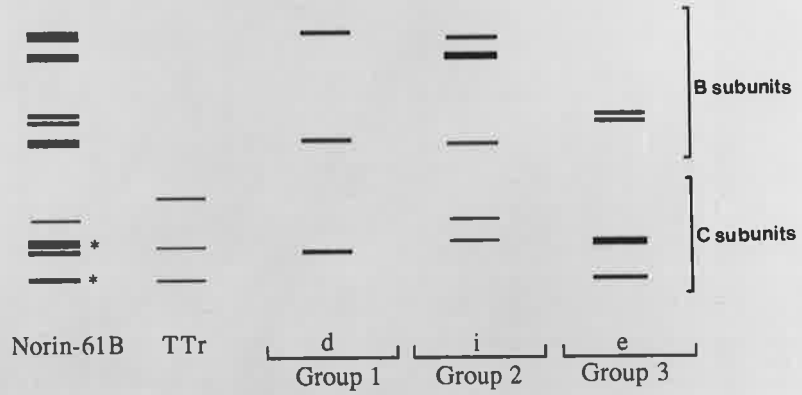
Δ = bands in group 2,

➤ = bands in group 3,

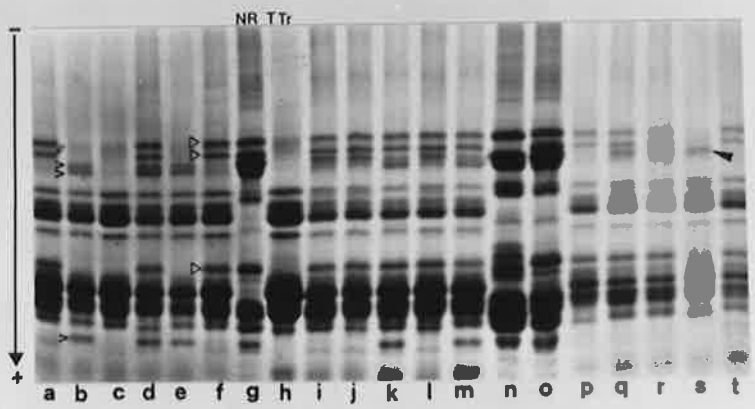
* bands not included in group 1, 2 or 3.


NR TTr
B subunits
C subunits
+
a b c d e f g h i j

A


B subunits
C subunits
Norin-61B
TTr
d
Group 1
i
Group 2
e
Group 3

B


NR TTr
+
a b c d e f g h i j k l m n o p q r s t

C

each other in pairs (Table 4.6, $\chi 2 = 4.86$, $P > 0.1$ and $\chi 2 = 4.35$, $P > 0.1$, respectively). In contrast, the observed joint segregation ratio of LMW subunits of glutenin in group 1 and

Table 4.6 Joint segregation of LMW subunits of glutenin among 122 test-cross seeds from [(Norin-61B × Triple translocation stock) × Triple translocation stock]

LMW subunit patterns (x) (y)	Observed frequency x y	x –	– y	– –	$\chi 2$ value (1 : 1: 1: 1)	Probability (df= 3)
Group 1 : Group 2	18	39	36	29	8.54	0.02-0.05
Group 1 : Group 3	26	28	41	27	4.86	0.1-0.25
Group 2 : Group 3	27	30	40	25	4.35	0.1-0.25

group 2 differed significantly from the expected two factor test-cross ratio of 1: 1: 1: 1 ($\chi 2 = 8.54$, $p < 0.05$). Partition of this $\chi 2$ value (Table 4.7) showed that this deviation was not due to the individual segregation ratios of group 1 and group 2 bands but was due to an excess of progeny with one or other of the group 1 and group 2 bands and a deficiency of those with both (Table 4.6), thus simulating linkage. A similar but non-significant trend occurred in the other two comparisons (Table 4.6).

Table 4.7 $\chi 2$ value for individual segregation (presence vs absence) of group 1 and group 2 bands and their independence with each other

LMW glutenin subunits in groups	$\chi 2$ value (1 : 1)	Probability (df= 1)
Group 1	1.60	0.2-0.3
Group 2	0.52	0.3-0.5
Group 1 : Group 2	6.42	0.01-0.02

Since the group 1 and group 2 markers did show segregation in the progeny, they could not be on the same chromosome arm and therefore could not be genetically linked. Moreover, patterns in group 1 and group 2 corresponded to patterns 1d and 2i in Orca and Norin-61A (see next section) which were controlled by chromosome arms 1AS and 1BS, respectively.

Thus, the significant linkage almost certainly was the result of the formation of quadrivalents due to pairing between the common rye arm 1RS joined to each of two group 1 chromosomes (1A, 1B). Whenever these quadrivalents would show a linear orientation at metaphase I (or as in Fig. 4.3b), an obligatory segregation of the wheat chromosome arms 1AS and 1BS to opposite poles would result, thus contributing to their non-independent segregation.

Gliadin patterns of the test-cross parents and test-cross seeds were also analysed as given in Fig. 4.11C. A total of nine bands was found to be segregating in the test-cross seeds and these could be divided into three groups labelled as group 1 (bands marked by >, Fig. 4.11C, slot b), group 2 (bands indicated by Δ, Fig. 4.11C, slot f) and group 3 (bands shown by ➤, Fig. 4.11C, slot s). As expected, these gliadins segregated as unit with the LMW subunits of glutenin in the appropriate three groups corresponding to the three group 1 chromosome arms segregating in this material.

In order to determine the allelic relationship of the above patterns with those of Chinese Spring, Norin-61 was crossed to Chinese Spring to produce an F_1 hybrid but the chosen seed was found to be an intra-varietal variant (Norin-61A). Its protein phenotype differed from biotype B for the LMW bands present in group 1 and group 3. It exhibited the pattern 1e, 2i and 3d instead of 1d, 2i and 3e.

4.3.3.2.2 Norin-61A

Forty test-cross seeds, produced by crossing the F_1 (Chinese Spring × Norin-61A) as female to the Triple translocation stock, were analysed by two-step SDS-PAGE. The joint segregation tests showed independent inheritance (a test-cross ratio of 1:1:1:1) of the Norin-61A and Chinese Spring band patterns: group 1 to group 2, $\chi 2 = 1.6$, $P > 0.5$; group 1 to group 3, $\chi 2 = 2.0$, $P > 0.5$ and similarly group 2 to group 3, $\chi 2 = 1.0$, $P > 0.75$. The band patterns within a group, viz. 2i and 2a (marked by Δ, Fig. 4.12A, slots g and i, respectively) and pattern 3d or 3a (marked by ▲, Fig. 4.12A, slots g and h, respectively) were, however, inherited as alternatives. None of the test-cross seeds revealed a non-parental combination. The population size was not large enough to detect rare recombinants and the upper limit for the recombination value (at 95% confidence limit, Hanson, 1959) was 7.2%. Because of the absence of recombinants, the genes controlling patterns 2i and 3d in Norin-61A were considered to be allelic to *Glu-B3a* and *Glu-D3a* in Chinese Spring, respectively and have

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Figure 4.12. Banding patterns of endosperm proteins extracted in 70% ethanol from the test-cross [(Chinese Spring × Norin-61A) × Triple translocation stock] and the parental seeds.

A. Two-step SDS-PAGE patterns of (d) Norin-61A, (e) Chinese Spring, (f) Triple translocation stock and (a-c, g-k) test-cross seeds.

B. Diagrammatic representation of the B and C subunits of Chinese Spring, Norin-61A and Triple translocation stock. The bands have been divided into three inheritance groups.

C. One-dimensional SDS-PAGE patterns of (a) Chinese Spring (b) Triple translocation stock, (j) Norin-61A and (c-i) test-cross seeds.

Symbols used in the figure ;

NR = Norin-61, CS = Chinese Spring, TTr = Triple translocation stock,

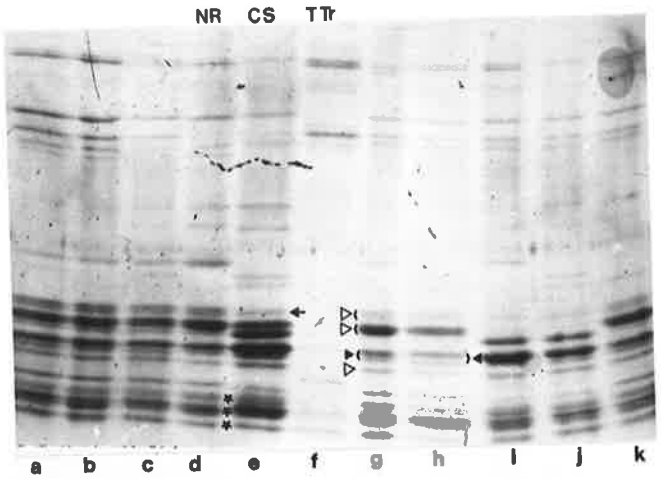
* bands which could not be scored in the test-cross seeds due to their presence in both of the F_1 parents

§ the top protion of this band is controlled by 1DS

Δ = bands controlled by 1BS

▲ = bands controlled by 1DS

→ = band suspected to be D subunit of glutenin and thus has been excluded from the analysis.


NR
CS
TTr
a
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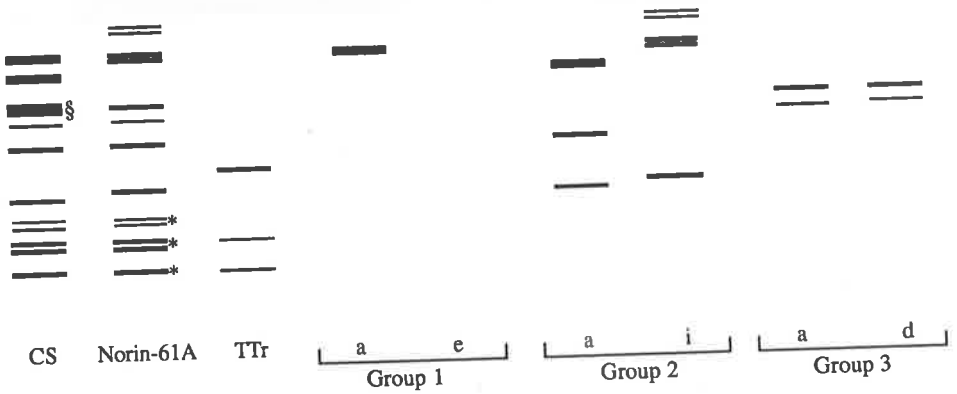
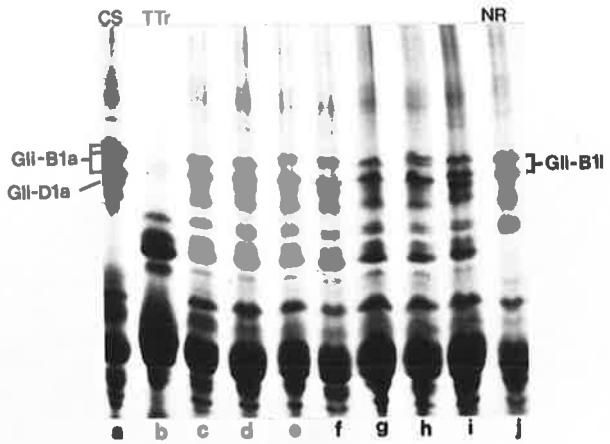
k

A


§
CS
Norin-61A
TTr
a
e
Group 1
a
i
Group 2
a
d
Group 3

B


CS
TTr
NR
Gli-B1a
Gli-D1a
Gli-B1l
a
b
c
d
e
f
g
h
i
j

C

been designated the *Glu-B3i* and *Glu-D3d* alleles. No clear band was detected in Norin-61A segregating as an alternative to the 1a pattern (Glu-A3a) of Chinese Spring (marked by →, Fig. 4.12A, slot e) among the progeny (Fig. 4.12A, slots i, j) indicating that it carried the null allele *Glu-A3e*.

Table 4.8 Segregation of LMW subunits of glutenin in test cross seeds from [(Chinese Spring × Norin-61A) × Triple translocation stock]

LMW glutenin pattern	Observed frequency	$\chi 2$ value (1 : 1)	Probability (df= 1)
a : i (group 2)	17 : 23	0.9	0.3-0.5
a : d (group 3)	21 : 19	0.4	0.5-0.7

Unreduced protein extracts from these test-crosses and the parental seeds were also analysed by 1-D SDS-PAGE and the gliadins were scored. Norin-61A contained some ω-gliadin bands (labelled as Gli-B1i in Fig. 4.12C) which were always co-inherited with the LMW glutenin bands Glu-B3i among the test-cross seeds, indicating a close genetic linkage. Moreover, the Gli-B1i phenotype was inherited as an alternative to the Gli-B1a phenotype in Chinese Spring, indicating their allelic relationship. There were no detectable gliadin bands in Norin-61A which segregated as alternatives to the Gli-A1a and Gli-D1a bands of Chinese Spring, suggesting that this biotype carried the null phenotypes for these gliadins.

4.3.3.3 Gabo and Insignia

The two-step SDS-PAGE banding patterns of seed proteins from the test-cross parents Insignia, Gabo and Triple translocation stock and some test-cross seeds are shown in Fig. 4.13. Gabo carried LMW subunit patterns 1b, 2b and 3b and Insignia had patterns 1e, 2c and 3c in group 1, 2 and 3, respectively. These patterns are shown digrammatically in Fig. 4.13C and the bands in these patterns within group 1, 2 and 3 are marked (Δ, ▲ and →, respectively, Fig. 4.13A, B).

The bands within a pattern were inherited together as a unit among 111 test-cross seeds suggesting that different bands in each of these patterns were controlled by closely linked genes. The joint segregation of these patterns indicated that group 1 patterns segregated

Figure 4.13. Two-step SDS-PAGE banding patterns of endosperm proteins extracted in 70% ethanol from test-cross [(Gabo × Insignia) × Triple translocation stock] and the parental seeds.

A. (a-c and g-l) test-cross seeds, (d) Insignia, (e) Gabo and (f) Triple translocation stock.

B. (a-i) test-cross seeds and (j) Gabo.

C. Diagrammatic representation of the B and C subunits of Gabo, Insignia and Triple translocation stock as deduced from their two-step banding patterns. The LMW subunits of Gabo and Insignia have been divided into three inheritance groups.

Symbols used in the figure;

INS = Insignia, GB = Gabo, TTr = Triple translocation stock,

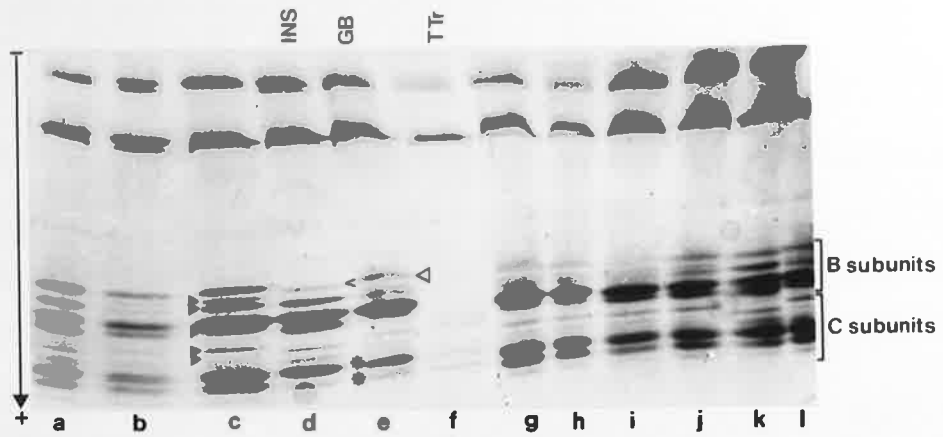
* bands which could not be scored in the test-cross seeds due their common mobilities in the parents.

> band suspected to be a D subunit of glutenin and is not included in the B subunits.

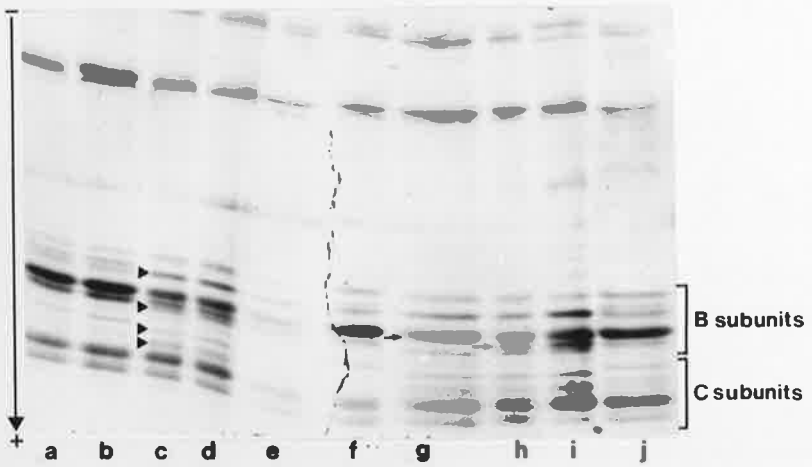
Δ = bands controlled by 1AS,

▲ = bands controlled by 1BS,

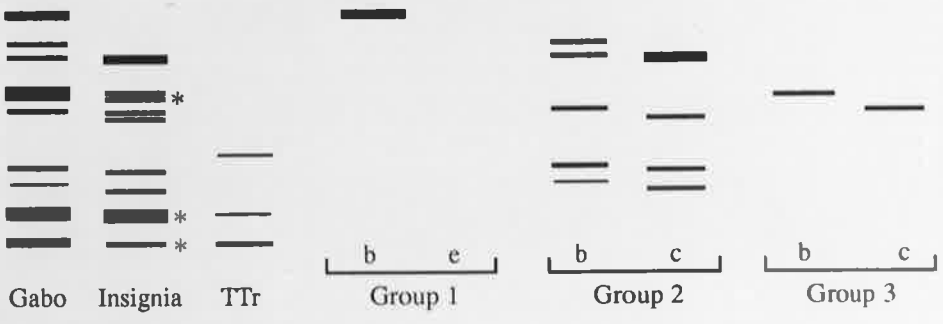
→ = bands controlled by 1DS.


INS
GB
TTr
B subunits
C subunits
+
a b c d e f g h i j k l

A


B subunits
C subunits
+
a b c d e f g h i j

B


Gabo
Insignia
TTr
b e
Group 1
b c
Group 2
b c
Group 3

C

independently of group 2 ($\chi 2$= 2.6, P > 0.25) and group 3 patterns ($\chi 2$= 4.4, P > 0.1) and similarly group 2 patterns were inherited independently of group 3 patterns ($\chi 2$= 1.5, P > 0.5) consistent with a 1:1:1:1 test-cross ratio (Table 4.9). The patterns within a group segregated as two mutually exclusive units in a ratio of 1:1 (Table 4.9). Since patterns 2b and 3b in Gabo were controlled by genes on 1BS and 1DS, patterns 2c and 3c in Insignia must also be controlled by genes on these same arms 1BS and 1DS, respectively and because of absence of recombinant phenotypes, the genes controlling these patterns in pairs (2b and 2c, 3b and 3c) were designated alleles *Glu-B3b* and *Glu-B3c* and alleles *Glu-D3b* and *Glu-D3c*. There was no clear band in Insignia segregating as an alternative to the band pattern 1b in Gabo controlled by chromosome arm 1AS and this indicated that Insignia possibly had the null phenotype 1e. The genes controlling these patterns have been designated *Glu-A3b* and *Glu-A3e*, respectively.

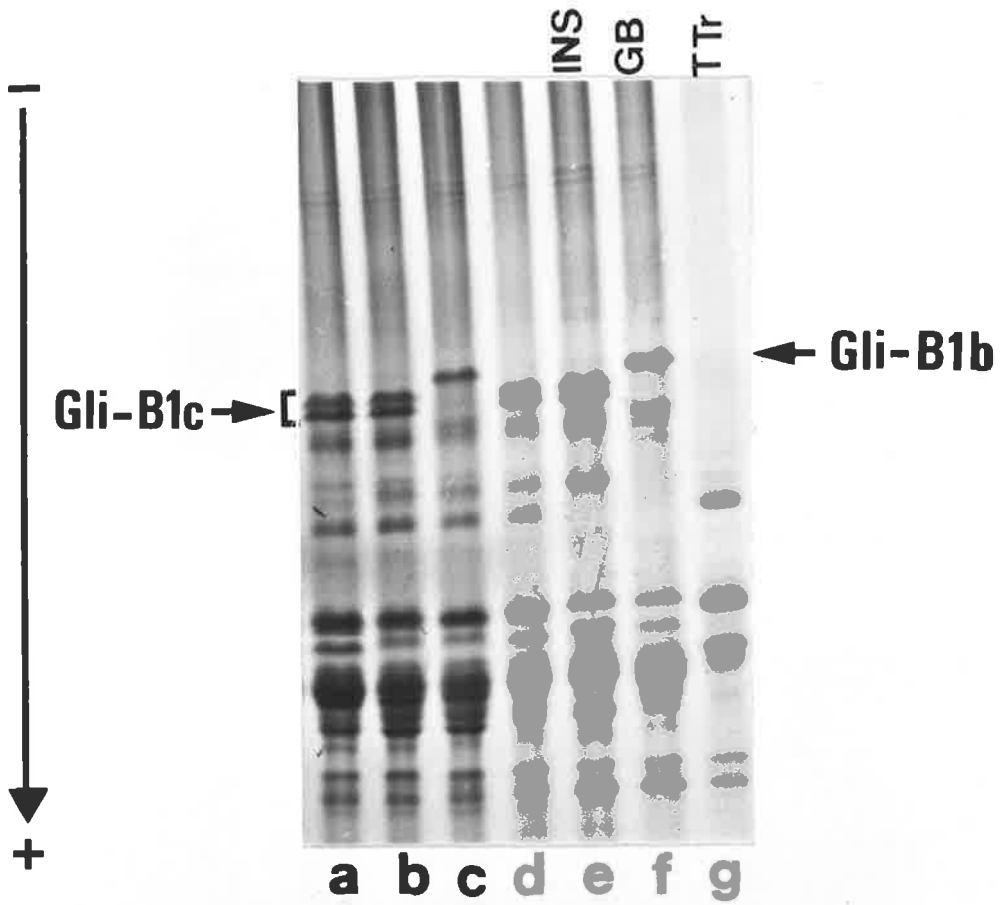
Table 4.9 Segregation of LMW subunits of glutenin in test cross seeds from [(Gabo × Insignia) × Triple translocation stock]

LMW glutenin pattern	Observed frequency	$\chi 2$ value (1 : 1)	Probability (df= 1)
b : c (group 2)	54 : 57	0.08	0.7-0.8
b : c (group 3)	50 : 61	1.09	0.2-0.3

The unreduced protein phenotypes of the parents and test-cross seeds, as determined by 1-D SDS-PAGE, revealed different ω-gliadin bands in Gabo and Insignia labelled as Gli-B1b and Gli-B1c (Fig. 4.14), respectively. These gliadin patterns were inherited with the LMW glutenin subunit patterns Glu-B3b and Glu-B3c without exception in the test-cross seeds indicating a tight linkage between the genes controlling these gliadins and the LMW subunits of glutenin on chromosome arm 1BS in these cultivars. The absence of any recombinant among 111 test-cross seeds gave an upper limit of 2.6% for the recombination value between *Gli-B1* and *Glu-B3* loci (at the 95% confidence limit, Hanson, 1959).

Figure 4.14. One-dimensional SDS-PAGE patterns of unreduced total proteins (a-d) test-cross seeds [(Gabo × Insignia) × Triple translocation stock], (e) Insignia, (f) Gabo and (g) Triple translocation stock.

INS = Insignia, GB = Gabo, TTr = Triple translocation stock


INS
GB
TTr
Gli-B1b
Gli-B1c
a b c d e f g

4.4 DISCUSSION

Twelve and 15 LMW subunits (B and C subunits) of glutenin were found in Chinese Spring and Gabo, respectively, which were controlled by genes on the short arms of group 1 chromosomes. In addition, these cultivars carried two LMW prolamin bands in the C subunit region of the gels which were not controlled by group 1 chromosomes. One of these in Chinese Spring was controlled by genes on chromosome arm 6DS. Because of their solubility in ethanol and disulphide linkage, these prolamin bands were considered to be LMW subunits of glutenin (C subunits). Thus these results indicate that the LMW subunits of glutenin in bread wheat are controlled by both group 1 and group 6 chromosomes. The translocation stocks further revealed that chromosome arm 1RS in Imperial rye also codes for a LMW glutelin band.

The 6DS- and 1RS-controlled prolamins were adjudged disulphide-linked (polymeric) because of their ability to form parallel lines in diagonal electrophoresis. The question arose as to how these three LMW prolamins could have appeared as parallel lines. There are two main ways by which parallel lines can be formed (Singh and Shepherd, 1985). Firstly, these prolamin bands may be present in a wide array of different-sized aggregates ranging from very large multimers to a small dimer involving just the smallest prolamin band. Secondly, occurrence of conformational isomers among the protein aggregates, due to differences in the number and sites of disulphide linkage between any two interacting subunits or to differences in degree of unfolding of a given dimer or multimer in the presence of SDS. Considering that the Triple translocation stock lacked all other bands from the LMW glutenin subunit region and contained only four HMW subunits of glutenin capable of forming disulphide linked aggregates, it is logical to assume that the three prolamin bands have formed aggregates among themselves as well as with the HMW subunits of glutenin. Such interactions, however, would not have generated continuous parallel lines because the aggregates would occur in a limited range of sizes which should have produced definite spots rather than parallel lines, upon reduction. Thus, it is likely that conformational changes were also involved in causing continuity of the parallel lines because the smallest aggregates formed in these cases are not expected to allow parallel lines to continue up to the diagonal line. However, such

conformational changes could not have produced parallel lines on their own and thus disulphide-linked aggregates are believed to be the main factor in their formation.

This study clearly showed that genes on the short arms of group 1 chromosomes control most of the LMW subunits of glutenin of Chinese Spring. Previously, Singh and Shepherd (1985) had located genes controlling 5 of these subunits by analysing aneuploid stocks by two-step SDS-PAGE. They were unable, however, to determine the genetic control of other bands, including those found in the present study to have multiple genetic control because of their identical mobility. The present results also largely agreed with those obtained by Jackson *et al.* (1983) in Chinese Spring using 2-D methods, with the following differences. First, they could assign genetic control of only 9 LMW subunits (B and C subunits) of glutenin to group 1 chromosomes. Second, they did not assign the genetic control of any LMW subunits of glutenin to group 6 chromosomes, probably because the C subunits were resolved as diffuse spots or streaks and were overlapped by some of the monomeric prolamins (gliadins) controlled by group 6 chromosomes. The C subunits present in the two-step patterns of the Triple translocation stock (that is the C subunits controlled by 1RS and 6DS) were not monomeric prolamins which would have been removed electrophoretically from the multimeric prolamins before separation in the second step of two-step electrophoresis. These bands, moreover, contrary to the characteristics of monomeric prolamins (Singh and Shepherd, 1985; Field and Shewry, 1987), appeared as parallel lines in diagonal electrophoresis. Thus, it is not likely that they were monomeric prolamins which were trapped in aggregates of glutenin.

The synthesis of a LMW glutelin band by chromosome arm 1RS in Imperial rye was not unexpected considering its homoeologous relationship with the short arms of group 1 chromosomes of wheat. This band was not seen in Imperial rye perhaps due to its synthesis in very small quantity. Similarly, the presence of some LMW subunits of glutenin in wheat controlled by group 6 chromosomes was not unexpected since some α-, β- and γ-gliadins are controlled by genes on group 6 chromosomes and these gliadins are biochemically related to LMW subunits of glutenin (Bietz and Wall, 1972; Shewry *et al.*, 1984a; Okita *et al.*, 1985; Kasarda *et al.*, 1987). It could be argued that these C subunits are in fact variant α- and β-gliadins controlled by chromosome 6D which have acquired aggregating properties possibly through a single base change TCT (serine) to TGT (cysteine) in the gene coding for it.

Recently Kasarda *et al.* (1987) have reported that certain α-type gliadins (including both α- and β-gliadins) were present in highly purified native glutenin and had an uneven number of cysteine residues which were probably responsible for their aggregation through intermolecular disulphide bonds. Such α-type gliadins would no longer be functionally different from the LMW subunits of glutenin which is the main basis of the distinction between gliadins and LMW subunits of glutenin at the practical level. Further work (e.g. protein sequencing) is required to obtain a better understanding of the relationship between the 1RS- and 6DS-controlled C subunits, gliadins and other LMW subunits of glutelin.

Since all of the B and most of the C subunits were absent in the Triple translocation stock, it served as a very useful third parent in the test-crosses produced for studying the inheritance of B and C subunits and their allelic relationships. Segregation data obtained from such test crosses, provided evidence for the allelic relationship between four of the five patterns '**a**', '**b**', '**d**' and '**e**' controlled by genes on chromosome arm 1AS (*Glu-A3*) and similarly for five of the nine patterns '**a**', '**b**', '**c**', '**d**' and '**i**' controlled by genes on 1BS (*Glu-B3*) and for all of the five patterns '**a**', '**b**', '**c**', '**d**' and '**e**' controlled by genes on 1DS (*Glu-D3*) in bread wheat. It is reasonable to assume that other patterns controlled by 1AS (pattern '**f**') and by 1BS (patterns '**e**', '**f**', '**g**' and '**h**') are also controlled by allelic genes at *Glu-A3* and *Glu-B3* loci, respectively. Hence, genes controlling all of the patterns on chromosome arms 1AS, 1BS and 1DS have been designated as alleles ***a*** to ***f***, ***a*** to ***i*** and ***a*** to ***e*** at *Glu-A3*, *Glu-B3* and *Glu-D3* loci, respectively.

All of the LMW glutenin patterns coded by the *Glu-B3* and *Glu-D3* loci and two patterns by *Glu-A3* loci consisted of two or more B and C subunits and the inheritance tests of several of these patterns clearly revealed that the bands within each of these patterns were inherited as a unit and thus are controlled by closely linked or allelic genes. Similarly, most of the gliadin bands (ω- and γ-gliadins) coded by *Gli-A1*, *Gli-B1* and *Gli-D1* loci were also inherited in clusters. Furthermore, they were always co-inherited with the LMW glutenin patterns controlled by same chromosome arms and gave no recombinant phenotypes in a total of 258 test-cross progeny analysed, thus setting an upper limit of 1.2% recombination value within the *Gli-1* or *Glu-3* loci or between *Gli-1* and *Glu-3* loci. This confirms the earlier work on gliadins (Sozinov and Poperelya, 1980) and LMW subunits of glutenin (Payne *et al.*, 1984e; Singh and Shepherd, 1988a) in bread wheat. The last two groups of authors studied

the inheritance of LMW subunits of glutenin and gliadins in their attempts to map the genes controlling them in some bread wheat cultivars. Payne *et al.* (1984e) scored the LMW subunits of glutenin in the overlapping background of gliadins in 1-D SDS-PAGE where these two groups of bands cannot be reliably distinguished from each other, without further tests. Only some of the B subunits occurred in positions that could be scored in 1-D gels. On the other hand, Singh and Shepherd (1988a) studied the segregation of only a few bands included in some of these LMW glutenin patterns. Thus, the present study is the first detailed study of the inheritance of LMW subunits of glutenin alone and also in combination with gliadins in bread wheat.

Although the B and C subunits within a pattern were inherited as a unit with no recombination, their differing isoelectric points (Jackson *et al.*, 1983) and mobilities indicate that they may be coded by two different gene sub-families. Further support for this notion came from the observations in the varietal survey that several band patterns controlled by chromosomes 1B and 1D have the same C subunits but different B subunits, and vice-versa, which is most likely the result of crossing over between the genes controlling these two groups of subunits. The tight linkage between genes on the short arms of group 1 chromosomes coding for gliadins and LMW subunits of glutenin in durum (Pogna *et al.*, 1988) and bread wheats (Payne *et al.*, 1984e; Singh and Shepherd, 1988a; Gupta and Shepherd, 1988) supports the hypothesis that gliadins and LMW subunits of glutenin have evolved from the same ancestral gene through duplication and divergence (see Kreis *et al.*, 1985 for a review; Bartels *et al.*, 1986; Colot *et al.*, 1989). This process, however, seems to have been present in the common progenitor of the Triticeae because both LMW subunits of glutenin and gliadins were present in the diploid *Triticum* species related to the A, B and D genome of wheat and also in other diploid wheat relatives (Chapter 3). This suggests that genes coding for gliadins and LMW subunits of glutenin have been present for a long period of time and it is possible that they may have been translocated together between group 1 and 6 chromosomes. However, only some of the genes on group 6 chromosomes are active and thus code for only few gliadins and LMW subunits of glutenin.

It has long been recognized that glutenin because of its aggregating nature is the main determinant of gluten elasticity (Dimler, 1965). The relative importance of the glutenin fractions containing HMW or LMW subunits of glutenin on gluten elasticity has not yet been

investigated. The novel translocation stocks produced in this work are believed to be valuable material for investigating this question. For example, the Triple translocation stock lacking most of the LMW subunits of glutenin can be used to analyse the effects of LMW glutenins as a group on the viscoelastic properties of gluten and dough. Similarly, the three double translocation stocks can be used to evaluate the role of specific LMW subunits of glutenin controlled by chromosome arm 1AS, 1BS and 1DS on gluten properties. In addition, the double translocation stocks can also be used for purifying specific LMW subunits of glutenin for protein sequencing. These stocks are currently being transferred into an agronomically superior Gabo background, to allow further work on these aspects.

Chapter 5

RELATIONSHIPS BETWEEN GLUTELIN SUBUNITS AND DOUGH PROPERTIES IN BREAD WHEAT

5.1 INTRODUCTION

It has long been known from flour reconstitution experiments that glutenin, a disulphide-linked protein, is the major determinant of the pasta- and bread-making qualities of wheat flours (see Chapter 2 for a review). Recent data from genetic reconstitution experiments have shown that individual subunits of glutenin are related to flour quality, viz. HMW subunits in bread wheat (see Payne *et al.*, 1984d for a review) and LMW subunits in durum wheats (Payne *et al.*, 1984b, Autran and Berrier, 1984). In bread, however, the relationship between LMW subunits and flour quality has not been established. Moreover, the relative importance of LMW and HMW subunits and the extent of their interaction in affecting the bread-making quality have not been assessed. In this chapter, data related to both these aspects of LMW and HMW subunits of glutenin are presented. Most of these data have been published (Gupta and Shepherd, 1987, 1988; Gupta *et al.*, 1989).

The 75 kd γ-secalins in cereal rye are also disulphide-linked proteins (Shewry *et al.*, 1983a), but their effect on bread-making quality is not known. They are coded by genes (*Sec*-2) on chromosome arm 2RS (Shewry *et al.*, 1984b) and this chromosome arm has been transferred from rye cultivar Imperial into wheat cultivar Timgalen by producing a 2BL-2RS translocation line (May and Appels, 1978). The fertility of this line was poor but has been improved by altering the genetic background e.g. Gabo, Spica (May and Appels, 1984). In the present study, this translocation in a mixed background of Timgalen and Gabo was further backcrossed into Gabo and highly fertile lines (chromotypes) with and without 75 kd γ-secalins (i.e. chromosome arm 2RS) were isolated. They were multiplied, grown in the field and dough properties were determined by the extensograph and the data are presented herein.

5.2 MATERIALS AND METHODS

5.2.1 Experimental materials

5.2.1.1 F_2-derived lines

The F_2 lines were derived from a cross between breeding line MKR/111/8 ={((Mexico 120 × Koda) × Raven) - selection 111/8} and the Australian bread wheat cultivar 'Kite'. These parents possess poor and medium physical dough properties (mainly dough resistance), respectively (A. J. Rathjen, personal communication). These parents have different gliadins and LMW and HMW glutenin subunit components (Table 5.1). The protein phenotypes of the parents and the detailed procedure for selecting and multiplying these F_2 lines are shown in Fig. 5.2. In outline, the F_2 seeds were screened by SDS-PAGE to select 20 individuals of each of the four phenotypic classes representing all combinations of the presence and absence of specific bands at the *Glu-A3* (LMW subunit) and *Glu-A1* (HMW subunit) loci. After further testing of their protein patterns during multiplication, the number of isolates in each family (phenotypic class) was reduced from 20 to 14 and these were included in field trials along with the parents.

5.2.1.2 Biotypes

Biotypes of cultivars BT-2288 (Tunisia) and Gamenya and Condor (Australia) with different LMW glutenin subunit patterns, detected during cultivar screening for LMW subunits of glutenin (Chapter 3), were isolated as single seeds and multiplied for field testing. The Condor biotypes A1 and A2 were isolated from the biotype A of Condor (Lawrence, 1986). Five isolates for each biotype were used for field planting.

5.2.1.3 2RS chromotypes

Thirty chromotypes viz. lines having either rye chromosome arm 2RS or its wheat homoeologue 2BS, were isolated from backcross F_2 population (BC_1F_2) between 2BL-2RS translocation in Timgalen and Gabo, the recurrent parent. The progeny seeds were screened by 1-D SDS-PAGE and the Sec-2 proteins (75 kd γ-secalins) coded by 2RS were scored. Ten isolates for chromotype 2RS (Sec-2^+) and 2BS (Sec-2^-) were selected and multiplied in

the glasshouse. Finally seven highly seed-fertile homogeneous lines for each chromotype, which were morphologically similar to each other, were chosen for field planting.

5.2.2 Field experiments

F_2-derived lines from Kite × MKR and biotypes of the three cultivars were planted in two replicates at two sites in the South Australian wheat belt [Roseworthy Agricultural College (RAC) and Bordertown (BTN)] in 1987 while the 2RS chromotypes were planted at RAC only. The seeds were planted at normal field sowing rates by a semi-automatic seeder in four-row plots with overall dimensions 0.6m × 4m. Neither nitrogen fertilizer nor irrigation was applied to these plots.

The experimental layout for the F_2 lines consisted of 14 randomized sub-blocks. Each sub-block contained 6 plots, 4 of them allocated to randomly selected F_2 derived lines representing all four phenotypic (protein) classes and the remaining plots were allocated to the two parents. Entries within each sub-block were randomized.

For the biotypes, ten isolates (5 isolates per biotype) from each cultivar were planted into 5 randomized sub-blocks, having one isolate from each of the biotypes. A total of 14 isolates of the 2RS chromotypes (7 with 2RS and other 8 with 2BS) were planted in a completely randomized block, with two replications.

5.2.3 Quality testing

5.2.3.1 Milling

Seed samples of 250 g from each line were tempered overnight to adjust their moisture content to 14% level before milling. Seeds were milled to an approximate 70% extraction rate using a Brabender Quadrumat Junior Experimental Mill fitted with a 0.255 mm aperture sieve. Flours were rested for a week before dough testing.

5.2.3.2 Flour protein content

The flour protein percentage was determined using a standard micro-Kjeldahl method and was expressed on flour dry weight basis (N × 5.7).

5.2.3.3 Extensograph tests

Dough was prepared by mixing 50 g of flour in a Brabender farinograph with the required amount of distilled water at 30°C, containing 1 g of common salt (NaCl), to give a final consistency of 500 Brabender units (BU) after 5 minutes mixing. A measured amount (75 g) of this dough was formed into a ball, then rolled and stored in a dough fermentation cabinet for 45 minutes at 30°C. The dough was then stretched at constant speed until breaking point. The maximum resistance to extension (R_{max} in BU) was obtained by measuring the maximum height of the extensograph curve (extensogram) while the extensibility (E in cm) was given by the length of the extensogram. The area (cm^2) under the curve reflects both dough resistance and extensibility and is a measure of dough strength (energy).

5.2.4 Protein extraction and electrophoresis

The total unreduced endosperm protein extraction, 1-D and two-step SDS-PAGE and staining and destaining of gels were carried out as described in Chapter 3.

5.2.5 Statistical analysis

Since extensograph tests require much labour, only one replicate of the F_2-derived lines and biotype from each location was analysed for R_{max} and E. Since 2RS chromotypes were planted at only one location, both replicates were analysed. The analyses of variance for F_2-derived progeny were performed on both individual site and pooled data. The 14 different sub-blocks containing isolates of each of the four phenotypes were treated as replicates in the single-site analysis of variance. The analysis of variance for biotypes was performed on only individual site data and the 5 isolates of each biotype were treated as replicates. The 14 isolates of 2RS chromotypes (7 lines with 2RS and the other 7 with 2BS) were subjected to one-way analysis of variance.

5.3 RESULTS

Results are presented in the following order: F_2-derived lines, biotypes and chromotypes.

5.3.1 F_2-derived lines from Kite × MKR/111/8

5.3.1.1 Isolation and characterization

The protein phenotypes of parents MKR/111/8 and 'Kite' are shown in Fig. 5.1 and the allelic differences for gliadins and LMW and HMW subunits of glutenin are summarized in Table 5.1.

Table 5.1 Allelic differences between parents for storage proteins and the location of genes controlling them

Protein class	Locus	Protein band differences in parents*	
		MKR/111/8	Kite
Gliadins	*Gli-A1*	Gli-A1c	Gli-A1e
LMW subunit of glutenin	*Glu-A3*	Glu-A3c	Glu-A3e (null)
HMW subunit of glutenin	*Glu-A1*	Glu-A1c (null)	Glu-A1b (2*)
	Glu-B1	Glu-B1b (7+8)	Glu-B1i (17+18)

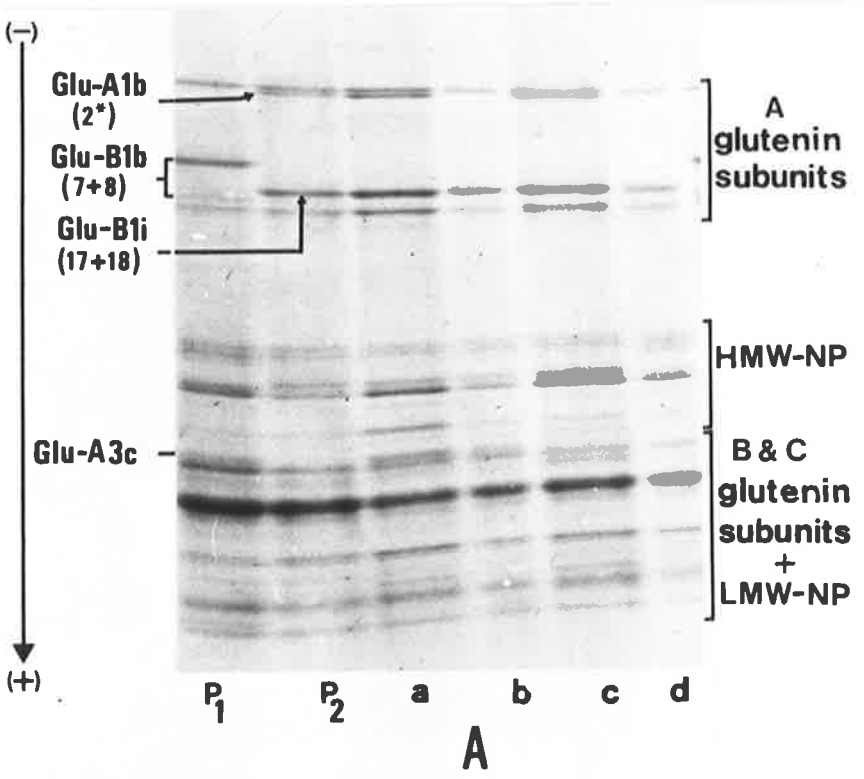
*In a previous publication (Gupta *et al.*, 1989), the symbols Glu-A3m, Glu-A3k, Gli-A1m and Gli-A1k were adopted from Singh and Shepherd (1988a) instead of Glu-A3c, Glu-A3e, Gli-A1c and Gli-A1e, respectively.

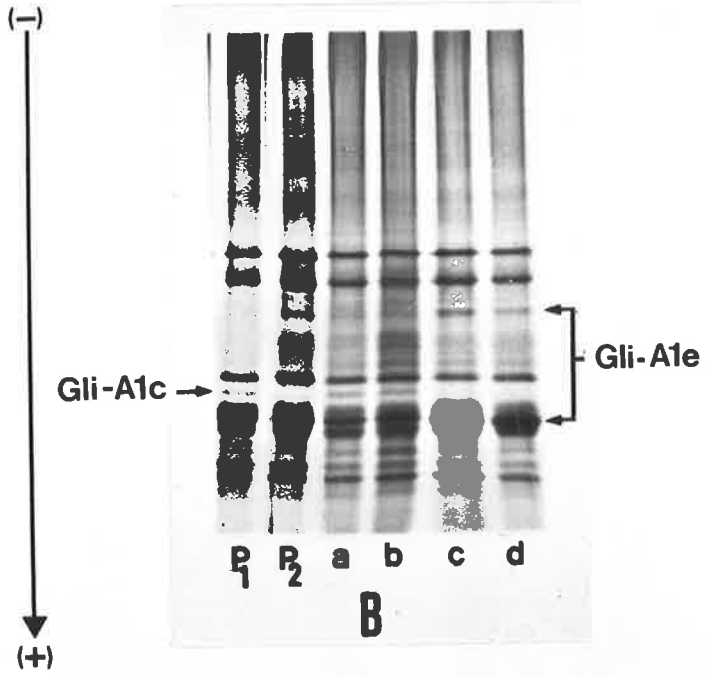
The LMW and HMW subunits of glutenin were scored by two-step SDS-PAGE (Fig. 5.1A) whereas the gliadins were examined by 1-D SDS-PAGE (Fig. 5.1B). There was no detectable LMW subunit in 'Kite' segregating alternatively to Glu-A3c band and similarly MKR/111/8 had no obvious HMW subunit band equivalent to Glu-A1b of 'Kite', and thus it was assumed that they carried null alleles (*Glu-A3e* and *Glu-A1c*, respectively) at these loci (Fig. 5.1A). In the analysis of F_2 seeds, the Glu-A3c band was always associated with Gli-A1c and similarly the null Glu-A3e was associated with Gli-A1e (Fig. 5.1B), indicating complete or close linkage between genes controlling these gliadins (*Gli-A1*) and LMW

Figure 5.1 A, B. SDS-PAGE patterns of parents (P1) MKR/111/8 and (P2) Kite and (a, b, c, d) the four F_6 families showing all main phenotypic classes.

A. Two-step SDS-PAGE patterns of total protein extracts. The LMW and HMW subunits of glutenin under study are labelled. NP = non-prolamins

B. One-dimensional SDS-PAGE patterns of unreduced protein extracts showing the differences in gliadin patterns of the same seeds.


(–)
Glu-A1b
(2*)
Glu-B1b
(7+8)
Glu-B1i
(17+18)
Glu-A3c
(+)
A
glutenin
subunits
HMW-NP
B & C
glutenin
subunits
+
LMW-NP
P1
P2
a
b
c
d
A


(–)
Gli-A1c
Gli-A1e
(+)
P1
P2
a
b
c
d
B

subunits of glutenin (*Glu-A3*). The Gli-A1 and Glu-A3 bands were found to be loosely linked (43.5% recombination) with the 2^* band which is known to be controlled by the *Glu-A1* locus on the long arm of chromosome 1A (Payne and Lawrence 1983) and thus it was concluded that all three of these loci are located on chromosome 1A. The control of these gliadins and LMW subunits by this chromosome is also verified by the genetic data from intervarietal substitution lines (Chapter 3). There was no difficulty in selecting F_2 seeds homozygous for the Glu-A3, Gli-A1 and Glu-B1 bands (7+8 and 17+18) due to the co-dominant expression of alleles. Homozygosity for Glu-A1b (band 2^*), however, could be determined only by progeny testing because there was no detectable band allelic to it. The linked gliadins were used to select seeds homozygous for the Glu-A3c band. For simplicity, only the detected bands are mentioned in the tables, figures and diagrams.

The main objective of the study was to investigate the effects of bands Glu-A3c and Glu-A1b on physical dough properties and thus the F_2 seeds were classified into the four main phenotypic classes, Glu-A3c$^+$ Glu-A1b$^+$; Glu-A3c$^+$ Glu-A1b$^-$; Glu-A3c$^-$ Glu-A1b$^+$; Glu-A3c$^-$ Glu-A1b$^-$. To minimize possible effects from the co-segregation of Glu-B1 bands, all combinations of these phenotypes (Glu-B1b, Glu-B1i and Glu-B1b/Glu-B1i) were included within each of the four main classifications as shown in Fig. 5.2. Thus, each main class was divided into three sub-classes based on their Glu-B1 patterns. The 80 selected F_2 seeds were individually multiplied for two further generations to produce F_4 families (Fig. 5.2) and the phenotypes of these families were checked on gels. Finally, 56 electrophoretically verified, morphologically similar, F_4 families (14 in each main class) were chosen and multiplied to develop 56 F_5 families. These families had no major differences in patterns of the gliadin and glutenin protein other than those already considered. Some minor differences in albumin components were noted but it was not known whether they have any effect on dough quality. Nevertheless, since they were randomly distributed among these families, any statistical effect these bands had on the physical dough properties could be attributed to experimental error.

One family from each of the four main classes with a common Glu-B1 band pattern was taken and planted along with the parents in a sub-block and thus a total 14 sub-blocks were required. These sub-blocks were treated as replicates in the analysis, so the effect of the Glu-B1 patterns contributed to the variance component attributable to the replication.

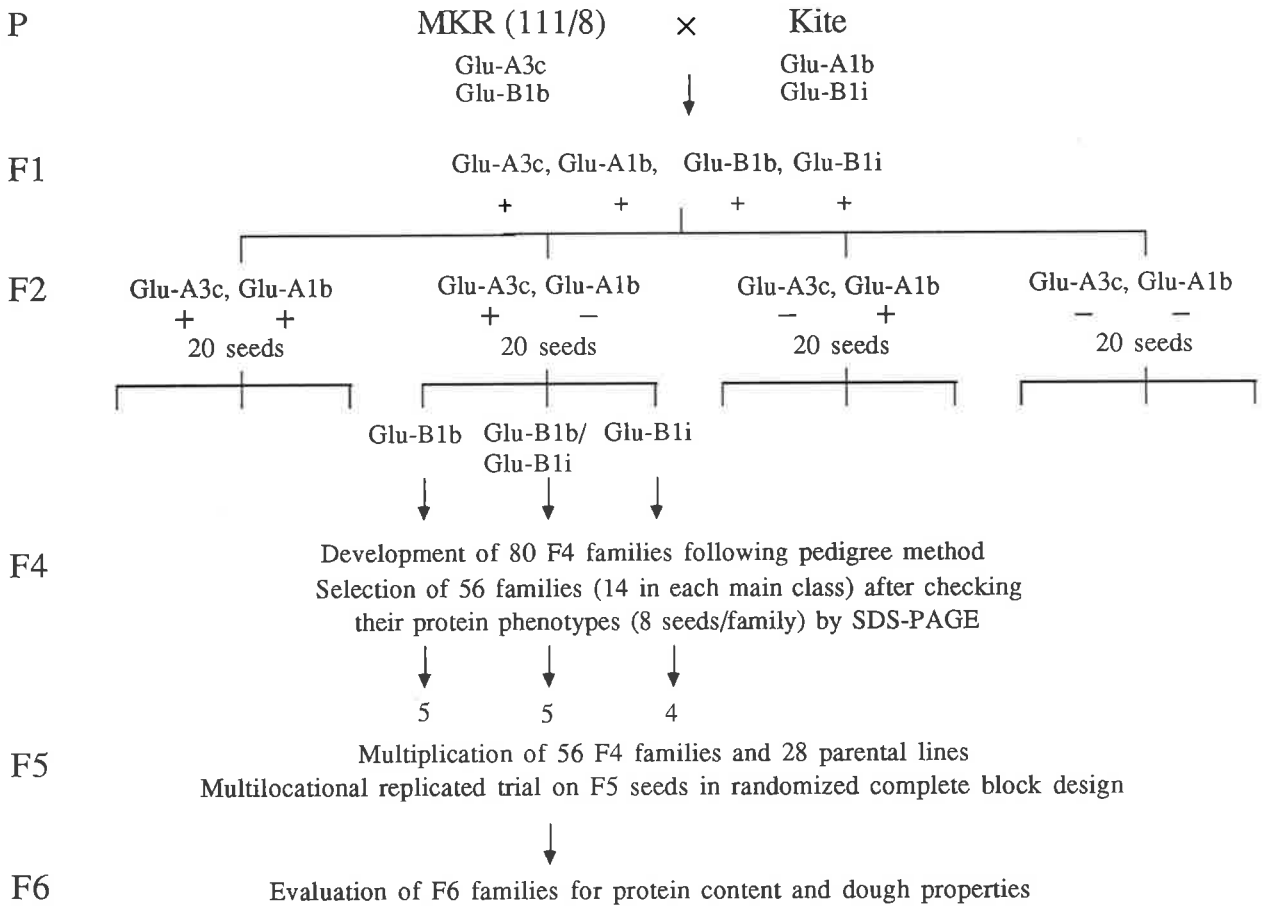


Figure 5.2. Procedure used to develop 56 F6 families from a cross between parents MKR/111/8 and Kite. The same procedure for selecting F2 seeds, dividing them into Glu-B1 subgroups and multiplying them, was used for all groups but is only shown in detail for the Glu-A3c+ Glu-A1b– group.

5.3.1.2 Effect of flour protein level on dough properties

The flour protein content of the parents and families were very different at the two sites (Table 5.2) with an average value of approximately 14% at RAC and 7% at BTN. Similarly, the average dough strength (both dough resistance and extensibility) of these parents and families was very different between the two sites, reflecting the differences in flour protein content (Fig. 5.3B). The effect of protein content on dough extensibility was relatively greater than on dough resistance. The cause of these changes has not been investigated. However, since the amount of glutenins and gliadins both were increased at higher total protein level (Tanaka and Bushuk, 1972) and they have causal relationships with dough visco-elasticity, these changes could be due to these proteins.

Table 5.2 Average flour protein content (mean of 14 entries) of parents and four types of families at two sites (RAC and BTN)

Phenotypic classes	Flour protein content (%)	
	RAC	BTN
Parents		
MKR/111/8	13.26	6.64
Kite	14.20	6.95
Families		
Glu-A3c$^+$ Glu-A1b$^+$	13.70	6.66
Glu-A3c$^+$ Glu-A1b$^-$	13.40	6.75
Glu-A3c$^-$ Glu-A1b$^+$	13.49	6.58
Glu-A3c$^-$ Glu-A1b$^-$	13.70	6.84
SED	0.24	0.16

5.3.1.3 Effect of allelic variation in protein bands on dough properties

The pooled ANOVA including all treatments (parents and F_6 families) showed significant interaction between treatments and sites for R_{max} and E but when the parents were excluded from the analysis, there was no interaction (Table 5.3). Similarly, there was a significant difference in the protein content of treatments over the sites, due to differences between the

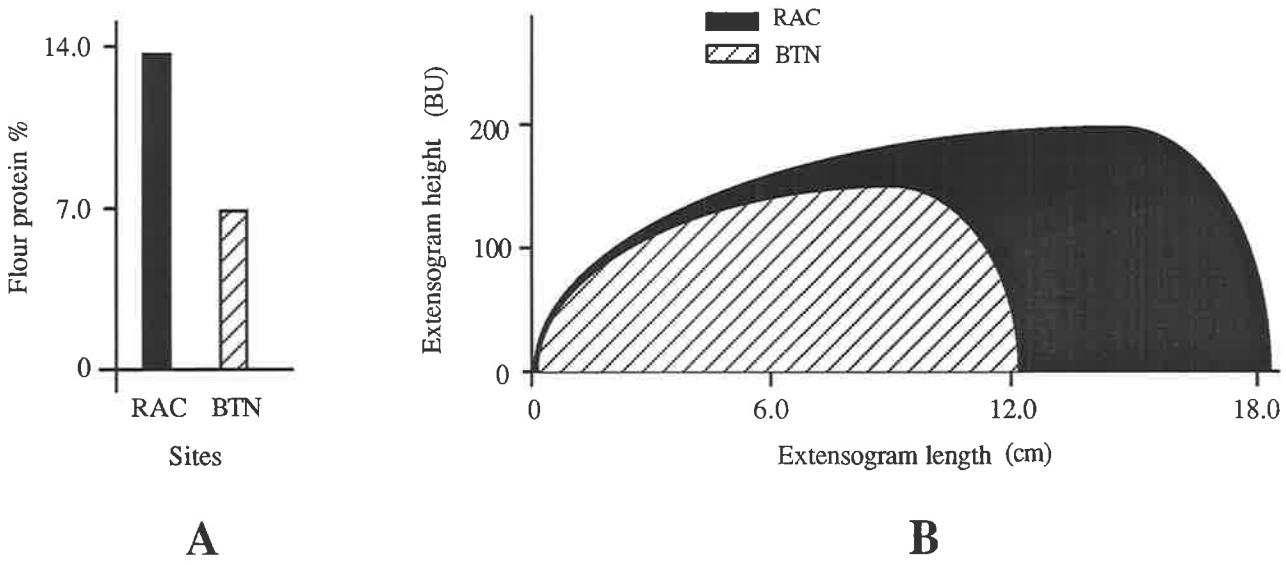


Figure 5.3 A, B. Overall mean values for quality characters of 56 F6 families and 28 parental lines grown at two sites (RAC = Roseworthy Agricutural College, BTN = Bordertwon). **A.** Flour protein content. **B.** Dough strength measured as dough resistance (extensogram height) and extensibility (extensogram length).

parents but not the F_6 families (Table 5.3). These differences in response shown by the parents were assumed to result from their different genetic backgrounds. Since the only valid comparisons were those within a similar genetic background, the data pertaining to parents were considered to be reference material only.

Table 5.3 Pooled analysis of variance for flour protein %, maximum resistance (R_{max}) and extensibility (E) in 56 F_6 families with and without 28 parental lines over two sites (RAC and BTN)

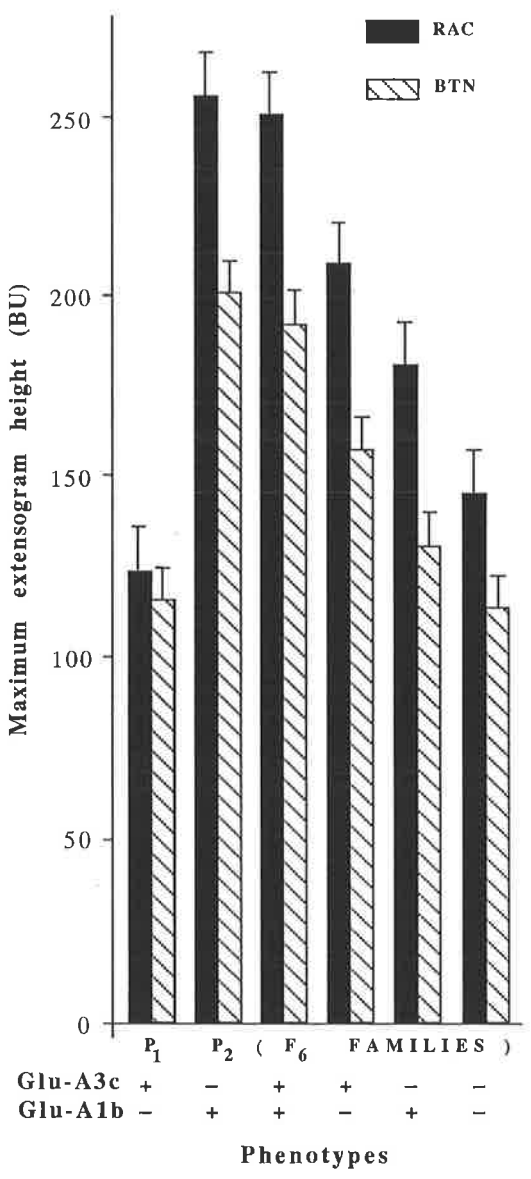
Character	Source of variation	F value and its significance level	
		With parents	Without parents
Flour protein %	Site	1350.5***	1212.8***
	Treatment	4.4***	1.0
	Site × Treatment	0.6	0.7
R_{max} (BU)	Site	48.2***	52.6***
	Treatment	75.7***	41.6***
	Site × Treatment	2.5*	0.19
E (cm)	Site	948.4***	935.4***
	Treatment	10.2***	14.6***
	Site × Treatment	2.2*	0.5

*, ***: significant at $P < 0.05$ and 0.001, respectively

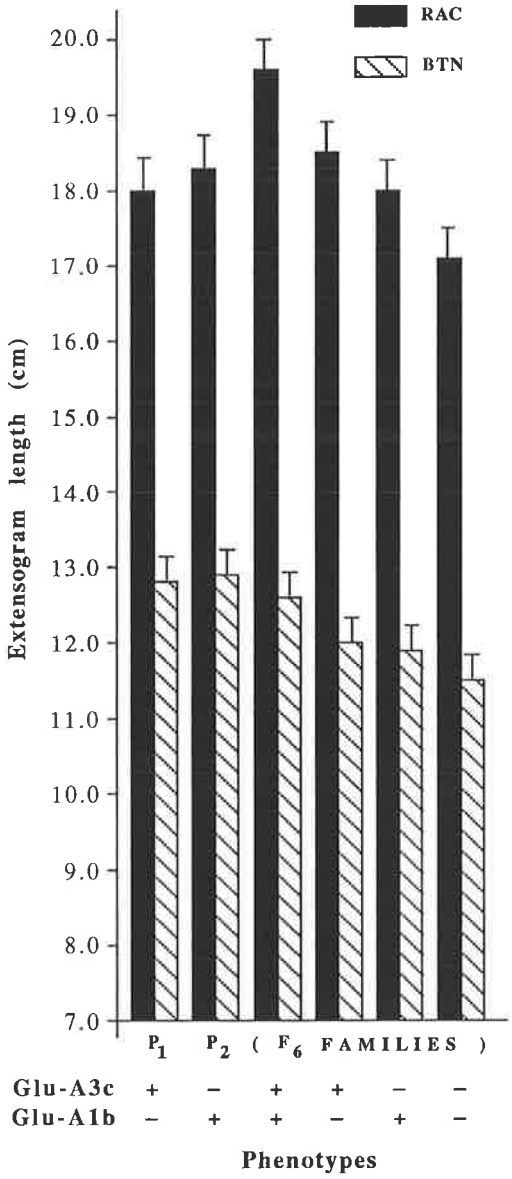
Although the data presented in Table 5.3 indicated that the site × treatment interaction for the F_6 families was insignificant and pooled ANOVA on them was sufficient, they were analysed separately for each site due to the large difference in the grain protein content between the sites (Table 5.2). This analysis showed that there were highly significant differences in the R_{max} and E values for the different F_6 families at both of the locations despite insignificant differences in their protein content (Tables 5.2, 5.4) indicating that these quality differences among the families were not due to the quantitative variation in their total flour proteins. The families lacking both LMW subunit band Glu-A3c and HMW subunit Glu-A1b had the lowest mean values for R_{max} (Fig. 5.4A) and extensibility (Fig. 5.4B) whereas families having both of these bands had the highest values for these characters. The families with only one of the bands gave intermediate values indicating the predominantly additive effects of these bands on the dough quality parameters. Families with LMW band

Figure 5.4 A, B. Mean values (average of 14 entries) for maximum extensogram height (**A**) and extensogram length (**B**) for both parents (P1 = MKR/111/8, P2 = Kite) and 56 F_6 families grown at two sites (RAC = Roseworthy Agricultural College, BTN = Bordertown). The standard error of differences (SED) of means are shown as bars.

Note: Apparent difference in the quality parameters (R_{max}, E) between 'Kite' type families (Glu-A3c$^-$ Glu-A1b$^+$ phenotype) and the parent 'Kite' (P_2) and similarly between MKR type families (Glu-A3c$^+$ Glu-A1b$^-$) and the parent MKR/111/8 (P_1) is not due to differences in their flour protein contents. It is rather due to their different genetic backgrounds (including differences in Glu-B1 bands) because the adjustment of protein content by co-variate analysis did not make any major change in the their relative physical dough characteristics.


RAC
BTN
Maximum extensogram height (BU)
250
200
150
100
50
0
P1 P2 (F6 FAMILIES)
Glu-A3c + – + + – –
Glu-A1b – + + – + –
Phenotypes

A


RAC
BTN
Extensogram length (cm)
20.0
19.0
18.0
17.0
16.0
15.0
14.0
13.0
12.0
11.0
10.0
9.0
8.0
7.0
P1 P2 (F6 FAMILIES)
Glu-A3c + – + + – –
Glu-A1b – + + – + –
Phenotypes

B

Glu-A3c gave significantly higher values for R_{max} at both sites than those with HMW band Glu-A1b.

Table 5.4 Between family variance ratios (F values) for flour protein %, R_{max} and E among 56 F_6 families at two sites (RAC and BTN)

Characters	RAC	BTN
Flour protein %	0.79	0.9
R_{max} (BU)	21.1***	20.0***
E (cm)	11.1***	4.6***

***: significant at $P < 0.001$

The families with the lowest dough strength (phenotype Glu-A3c$^-$ Glu-A1b$^-$) had the null alleles *Glu-A3e* and *Glu-A1c* but had two prominent gliadin bands controlled by the *Gli-A1e* allele (Fig. 5.1B, slot d). The families with phenotype Glu-A3c$^+$Glu-A1b$^-$ differed from the above by two extra bands (LMW glutenin band Glu-A3c and gliadin band Gli-A1c), and had significantly higher R_{max} at both locations and higher E at RAC. Based on the aggregating ability of LMW subunits of glutenin and the evidence of their causal relationship with dough property (Pogna *et al.*, 1988), it is suggested that it was the LMW subunit of glutenin rather than the extra gliadin band which was mainly responsible for the improved dough properties and that allele *Glu-A3c* is better that its null allele *Glu-A3e* for dough properties. Similarly, comparison of the dough quality of families possessing the *Glu-A1b* allele (phenotype Glu-A3c$^-$ Glu-A1b$^+$) and those having allele *Glu-A1c* (phenotype Glu-A3c$^-$ Glu-A1b$^-$) indicated that *Glu-A1b* was associated with significantly higher R_{max} at both locations and E at RAC than the null allele *Glu-A1c*. The higher dough strength associated with Glu-A3c and Glu-A1b bands over their null phenotypes may have been due to the quantitative differences in the glutenin concentration.

5.3.1.4 Relative effects of Glu-A3c and Glu-A1b on dough quality

The average effect on R_{max} and E of either LMW allele *Glu-A3c* or HMW allele *Glu-A1b* was highly significant ($F > 5.53$, $P < 0.05$) at both sites (Table 5.5). The LMW allele *Glu-*

A3c was, however, associated with a relatively larger effect on R_{max} than HMW allele *Glu-A1b* at both the sites (Table 5.5). The two alleles had the same relative magnitude of effects on dough extensibility. The presence of both alleles together gave higher R_{max} and E values than either one separately, suggesting that these alleles at the *Glu-A3* and *Glu-A1* loci act in an additive manner on quality attributes at both high and low levels of flour protein (Fig. 5.4A, B). The combined effect of these alleles did not differ significantly ($F < 2.05$, $P > 0.05$) from the simple sum of their individual effects, confirming their additive behaviour.

Table 5.5 Average effects of bands Glu-A3c and Glu-A1b on R_{max} and E at two sites (RAC and BTN)

Type of effect	Protein bands	R_{max} (BU)		E (cm)	
		RAC	BTN	RAC	BTN
Individual	Glu-A3c	63.8	44.0	1.4	0.5
	Glu-A1b	35.5	17.1	0.8	0.4
	SED	9.8	7.6	0.32	0.24
Observed combined	Glu-A3c & Glu-A1b	109.3	79.1	2.6	1.2
Expected combined[a]	Glu-A3c + Glu-A1b	99.3	61.1	2.2	0.9
Observed – Expected		10.0	18.0	0.4	0.3

[a] Based on additive model.

5.3.2 Biotypes of Condor, Gamenya and BT-2288

The biotypes within Condor, Gamenya and BT-2288 cultivars were found to be agronomically identical and electrophoretically they had no protein differences other than those already considered so they can be assumed to have almost identical genetic constitutions. The biotypes in Condor and Gamenya had the same LMW glutenin subunit/gliadin differences (Fig. 5.5A, B). Biotypes Condor A2 and Gamenya A contained LMW band Glu-A3b (marked by ➤, Fig. 5.5A, slots b, c) and gliadin Gli-A1b (marked by ➤, Fig. 5.5B, slots c-d, e-f) while Condor A1 and Gamenya B carried the LMW band Glu-A3c (marked by Δ, Fig. 5.5A, slots a, d) and gliadin Gli-A1c (marked by Δ, Fig. 5.5B, slots a-b, g-h). Since these cultivars have quite unrelated pedigrees (Moss and Wrigley, 1974), these biotypes provided

Figure 5.5 A, B, C. SDS-PAGE patterns of SDS-extracted proteins from biotypes in cultivars Condor, Gamenya and BT-2288.

A. Two-step SDS-PAGE patterns of biotypes (a) Condor A1, (b) Condor A2, (c) Gamenya A, (d) Gamenya B, (e) BT-2288 A and (f) BT-2288B.

B. One-dimensional SDS-PAGE patterns of biotypes (a, b) Condor A1, (c, d) Condor A2, (e, f) Gamenya A and (g, h) Gamenya B.

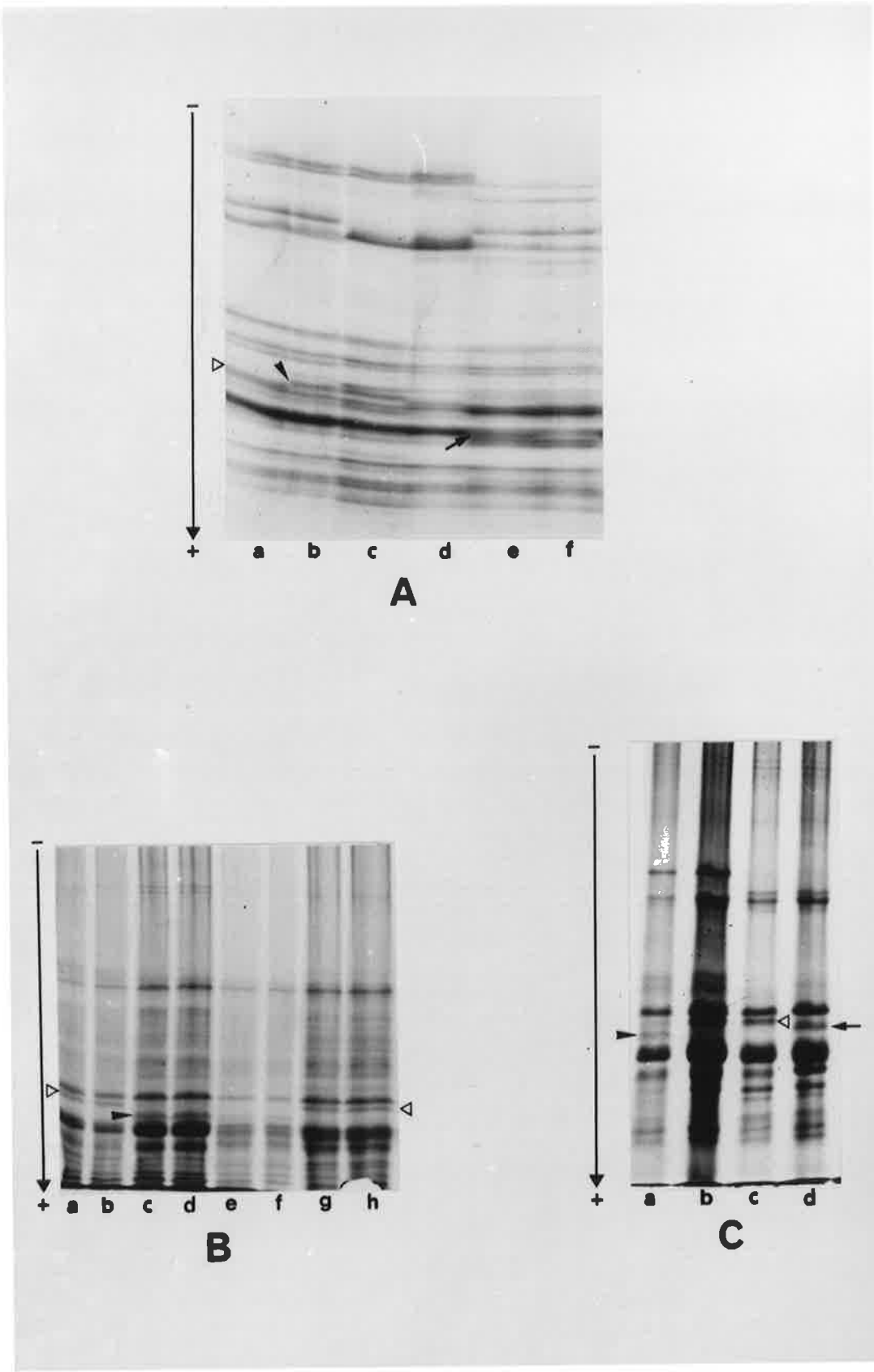
C. One-dimensional SDS-PAGE patterns of biotypes (a) Condor A2, (b) Condor A1, (c) BT-2288B and (d) BT-2288A.

Symbols used in the figure;

➤ = Glu-A3b and Gli-A1b bands,

Δ = Glu-A3c and Gli-A1c bands,

→ = Glu-A3f and Gli-A1f bands.


−
+
a b c d e f
A
−
+
a b c d e f g h
B
−
+
a b c d
C

an opportunity to analyse the relationship between these protein bands and physical dough properties in two diverse genetic backgrounds.

Biotypes A and B of BT-2288 were distinguished by LMW subunit/gliadin bands Glu-A3f/Gli-A1f (band marked by →, Fig. 5.5A, slots e; Fig. 5.5C, slot d) and Glu-A3c/Gli-A1c (see gliadin band indicated by Δ, Fig. 5.5C, slot c), respectively. The presence of LMW subunit band Glu-A3c in biotype B (Fig. 5.5A, slot f), which was overlapped by a Glu-B3 band, was inferred from the presence of the Gli-A1c gliadin band linked to it (Gupta *et al.*, 1989). It is clear that biotype B carried the gliadin band Gli-A1c present in Condor A1 and Gamenya B biotypes (Fig. 5.5C) which also had the Glu-A3c band. Gliadin band Gli-A1f occupied an intermediate position between Gli-A1c and Gli-A1b bands (Fig. 5.5C).

The dough quality and flour protein data from these biotypes at two sites are presented in Table 5.6.

Table 5.6 Dough properties of the biotypes differing in Glu-A3/Gli-A1 bands

Biotypes	LMW subunit band patterns	Mean values and significance level for:					
		R_{max} (BU)		E (cm)		Protein %	
		RAC	BTN	RAC	BTN	RAC	BTN
Condor A2	Glu-A3b	220*	246	19.9*	21.6***	11.0	11.0
Condor A1	Glu-A3c	200	250	17.9	19.5	10.8	11.1
Gamenya A	Glu-A3b	234	285	17.6	18.8*	9.0	10.9
Gamenya B	Glu-A3c	206	297	15.7	16.6	8.8	10.9
BT-2288 B	Glu-A3c	268	285	16.3*	17.6**	9.4	10.6
BT-2288 A	Glu-A3f	243	281	15.1	15.9	8.9	10.6

*, **, ***: lower member of the pair is different from upper member at $P < 0.05$, 0.01 and 0.001, respectively

Condor and Gamenya biotypes having Glu-A3b/Gli-A1b bands had significantly higher dough extensibility (E) at both the sites than the biotypes having bands Glu-A3c/Gli-A1c (the difference between Gamenya biotypes for E was close to the significant level ($P= 0.09$) at RAC). The difference in R_{max} was significant between biotypes only for Condor at RAC. Since in both cultivars, greater extensibility was associated with Glu-A3b/Gli-A1b bands than

with Glu-A3c/Gli-A1c, it is most likely that this association is not due to any spurious correlation or background genes.

Similarly, biotype B of cultivar BT-2288 with bands Glu-A3c/Gli-A1c gave significantly higher dough extensibility at both locations than the biotype A having Glu-A3f/Gli-A1f bands (Table 5.6) indicating that Glu-A3c/Gli-A1c confer greater extensibility than the bands allelic to them.

Since flour protein concentration did not differ significantly between biotypes within a cultivar, the differences in extensibility can be accounted for by the allelic variation at the *Glu-A3/Gli-A1* loci.

5.3.3 2RS chromotypes

The banding patterns of the total protein extracts from wheat cultivars Timgalen and Gabo, rye cultivar Imperial and 2RS chromotypes (Fig. 5.6) showed that total protein extracts from 2RS chromotypes were identical to each other and to Gabo except for presence of 75 kd γ-secalin bands coded by *Sec-2* locus on 2RS. The *Sec-2* locus codes for at least three bands (see bands shown by a bracket, Fig. 5.6, slot b) but only the fastest band could be scored easily because the others had the same mobility as HMW subunits 17+18 and 12 of Gabo.

Timgalen (Fig. 5.6, slot a), the original background parent for this translocation, has Gabo in its parentage and had the same band patterns as Gabo (Fig. 5.6, slot c) for gliadins (Wrigley *et al.*, 1982b), LMW subunits (see Appendix Table) and showed only a few differences in HMW subunits of glutenin (Lawrence, 1986). Thus uniformity for proteins was quickly attained among these backcross lines (chromotypes). Moreover, since these lines were isolated from limited backcrossing, the lines which were similar in plant morphology were chosen to increase the other genetic similarity. Nevertheless, several lines (7 isolates of each chromotype) were selected to accommodate the effects of background gene segregation on data. The chromotypes were similar to each other ~~other~~ and to Gabo morphologically except that, like Timgalen, they had long awns. These lines, from two replications, were analysed for protein content, grain yield and dough properties (Table 5.7). The data from both the replications were pooled for analysis of variance because the differences between the replications were insignificant ($P > 0.08$) for the parameters measured.

Figure 5.6. One-dimensional SDS-PAGE patterns of total reduced proteins from (a) Timgalen, (b) Gabo, (c) Imperial rye, (d, e, f) chromotypes 2BL– 2RS and (g, h, i) 2BL-2BS. 75 kD γ-secalins controlled by chromosome arm 2RS are labelled. ➤ = one of the 75 kD γ-secalin bands which is clearly resolved in Gabo background.

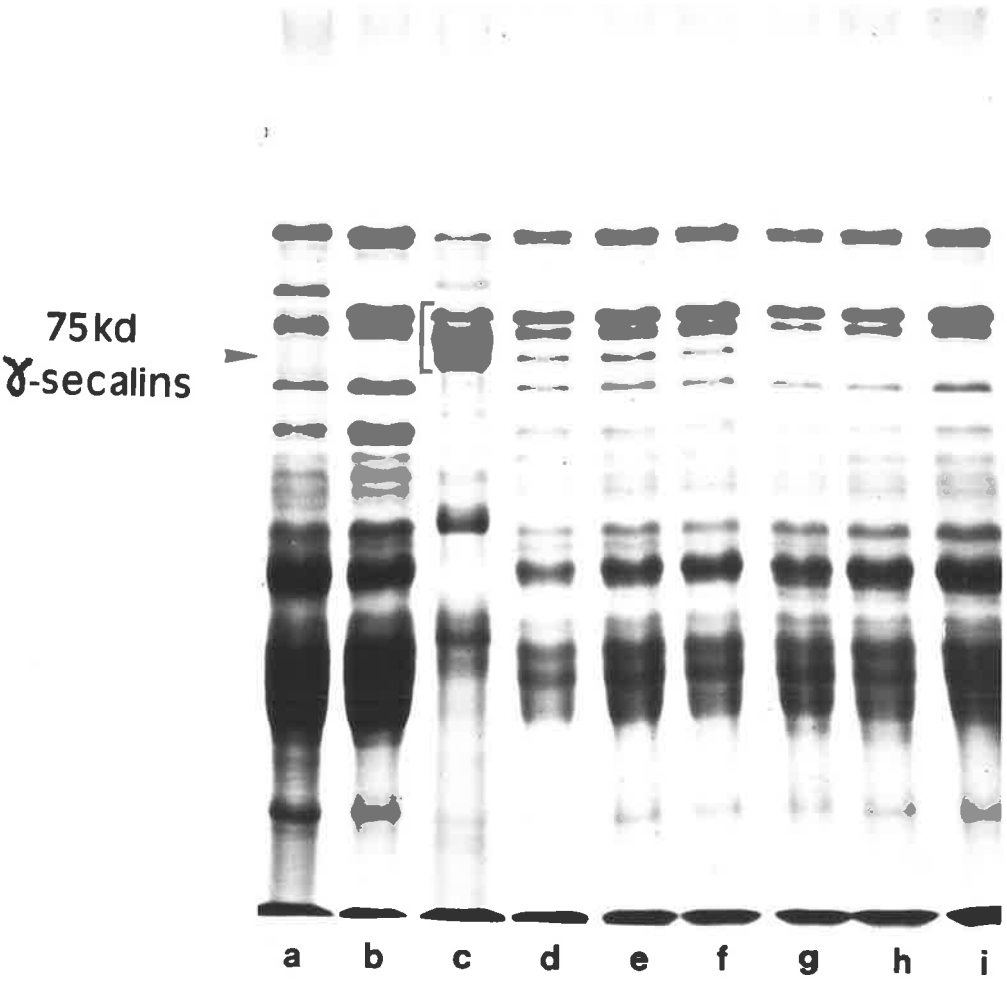

75kd
γ-secalins
a
b
c
d
e
f
g
h
i

Table 5.7 Grain yield and quality attributes of 2RS chromotypes (mean of 14 lines/chromotypes)

Chromotypes	Protein bands	Mean values			
		R_{max} (BU)	E (cm)	Protein %	Grain yield (g)
2BL-2RS	Sec-2^{+}	298	17.4	13.8	330
2BL-2BS	Sec-2^{-}	271	17.0	11.8	372
Significance (P)		0.006	0.432	0.001	0.059

The 2BL-2RS lines gave significantly higher flour protein (2% more) and dough elasticity than those with 2BS (i.e. lacking 2RS) (Table 5.7). These increases in flour quality can be at least partly attributed to Sec-2 proteins (75 kd γ-secalins) because they were additional to those proteins present in normal line. The 2BL-2RS lines, however, had significantly lower grain yield. Nevertheless, these data clearly demonstrated that presence of 75 kd γ-secalins was not associated with negative effects on dough visco-elasticity, as earlier suggested by Field and Shewry (1987).

5.4 DISCUSSION

Since the amounts of flour protein at the two sites (RAC and BTN) were very different, the effects of allelic variation at the *Glu-A3/Gli-A1* and *Glu-A1* loci on dough quality could be tested at two extremes of flour protein. The LMW subunit allele *Glu-A3c* and HMW subunit allele *Glu-A1b* were associated with larger effects on dough parameters than the null alleles *Glu-A3e* and *Glu-A1c* and this could possibly be due to the greater amount of glutenin in lines having Glu-A3c and Glu-A1b bands. Moreover, these bands had cumulative effects on R_{max} and E at both protein levels (Gupta and Shepherd, 1987; Gupta *et al.*, 1989). Similarly, work of Payne *et al.* (1987a) indicated additive effects of a LMW subunit (corresponding to band Glu-A3a of Gupta and Shepherd, 1988 and Chapter 3) and a HMW subunit (Glu-A1a) on bread-making quality (as measured by sedimentation test and extensometer), in the progeny of a cross between Chinese Spring and Chinese Spring (Hope 1A) substitution line. Since cumulative effects were observed in these two studies with different alleles (at the same loci) and different genetic backgrounds, these results highlight the combined role of LMW and HMW glutenin subunits in affecting bread-making quality. This suggests that combination of different glutenin bands having positive effects will be more effective and important than individual glutenin bands in improving the bread-making quality of wheat.

Another interesting finding of the present study was that LMW glutenin band Glu-A3c was associated with significantly greater dough resistance than the HMW glutenin band Glu-A1b. In contrast, Payne *et al.* (1987a) detected no difference in the relative effects of another pair of LMW and HMW glutenin bands (Glu-A3a and Glu-A1a) on sedimentation volume and dough elasticity in their study. Visual assessment of the staining intensity of the LMW subunit Glu-A3c and HMW subunit Glu-A1b did not indicate any major difference in the amounts of these bands, thus it is speculated that differences in the number and positions of cysteine residues (Moonen *et al.*, 1985) in their polypeptide chains could be responsible for the observed differences in dough viscoelasticity. However, a precise measurement of the protein content of bands is required to validate this suggestion.

The observed strong positive effect of flour protein quantity on physical dough properties is in accordance with its positive effect on other quality parameters viz. bread loaf

volume and sedimentation volume (Bailey and Sherwood 1926; Finney and Barmore 1948; Bushuk *et al.*, 1969). Of particular interest is the much larger effect of protein concentration on dough extensibility than on dough resistance. Although this indicates that dough extensibility can easily be improved by increasing grain protein level, the well known inverse relationship between grain protein and grain yield makes it difficult. The significant cumulative effects of allelic variation at the *Glu-A3* and *Glu-A1* loci on dough extensibility at two given protein levels (7% and 14%) (Table 5.4) suggested that selection of such alleles will help improve extensibility regardless of the grain protein level. This is illustrated by progeny carrying *Glu-A3c* and *Glu-A1b* alleles having the largest E while progeny with null alleles *Glu-A3e* and *Glu-A1c* have the lowest E at both protein levels (Fig. 5.4B). The data from biotypes differing in LMW subunit composition also revealed that LMW subunit alleles are associated with differences in the dough extensibility. The relative performance of various alleles at the *Glu-A3* locus for dough extensibility can be summarised as follows;

$$Glu\text{-}A3b > Glu\text{-}A3c > Glu\text{-}A3f > Glu\text{-}A3e < Glu\text{-}A3a.$$

In contrast to dough extensibility, higher dough resistance cannot be guaranteed by an increased level of grain protein concentration and the presence of suitable types (and amounts) of glutenin subunits is essential. For example, the progeny having alleles LMW *Glu-A3c* and HMW *Glu-A1c* (phenotype Glu-A3c^{-} Glu-A1b^{-}) at 14% protein level had a much lower dough resistance than those having alleles *Glu-A3c* and *Glu-A1b* (phenotype Glu-A3c^{+} Glu-A1b^{+}) at the 7% protein level (Fig. 5.4A).

The data clearly indicated that LMW and HMW subunits of glutenin contribute to both dough resistance and extensibility. The association found between HMW subunits of glutenin and dough visco-elasticity can almost certainly be attributed to the bands themselves as these are not known to have tight linkage with any other storage protein markers. In contrast, whether the quality differences associated with LMW subunits of glutenin are due to these subunits or due to the genetically linked gliadins is difficult to determine. A similar dilemma may also be seen in earlier reports of associations between certain gliadins and bread wheat quality with unknown (Sozinov and Poperelya, 1980; Wrigley *et al.* 1981; Branlard and Dardevet, 1985a; Campbell *et al.*, 1987) or known linkages with LMW subunits (Payne *et al.*, 1987a). Although aggregating ability of glutenin subunits is helpful in relating the dough

quality differences to glutenin bands (Autran *et al.*, 1987; Payne *et al.*, 1987a), it does not provide conclusive evidence. Thus partitioning of the individual effects of LMW subunits and gliadins is essential to prove which one of them is the causal factor for extensibility in bread wheat. This could be achieved in the future by breaking the genetic linkage between these two types of proteins, by directly adding (reconstituting) these proteins to wheat flours after purifying them chemically (MacRitchie, 1978, 1987) or by synthesizing them *in vitro* after cloning the genes coding for them. For example, Pogna *et al.* (1988) found a durum wheat which possessed gliadin band 42 and LMW glutenin band LMW-2 and showed that the gluten elasticity of this naturally occurring recombinant line was equal to high quality durum wheats having gliadin band 45 and LMW-2 and thus provided evidence for causal relationship between the LMW glutenin bands and gluten elasticity rather than between the gliadins and gluten elasticity. Hence, it can be suggested that association observed between LMW subunit of glutenin/gliadins and dough resistance in bread wheat is also due to the LMW subunits.

These data do not, however, exclude gliadins as being the factor responsible for changes in the dough extensibility in bread wheat. Indeed, since gliadin as a whole is a viscous mass and glutenin is elastic (Dimler, 1965), these two quality parameters may be affected simultaneously where variation in LMW glutenin subunits is linked with gliadin variation. On the basis of size and aggregation behaviour, LMW subunits of glutenin are believed to influence both elasticity and viscosity whereas gliadins are thought to influence only viscosity (Shewry *et al.*, 1984c). All these data suggest that elasticity associated with gliadins/LMW subunits could be related to LMW subunits of glutenin. Whether the extensibility differences are the results of differences in both gliadins/LMW subunits or in only one of these, cannot be determined without partitioning their effects. This applies to the data from biotypes where gliadins/LMW subunits were associated with dough extensibility only.

Rye glutelins (75 kd γ-secalins) also had a highly significant positive effect on dough resistance but the increase in dough extensibility was not significant. These data were inconsistent with those of Field and Shewry (1987) that incorporation of these proteins may bring deleterious effects on dough visco-elasticity. Their conclusions, however, were based on data obtained using gel filtration chromatography and they argued that 75 kd γ-secalins, due to the formation of alcohol-soluble oligomers and polymers, would decrease the amount of insoluble glutelins (aggregates of very large sizes) which are correlated with good bread-

making properties (Field *et al.*, 1983) and hence would cause deterioration in the physical dough quality. The data from the present study might imply that the overall glutelin in wheat flour is more important for dough elasticity than just the glutenins of very high molecular weights. Since these rye glutelins appeared to be in large quantity, it is likely that their presence in the translocation line might have increased total glutelins in the flour and hence its dough resistance. The observed quality effect could also be explained if these secalins might have increased the types of subunits present in the glutelin which in turn might have improved the functionality of glutelin. In fact, it has recently been shown that these secalins, like HMW subunits of glutenin, form β-turns (P R Shewry, personal communication) which are important for elasticity (Shewry *et al.*, 1984c; Tatham *et al.*, 1985, 1987).

The data from 2RS chromotypes also showed that the chromosome arm 2RS (coding for 75 kd γ-secalins) was associated with significantly higher (2% more) flour protein level and supported that grain protein level can be increased in bread wheat by the incorporation of this arm (Jagannath and Bhatia, 1972; May and Appels, 1978, 1984). Since the yield of the 2BL-2RS lines was significantly lower, however, this strategy may be of limited use from the breeding point of view. It cannot be determined from the data presented in this thesis whether the increase in the flour protein content was due to a direct effect of *Sec-2* genes, other genes on 2RS probably coding for other proteins such as albumins, globulins or due to indirect effects (physiological changes such as increased nitrogen uptake or translocation into the grains, reduced grain size, less starch accumulation in the grain). It is likely, however, that at least a part of the increase in the protein level is due to the presence or absence of *Sec-2* genes. It is also not known whether the yield decrease was associated with *Sec-2* genes or with some other genes on chromosome 2RS of rye. If the other genes were responsible, they could be eliminated in the long term while the *Sec-2* genes were retained by inducing homoeologous pairing (Koebner and Shepherd, 1985, 1986) between chromosome arms 2RS and 2BS.

The data presented in this chapter thus highlight the agronomic significance of disulphide-linked proteins (LMW and HMW subunits of glutelin, 75 kd γ-secalins) and point to the need for more detailed analyses of the associations between the extensive allelic variation detected in LMW glutenin subunits (Chapter 3) and bread-making quality. Some work is in progress regarding the evaluation of effects of LMW subunits controlled by *Glu-D3* genes on dough quality. Isogenic lines with null, *Glu-D3a* or *Glu-D3b* alleles are being

produced by backcrossing the wheat-rye recombinant lines of Koebner and Shepherd (1986) into Gabo wheat. Biotypes differing at the *Glu-B3* and *Glu-D3* loci are being multiplied for dough testing.

Chapter 6

GENETICAL AND BIOCHEMICAL STUDIES OF THE NON-PROLAMINS IDENTIFIED BY TWO-STEP SDS-PAGE

6.1 INTRODUCTION

Gliadins and glutenins are believed to be held together by both covalent and noncovalent forces in gluten and they form the major portion of the gluten, responsible for its unique visco-elastic property (Dimler, 1965; Khan and Bushuk, 1979; Shewry *et al.*, 1984c). Some albumins and globulins with molecular weights of 30 to 70 kd often have also been reported to be associated with glutenin (Rothfus and Crow, 1968; Huebner *et al.*, 1974; Cole *et al.*, 1981; Graveland *et al.*, 1982) even after exhaustive extraction and thus they have been considered as an integral part of the glutenin complex (Khan and Bushuk, 1979). Payne *et al.* (1985) also found some albumins and globulins of similar sizes which were present in gluten. Their presence with gluten was suggested to be due to their denaturation (hence insoluble in water or salt solutions) during gluten preparation and not because of any covalent linkages between them and the gluten proteins. Singh and Shepherd (1985), however, showed that some globulins (triplet bands) controlled by the short arms of chromosomes 1A and 1D, were covalently linked with glutenins. Influence of such albumins and globulins on gluten properties is not clearly known but Graveland *et al.* (1982) have shown that the addition of certain protein fractions (glutelin I and II) carrying such albumins and globulins bands of 30-95 kd to wheat flour were detrimental to dough quality.

As described in Chapter 3, some ethanol insoluble bands (hence called non-prolamins) were also separated with the glutenin subunits when fractionated by two-step SDS-PAGE. The presence of these non-prolamin bands in 2-step gels raised several questions about their physical relation with glutenin. For example, whether they were associated with the glutenin by covalent or noncovalent bonds or were they merely trapped within the glutenin. Besides, their genetic control, solubility and biological role in wheat endosperm were not known, so these aspects were investigated in the present chapter.

6.2 MATERIALS AND METHODS

6.2.1 Plant materials

Seeds of Chinese Spring (CS), CS nullisomic-tetrasomic lines 4D-4A, 5A-5D, 5B-5A, 5D-5B; CS tetrasomics 4D, 5A, 5D; CS ditelocentrics 4DL, 5AL, 5BL, 5DL (Sears, 1954, 1966; Sears and Sears, 1978), CS-*Triticum umbellulatum* amphiploid (Kimber, 1967), *T. umbellulatum*, CS-*T. umbellulatum* substitutions 5U(5A), 5U(5B) and 5U(5D) (Chapman and Riley, 1970) and Gabo and Gabo-Imperial rye translocation 1BL-1RS/1DL-1RS (Shepherd, unpublished) were analysed. Seeds of the Triple translocation stock (Chapter 4) were also analysed.

Protein bodies, isolated by Singh and Shepherd (1987) from the developing endosperm (about 21 days after anthesis) of Chinese Spring wheat using the sucrose density gradient ultracentrifugation procedure of Miflin *et al.* (1981), were also analysed.

6.2.2 Protein extraction and electrophoresis

The unreduced and reduced total proteins or prolamins from endosperm were extracted as described in Chapter 3. Similarly, total reduced protein extracts from the germinating seeds (endosperms collected from two seeds were pooled at each stage) and from the different organs of the Gabo wheat plant (50 mg of fresh tissue) were also obtained by using 1 ml of Tris-HCl buffer containing SDS.

The albumins were extracted by dissolving a portion of seed endosperm in 1 ml distilled water for 20 hours at room temperature and the top 50-70% portion of the supernatant was collected after 10 minutes of centrifugation at 10,000 rpm. The supernatant was dried by allowing the water to evaporate at 80°C and the residue was dissolved in 100 µl of Tris-HCl buffer containing 4% SDS (with 1.5% 2-ME v/v for reduced albumins). Aliquots of 20 and 70µl of this solution were loaded into each slot for 1-D and 2-D gel electrophoresis, respectively.

The 1-D, 2-D and two-step electrophoresis and staining and destaining of gels were conducted as described in Chapters 3 and 4.

6.2.3 Molecular weight calibration

The molecular weights of wheat proteins were estimated by comparison with the mobilities of the following proteins used as standards (Sigma): β-Galactosidase (116,000), Phosphorylase B (97,000), Bovine albumin (66,000), Egg albumin (44,000) and Carbonic anhydrase (29,000).

6.3 RESULTS

6.3.1 Genetic control of non-prolamins

When SDS extracts from the Triple translocation (TTr) stock were fractionated by the 2-step SDS-PAGE (Fig. 6.1B, slot a), at least 8 different bands (excluding HMW subunits of glutenin) were detected and they were controlled by genes on chromosome arms other than the 1AS, 1BS and 1DS missing from this stock. Ethanol extracts from the TTr stock revealed only three of these 8 bands (Fig. 6.1A) and they were found to be the C subunits of glutenin controlled by genes on 1RS and 6DS (Fig. 6.1A, see Chapter 4 for details). The remaining five bands were insoluble in ethanol and hence were classified as non-prolamins (Fig. 6.1B).

The genetic control of these five non-prolamin bands was ascertained by analysis of nulli-tetrasomic stocks of Chinese Spring and the substitution of *T. umbellulatum* chromosome 5U for group 5 chromosomes of Chinese Spring. The slowest moving non-prolamin band (seen as a doublet in Fig. 6.1B and 6.4A and hence considered as two bands) with apparent MW of 69 kd was absent in NT 4D-4A (Fig. 6.1C, slot a) but was present in Dt 4DL (Fig. 6.1C, slot b) indicating that this band was controlled by chromosome arm 4DL. A band of similar electrophoretic mobility to this band had been assigned to chromosome arm 4DL but this was described as a subunit of glutenin (Bietz *et al.*, 1975). The two bands adjacent to the 4DL band, with greater mobility (63, 60kd), were absent in CS-*T. umbellulatum* substitution lines 5U (5B) (Fig. 6.1C, slot g) and 5U (5A) (Fig. 6.1C, slot f), respectively, indicating that the genes controlling them are located on chromosomes 5B and 5A. Corresponding analysis of the 5U (5D) stock (Fig. 6.1C, slot h) revealed that a fast moving band (45 kd) was controlled by a gene(s) located on chromosome 5D.

Nullisomic-tetrasomic, ditelocentric and tetrasomic stocks for group 5 chromosomes in Chinese Spring, were also analysed by two-step and 1-D SDS-PAGE and these confirmed the above results. Moreover, they revealed that bands controlled by chromosomes 5A, 5B and 5D were present in Dt 5AL (Fig. 6.1D, slot n), 5BL and 5DL (Fig. 6.1D, slot s) stocks (two-step patterns not shown) indicating that they are controlled by the long arms of the respective chromosomes. The presence or absence of the 5BL-controlled band could not be determined

Figure 6.1. SDS-PAGE patterns of endosperm proteins extracted in (**A**) 70% ethanol and (**B**, **C**, **D**) Tris-HCl buffer containing SDS.

A. Two-step pattern of Triple translocation stock (TTr).

B. Two-step pattern of (a) TTr and (b) Chinese Spring (CS).

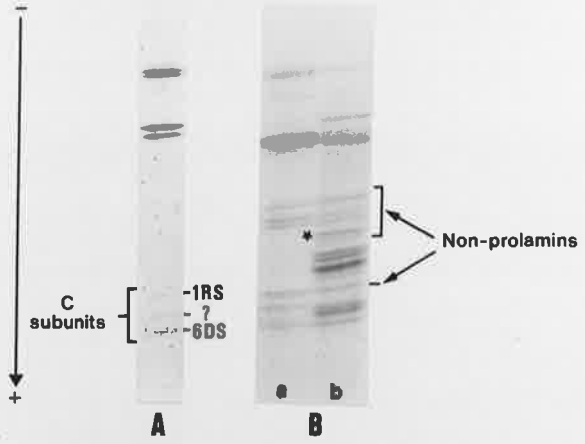
C. Two-step pattern of (a) CS nullisomic-tetrasomic (NT) 4D-4A, (b) CS ditelocentric (Dt) 4DL, (c) CS, (d) *T. umbellulatum*, (e) CS-*T. umbellulatum* amphiploid, and (f, g, h) CS-*T. umbellulatum* substitution lines 5U (5A), 5U (5B) and 5U (5D), respectively.

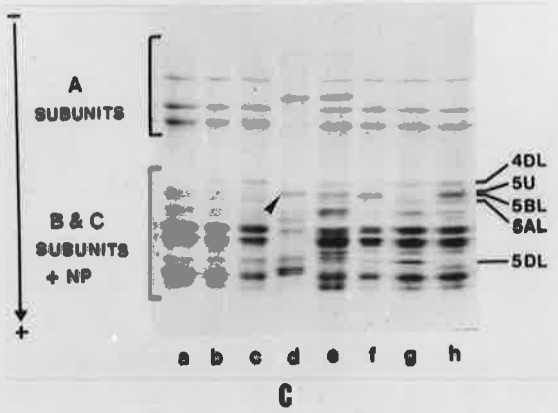
D. One-dimensional pattern of (a) TTr, (b) Molecular weight markers, 1= β-Galactosidase (116, 000), 2 = Phosphorylase B (97, 000), 3 = Bovine Albumin (66, 000), 4 = Egg albumin (44, 000) and 5 = Carbonic Anhydrase (29, 000), (c) CS, (d) CS NT 4D-4A, (e) CS Dt 4DL, (f) CS tetrasomic (Tetra) 4D, (g) CS, (h) *T. umbellulatum*, (i) CS-*T. umbellulatum* amphiploid, (j, k, l) CS-*T. umbellulatum* substitution lines 5U (5A), 5U (5B) and 5U (5D), respectively, (m) CS NT 5A-5D, (n) CS Dt 5AL, (o) CS tetra 5A, (p) CS NT 5B-5A, (q) CS Dt 5BL, (r) CS NT 5D-5B, (s) CS Dt 5DL, and (t) CS tetra 5D.

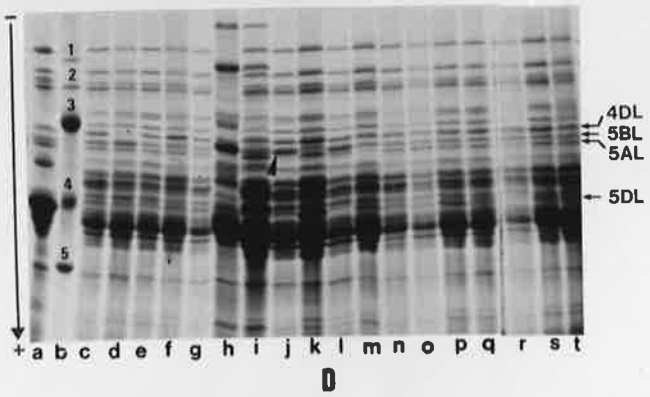
Symbols used:

* denotes triplet subunits,

➤ = band controlled by chromosome 5U.


C subunits
-1RS
- ?
-6DS
Non-prolamins
a b
A
B


A SUBUNITS
B & C SUBUNITS + NP
4DL
5U
5BL
5AL
5DL
a b c d e f g h
C


1
2
3
4
5
4DL
5BL
5AL
5DL
a b c d e f g h i j k l m n o p q r s t
D

in the 1-D separation of total protein extract (Fig. 6.1C), because there was another band in Chinese Spring which overlapped in mobility.

During the genetic analysis of non-prolamin bands in wheat using CS-*T. umbellulatum* substitution lines, an accession of *T. umbellulatum* and Chinese Spring and the amphiploid between them were also analysed by the two-step procedure. The amphiploid (Fig. 6.1C, slot e) carried an extra band to those in Chinese Spring (Fig. 6.1C, slot c) in the HMW non-prolamin region and this band was also present in *T. umbellulatum* (marked by ➤, Fig. 6.1C, slot d). Since this band was not seen in the ethanol extract of the same stocks (Chapter 3), it was also a non-prolamin band. Interestingly, this band was found to be expressed in 5U (5D) (Fig. 6.1C, slot h) and 5U (5A) (Fig. 6.1C, slot f) but not in 5U (5B) (Fig. 6.1C, slot g). Since homoeologous recombination is known to occur in the 5U (5B) stock due to the absence of chromosome 5B (Chapman and Riley, 1970), the seed tested may have been deficient for the gene(s) on 5U responsible for the synthesis of this band and hence did not show the band. This band was not resolved very well in the 5A (5U) stock in the two-step procedure but it could be seen clearly in the 1-D pattern (indicated by ➤, Fig. 6.1D, slot j). Thus, it can be concluded that this band was controlled by gene(s) on chromosome 5U. This band probably corresponds to a band of similar mobility reported by Lawrence and Shepherd (1981a) to be controlled by chromosome 5U in total protein extracts of 5U addition lines in Chinese Spring.

6.3.2 Aggregation behaviour of non-prolamin bands

The absence of these non-prolamin bands, while glutenin subunits were present, in the two-step gel patterns of ethanol extracts (Fig. 6.1A) suggested that they were possibly not linked to glutenins through disulphide bonds (covalent bonds). If they had been covalently linked to glutenin or trapped within it, they would be expected to be resolved along with the subunits of glutenin in the two-step separation. Thus, this indicated that they were not covalently linked with the glutenin or the subunits of the glutenin. Furthermore, if they had been associated with glutenin by other than covalent forces, they should have been released from glutenin when extracted in the presence of strong dissociating reagents such as SDS and consequently they should not have been resolved in 2-step gels. Their resolution with glutenin subunits in the absence of covalent association, however, could have been due to disulphide linkages

among their own molecules which become so large that they were present in the streaky portion of the first step gels, like native glutenin.

To investigate this possibility, SDS extracts of the Triple translocation stock were fractionated by non-reducing × reducing diagonal electrophoresis. In this procedure, bands forming parallel lines off the diagonal were considered to be present as originally disulphide-linked aggregates (polymers) and those on the diagonal lines as monomers (Wang and Richards, 1974; Singh and Shepherd, 1985). The non-prolamin bands gave clear parallel lines which extended onto the diagonal line (lines marked by →, Fig. 6.2A, c). It was concluded that these protein bands occurred in both polymeric and monomeric forms. The subunits which were not used in the polymer formation were present as monomers.

The other parallel lines in Fig. 6.2A with greater mobility than the non-prolamins corresponded to the C subunits of glutelin, but it cannot be excluded that some of them were also non-prolamins of overlapping mobility. Other known non-prolamins such as triplet subunits were not seen in the SDS extract of the Triple translocation stock because of their control by genes on 1AS and 1DS (Singh and Shepherd, 1985) which are lacking in this stock. The triplet bands could be observed in two-step patterns of the SDS-extracted proteins from the wheat cultivars or genetic stocks carrying these chromosome arms (bands marked by *, Fig. 6.1; Singh and Shepherd, 1985) but they were absent from two-step band patterns of ethanol-extracted endosperm proteins (Chapter 3). Considering that these subunits have been shown to be present in the form of disulphide-linked aggregates (Singh and Shepherd, 1985), it is suggested that aggregates of triplet bands like the other non-prolamin aggregates examined here, may also be linked with glutenins by noncovalent forces. Moreover, the present results do not support the hypothesis of Singh and Shepherd (1985) that these subunits are linked with the subunits of glutenin in the formation of polymers.

6.3.3 Solubility behaviour of non-prolamin bands

Although the non-prolamin bands (excluding triplet subunits) had not been analysed previously by the two-step method, they have been observed in other electrophoretic systems. Their solubility behaviour, however, was not clearly defined. For example, the 4DL band had variously been classified as a HMW subunit of glutenin (Bietz *et al.*, 1975), a HMW globulin band (Brown and Flavell, 1981) and then tentatively as an albumin band (Garcia-

Figure 6.2. SDS-PAGE patterns of endosperm proteins from Triple translocation stock (TTr).

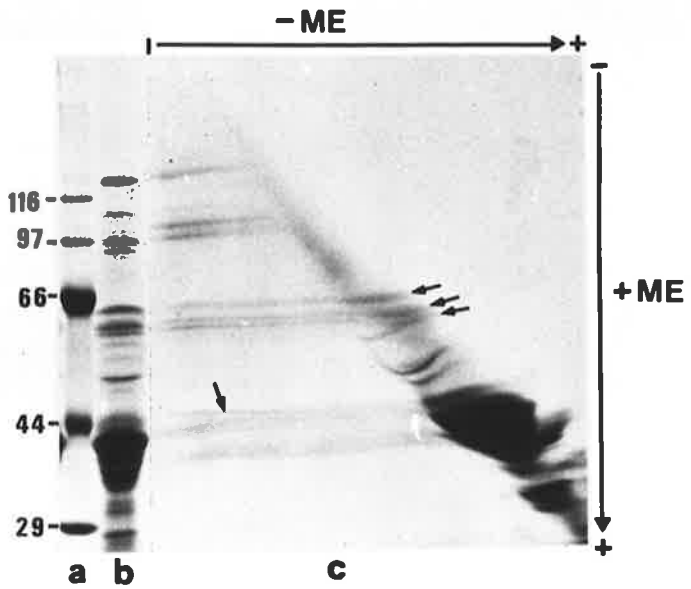
A . One-dimensional patterns of (a) molecular weight markers in kilodaltons, β-Galactosidase (116), Phosphorylase B (97), Bovine Albumin (66), Egg albumin (44) and Carbonic Anhydrase (29), (b) reduced SDS extract from the TTr stock and (c) Diagonal (unreduced × reduced) electrophoresis of SDS extract from TTr.

B . One-dimensional patterns of reduced (a) distilled water extracts after 20 hours treatment, (b) SDS extracts from TTr stock, (c) molecular weight markers as above (*1* = 116 kd, *2* = 97 kd, *3* = 66 kd, *4* = 44 kd, *5* = 29 kd), (d) distilled water extract after 40 hours treatment treated and (e) Diagonal (unreduced × reduced) electrophoresis of water extract (40 hours treatment) from the TTr stock.

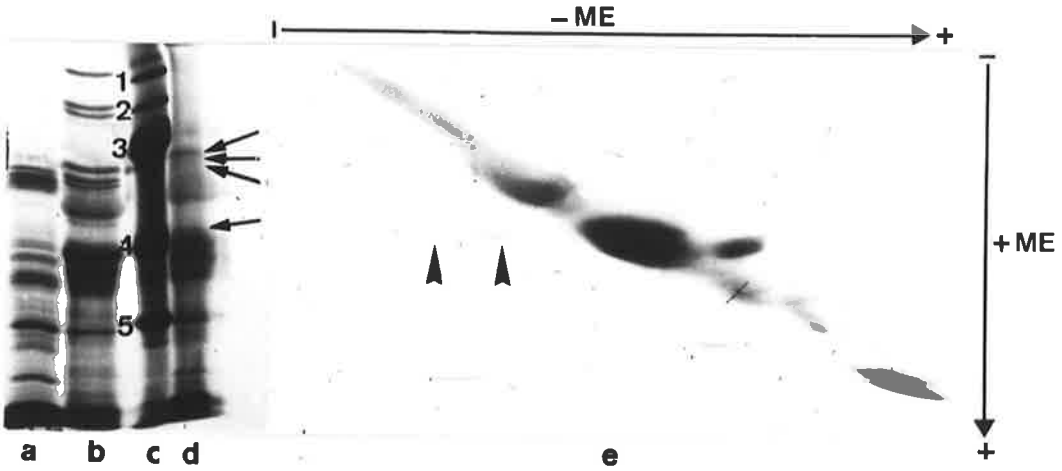
Symbols used:

→ = Non-prolamin bands controlled by 4DL, 5BL, 5AL and 5DL,

➤ = Off-diagonal spots with similar mobility to 5DL-controlled band.



A



B

Olmedo *et al.*, 1982). Three heavy streaks similar in electrophoretic mobility to these 4DL, 5BL and 5AL bands were identified using 2-D methods and characterized as HMW globulins (Payne *et al.*, 1984d) and then as HMW albumins (Payne *et al.*, 1985).

Since these non-prolamins were present in the water extract (Fig. 6.2B, slot a), they could be classified as albumins. It should be noted that the 4DL-controlled albumin band usually took longer to be extracted sufficiently in the water (Fig. 6.2B, slots a, d).

To investigate whether both monomeric and polymeric albumins were soluble in water in the native state (i.e. in the absence of the disulphide bond reducing reagent, 2-ME), the water extract from the endosperm of Triple translocation stock was analysed by diagonal electrophoresis (Fig. 6.2B, e). The extract exhibited spots on the diagonal line corresponding to the position of these albumins but did not show any clearly defined parallel line off the diagonal except for two diffuse spots (marked by ➤) identical in mobility to the 5DL band. This suggested that some dimer, trimer or small size polymers of this albumin band were formed and they were soluble in water in their native state. The lack of parallel lines indicated that large polymers which were SDS-soluble (Fig. 6.2A) were not solubilized in water. Since the mobility and genetic control of the water soluble bands were the same as the SDS-soluble bands in two-step separation, they were considered to be the same albumins. These results thus confirmed the recent conclusion of Payne *et al.* (1985) that they are albumins.

6.3.4 Degradation of albumin bands during seed germination

The apparent disulphide-linkage of these non-prolamins indicated that they were different from the usual albumins and thus they required further investigation.

The electrophoretic patterns of total reduced proteins from germinating seeds of Gabo were analysed by 1-D SDS-PAGE to investigate their sequential degradation during seed germination and early seedling growth and the results are shown in Fig. 6.3A. These albumin bands (marked by ➤) disappeared rapidly during germination. Their intensity was markedly reduced only 3 days after imbibition (or 2 days after germination) and they could not be detected 5 days after germination. These changes in albumins were accompanied by parallel changes in HMW subunits (A subunits) of glutenin and gliadins (e.g. ω-gliadin). In contrast, one protein band (marked by ➤, Fig. 6.3A) did not show any significant decrease in intensity and remained prominent until 9 days after imbibition while another protein band (marked by

Figure 6.3. One-dimensional SDS-PAGE patterns of SDS extracted proteins.

A . Protein extracts from the endosperm of mature (M) and germinating seeds of Gabo and mature endosperm of Triple translocation (TTr) stock.

DAI = days after imbibition, MW = molecular weight markers in kilodaltons namely, β-Galactosidase (116), Phosphorylase B (97), Bovine Albumin (66), Egg albumin (44) and Carbonic Anhydrase (29).

B . Protein extracts from leaf (from 5 day-old seedling), root (from 3 day-old seedling) and undifferentiated shoot (from 3 day-old seedling) of the Gabo seedling and the mature endosperm of TTr.

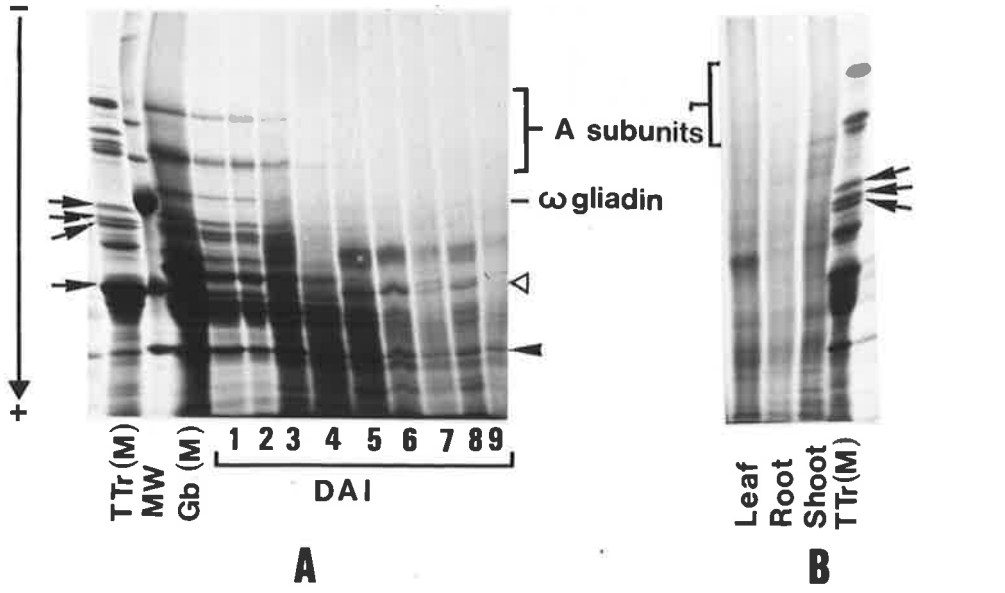
C . Fifteen sucrose density gradient fractions (c-q) from the developing endosperms of Chinese Spring wheat isolated by Singh and Shepherd (1987). The density of fractions corresponding to protein bodies (e, f, g) was 1.28, 1.27 and 1.26 g/ml, respectively. The density of first (c) and last fraction (q) was 1.29 and 1.08 g/ml. Endosperm protein extracts from the mature seeds of Chinese Spring (b) and TTr stock (r) and the molecular weight markers (a) are also given for comparison.

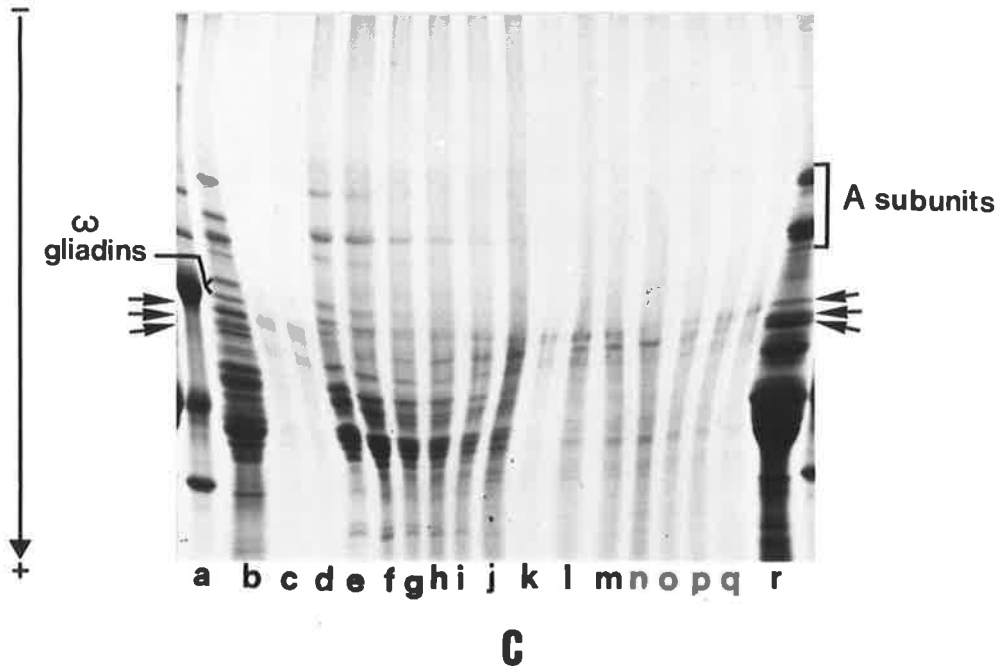
Symbols used:

→ = albumin bands,

➤ = a structural protein band,

Δ = a metabolic protein band.


A subunits
ω gliadin
TTr (M)
MW
Gb (M)
1 2 3 4 5 6 7 8 9
DAI
A
Leaf
Root
Shoot
TTr(M)
B


ω
gliadins
A subunits
a b c d e f g h i j k l m n o p q r
C

Δ, Fig. 6.3A) did not appear until 3 days after imbibition and was present until 8 days. These two bands could be structural and metabolic (newly synthesized enzyme protein e.g. α-amylase) proteins, respectively. Since proteins which are rapidly degraded and utilised during germination are considered to be storage proteins (Murray, 1984), e.g. HMW subunits of glutenin, gliadins (Coulson and Sim, 1965; Dell'Aquilla *et al.*, 1983) and triplet bands (a globulin protein; Singh and Shepherd, 1987), it is suggested that these albumin proteins could also be storage proteins. Furthermore, as shown in Fig. 6.3B, these proteins were not present in 3 day-old root, undifferentiated shoot and 5 day-old leaf tissues suggesting that they were seed specific like the other wheat storage proteins.

To determine whether these albumins also resemble storage proteins in their subcellular localization in the endosperm, the fifteen sucrose gradient fractions (density ranging from 1.29 to 1.08 g/ml) isolated from the developing wheat endosperms of Chinese Spring by Singh and Shepherd (1987) were analysed by 1-D SDS-PAGE after dissolving them in Tris-HCl buffer containing SDS and 2-ME. The albumin bands (denoted by ➛, Fig. 6.3C) were not present in the fractions (Fig. 6.3C, slots e, f, g) having the average density (1.27 g/ml) corresponding to the protein bodies (Singh and Shepherd, 1987). These fractions however, contained the HMW subunits of glutenin, gliadins (Fig. 6.3C) and triplet bands (Singh and Shepherd, 1987). Trace amounts of these storage proteins appeared in the three subsequent fractions (Fig. 6.3C, slots h, i, j) and one band similar in mobility to the 5AL-controlled albumin was also present in these as well as in the fractions lower in density (Fig. 6.3C, slots k-o). A band similar in mobility to the 4DL-controlled albumin band was present in all the fractions but this band did not appear to represent a protein band as it appeared in control samples lacking any protein and having SDS buffer + 2-ME.

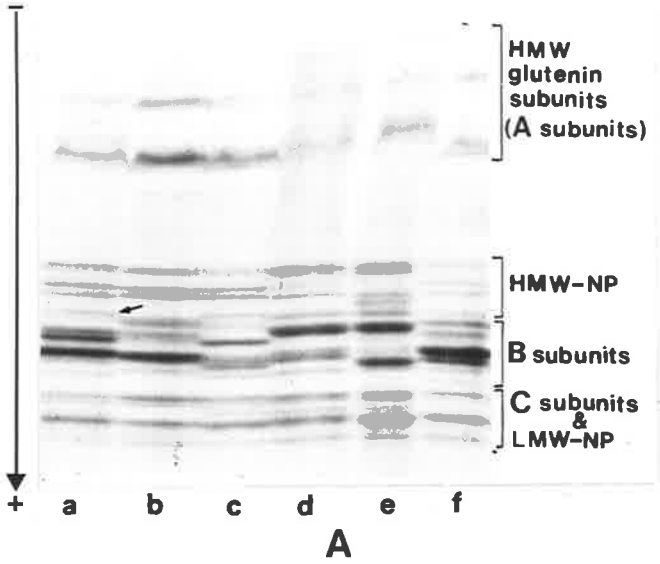
6.3.5 Variation in non-prolamin bands

During the examination of the B and C subunits of glutenin in bread wheat, a limited amount of variation in the non-prolamin band patterns was also recorded (Fig. 6.4A). The band patterns observed could be divided into groups on the basis of their alternative (mutually exclusive) behaviour among different bread wheat cultivars (Fig. 6.4B). Since one of the patterns in each group was from Chinese Spring with known genetic control, the chromosomal control of the other patterns could be inferred. Thus, two different band

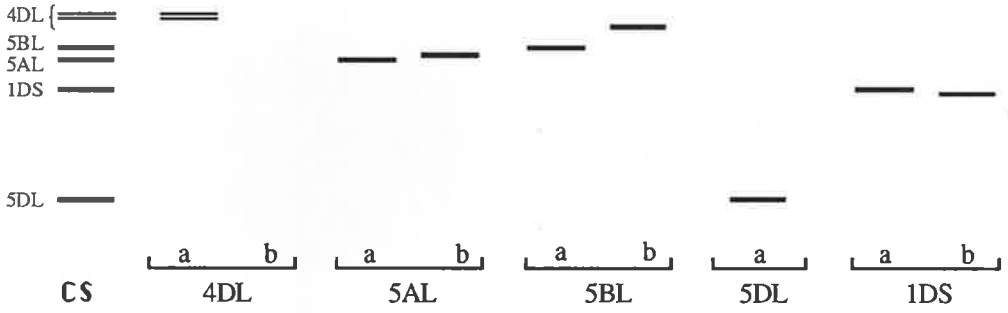
Figure 6.4. Two-step banding patterns of non-prolamins in bread wheat.

A . Endosperm proteins extracted in Tris-HCl buffer containing SDS from bread wheat cultivars; (a) Chinese Spring, (b) Gabo, (c) Halberd, (d) Norin-61A, (e) Norin-61B, and (f) Kalyansona-227. → = Triplet 1D subunit in Chinese Spring.

B . Diagram showing the two-step band patterns of non-prolamins. The Chinese Spring non-prolamin bands are included for reference.


HMW
glutenin
subunits
(A subunits)
HMW-NP
B subunits
C subunits
&
LMW-NP
+
a
b
c
d
e
f

A


4DL
5BL
5AL
1DS
5DL
a
b
a
b
a
b
a
a
b
CS
4DL
5AL
5BL
5DL
1DS

B

patterns controlled by each of 4DL, 5BL and 5AL were detected, but no variation among cultivars was recorded for the 5DL-controlled band. The band pair controlled by 4DL was absent from some cultivars and these cultivars probably carried a null phenotype.

Variation in the non-prolamin bands viz. the large triplet subunits known to be controlled by the short arms of chromosomes 1A and 1D (Singh and Shepherd, 1985) were also analysed during the varietal survey for B and C subunits. The subunits controlled by 1DS showed two types of patterns (Fig. 6.4B, Chinese Spring pattern is marked by →) but the subunits controlled by 1AS could not be scored reliably enough, since they co-migrated with the B subunits of glutenin. The band patterns controlled by 1DS were inherited as alternatives among 78 test-cross progeny from [(Gabo × Insignia) × Triple translocation stock] indicating that they were controlled by allelic genes (Gupta and Shepherd, 1987). The small subunits of triplet proteins were much faster in mobility than the B and C subunits (Singh and Shepherd, 1985) and hence were not analysed for variation in this study.

6.4 DISCUSSION

The results presented in this chapter indicate that the albumin bands appearing in two-step SDS-PAGE are most likely disulphide-linked subunits and that they are degraded during seed germination like storage proteins. Hence, they are considered to be a new class of albumins. These subunits, in order of their size (45, 60, 63 and 69 kd), are controlled by genes on the chromosome arms 5DL, 5AL, 5BL and 4DL in Chinese Spring wheat. Chromosome 5U of *T. umbellulatum* also carried genes for a non-prolamin band. The presence of genes controlling non-prolamin bands on chromosome 5A, 5B and 5D of bread wheat and 5U of *T. umbellulatum* further supported the homoeologous relationship between group 5 chromosomes of these species (Chapman and Riley, 1970).

It is likely that these albumin bands correspond to some of the albumin and globulin bands found in reduced glutenin (Rothfus and Crow, 1968; Huebner *et al.*, 1974; Khan and Bushuk, 1979; Cole *et al.*, 1981) as they were similar in size (64-70kd, 40kd) as judged by electrophoretic mobility. Bands with similar properties, soluble in SDS solution but insoluble in 70% ethanol, were also detected in the glutelin I and II fractions by Graveland *et al.* (1982) and were described as β- and γ-subunits. Some non-prolamins present in wheat endosperm have been classified as ~~protein~~ protease inhibitors (Shewry *et al.*, 1984d) but since they are much smaller (< 20kd) than the proteins detected here, it is unlikely that these albumin bands belong to this class.

The formation of parallel lines away from the diagonal lines and the presence of spots on the diagonal suggest that the albumin bands extracted in Tris-HCl buffer having SDS, are present in both multimeric as well as monomeric forms. Furthermore, the presence of these albumin proteins (extracted in water) as diagonal spots and some off-diagonal spots similar in mobility to the 5DL band suggested that only monomers and some oligomers formed by the 5DL-controlled band were soluble in water at room temperature. The large sizes of multimeric proteins could be responsible for their low solubility in water and thus for their presence in the gluten ball and in glutenin preparations. Any non-covalent association between the aggregates of these two groups of proteins would contribute to their insolubility in water. In view of these data, it seems that the denaturation of these proteins during extraction and dough mixing

may not be the main cause of their association with gluten as suggested by Payne *et al.* (1985).

The separation of these albumin subunits in two-step gels (when extracted with Tris-HCl buffer containing SDS) along with the subunits of glutenin and triplet bands indicated that they all contribute to streakiness near the origin in unreduced 1-D gels. Nevertheless, mutually exclusive separation of the non-prolamin subunits and the glutenin subunits in 2-step electrophoresis when extracted with 70% ethanol indicated that the non-prolamin subunits or the aggregates were probably non-covalently linked with the glutenin subunits or the aggregates. Within the limitation of gel concentration used (7.5–15%), it seemed that the non-prolamin subunits were involved with the subunits of glutenin in the formation of disulphide-linked mixed polymers. It is emphasized that the different solvents used (SDS or ethanol) should not have caused a major difference in the size of aggregates entering the same pore-size gel. On the other hand, they may have extracted aggregates similar in size but different in subunit composition. For example, mixed polymers produced between the subunits of glutenin and non-prolamins may not be solubilised in 70% ethanol due to the hydrophobicity of non-prolamins, but they could be soluble in the solvent containing SDS. Thus, the possibility cannot be ruled out that subunits of glutenin and non-prolamins may be involved together in the formation of disulphide-linked polymers of larger sizes not able to penetrate the gel pore-size used.

The disappearance of albumin subunit proteins during seed germination suggested that they behaved biologically like storage proteins and hence were inconsistent with the traditional assumption of an entirely non-storage function for albumins. Similarly, the study by Singh and Shepherd (1987) showed that some globulin proteins (triplet bands) in wheat are storage proteins. Nevertheless, unlike triplet bands or other known storage proteins viz. glutenin subunits and gliadins, these albumins were not found in the developing wheat endosperm fractions representing protein bodies and this was in agreement with the data by Payne *et al.* (1986). Some of these albumins were, however, seen in the fractions with smaller densities. Since two size types of protein bodies have been reported in wheat (Graham *et al.*, 1963) and in rice (Tanaka *et al.*, 1980) and it has been shown that amino acid composition of small protein bodies resembled albumins and globulins in wheat (Jennings and Morton, 1963) and globulin in rice (Tanaka *et al.*, 1980), the possibility of the presence of these albumins in the

small protein bodies cannot be ruled out. Certainly, further work on the subcellular localization of these albumins in wheat endosperm is needed.

Moreover, these non-prolamins may have some effect on the functionality of glutenin and thus on dough quality because they are polymeric proteins (glutelins) and are tenaciously associated with the glutenin, responsible for dough elasticity. Furthermore, they appear to make up a significant proportion of seed proteins and thus of gluten. Some initial data have suggested that addition of the protein fractions, containing some of these non-prolamin bands, to dough causes a decrease in the dough quality (Graveland *et al.*, 1982; Forsyth *et al.*, 1987; MacRitchie, 1987). However, since these fractions also contained other non-prolamins, it cannot be determined whether the negative effect on dough quality was due to these proteins or due to others. Thus, further investigations on the functionality of these aggregating non-prolamins are required.

Chapter 7

GENERAL DISCUSSION

Wheat endosperm proteins can be classified into prolamins (gliadins, glutenins) and non-prolamins (albumins and globulins) on the basis of their solubility in alcohols (Shewry *et al.*, 1986b). Traditionally, prolamins have been recognized as the storage proteins and non-prolamins as the non-storage proteins in wheat (Shewry and Miflin, 1985 for a review). Moreover, albumins and globulins have been regarded as simple polypeptides. The recent data, however, have indicated that certain globulins (Singh and Shepherd, 1987) and albumins (Chapter 6) are disulphide-linked proteins and are storage proteins. Thus, these non-prolamins resemble the glutenins for these two properties. Interestingly, storage globulins (called triticin) have been shown to be considerably similar to HMW subunits of glutenin in solubility, amino acid sequences and immunological properties and it has been suggested that they might be evolutionarily related to these glutenin subunits (Singh *et al.*, 1988). The triticins, glutenins and gliadins are also cytogenetically similar, as they are controlled, at least in part, by group 1 chromosomes. The genes encoding the polymeric albumins are not yet known to be located on group 1 chromosomes. Nevertheless, Kreis *et al.* (1985) have suggested that some albumins (termed trypsin inhibitors, CM proteins) are evolutionarily related to prolamins and all of these have been derived from a common ancestral gene. On this basis, the existing differences among these proteins could be explained by postulating that genes encoding them have undergone extensive divergence since their separation.

Among the disulphide-linked proteins of wheat (HMW and LMW subunits of glutenin and storage non-prolamins), the HMW subunits (A subunits) have been studied most extensively for their genetic and molecular properties and for their importance in determining flour quality (see review by Payne, 1987). Such studies on LMW subunits of glutenin have only recently begun (Payne, loc. cit; Chapters 3, 4, 5). The non-prolamins have also been the subject of recent biochemical and genetical investigations (Chapter 6; Gupta and Shepherd,

1987; Forsyth *et al.*, 1987; Payne *et al.*, 1985, 1986; Singh *et al.*, 1988) but their effect on flour quality is not clearly known yet.

Extensive genetic variation of LMW subunits (B and C subunits) of glutenin has been found in hexaploid and tetraploid wheat (Chapter 3). Variation in cultivated and wild diploid *Triticum* species and *Elytrigia* species has also been recorded. Since B and C subunits exhibit different isoelectric points (Jackson *et al.*, 1983), electrophoretic mobility and form different combinations between each other (Chapter 3), they might belong to two gene sub-families. The inheritance studies of differences between these subunits, however, have shown that they are controlled by tightly linked genes which have been derived from a common ancestral gene through duplication and divergence (Chapter 4). The presence of B and C subunits in all the diploid and non-domesticated tetraploids species analysed, suggests that duplication of the ancestral gene must have occurred prior to the evolution of the A, B and D genome diploid species. These two gene sub-families seem to have since diversified into many allelic forms, giving rise to the extensive variation in the patterns observed.

Recently, using a group 1 chromosome-specific cDNA clone, pTag 544 from wheat endosperm, the nucleotide sequence of two major LMW subunit genes has been determined (Colot *et al.*, 1989). Quantitative Southern hybridizations to restriction enzyme digested shoot DNA have provided estimates that these genes are present in 10–15 copies per haploid genome (Harberd *et al.*, 1985), that is about 3–5 genes per *Glu-3* locus. These gene numbers could account for the number of LMW subunits found to be controlled by *Glu-B3* and *Glu-D3* loci. The number of genes at the *Glu-A3* locus, however, is greater than the number of LMW subunits (maximum three) coded by it, indicating that some of the genes at this locus are not expressed. Moreover, some cultivars do not have any bands controlled by this locus suggesting that none of the LMW genes is expressed. This suggests that genes at the *Glu-3* loci have undergone non-random diploidization, a phenomenon proposed earlier to account for variation at the *Gli-1* and *Glu-1* loci (Galili and Feldman, 1983b, c, Galili *et al.*, 1988). Since it is not known with certainty whether the cDNA clone represents a γ-gliadin or LMW subunits (Bartels *et al.*, 1986; Colot *et al.*, 1989), the validity of the conclusions above depends upon the accuracy of the estimate of gene number per *Glu-3* locus. It is not known whether the quantitatively minor subunits coded by these loci are the products of different genes and are expressed in small quantity or whether they are simply post-translational

modifications of major proteins. Some faintly stained HMW subunits have been suggested to be derived from the proteolytic cleavage of the major HMW protein (Holt *et al.*, 1981; Galili and Feldman, 1983b). Considering only the major LMW subunits and assuming that each major subunit is a product of a single gene, it can be suggested that the LMW subunits are coded by 3 to 8 different genes active among the different cultivars.

The molecular nature of the allelic variation in the LMW subunits of glutenin has not yet been studied. Studies on DNA for HMW subunit from various intervarietal chromosome substitution lines have shown that these subunits contain a repetitive domain of variable sizes and the electrophoretic mobilities of the subunits in SDS-PAGE are positively related to the length of the domain (Harberd *et al.*, 1986). If a similar situation exists with the LMW subunits of glutenin, it could be suggested that unequal crossing over has occurred between these domains and created the subunits of different mobilities. Differences between these subunits due to charge have also been recorded (Payne *et al.*, 1984b, 1987a) and these could arise from point mutations in the genes coding for them.

The data in this thesis have clearly demonstrated that the majority of the LMW subunits of glutenin are controlled by group 1 chromosomes but some LMW subunits (C subunits) appear to be controlled by genes on group 6 chromosomes (particularly 6DS). These newly discovered bands are regarded as glutelin subunits on the basis of their ethanol solubility and capacity to form parallel lines in diagonal electrophoresis (Chapter 4). Other LMW prolamin bands forming parallel lines were also detected but their chromosomal control ~~have~~ has not been determined due to their overlap with major C subunits.

It may be argued that the parallel lines in diagonal electrophoresis could also be formed if these bands were simply trapped within the different sized glutenin aggregates and were released only after the dissociation of polymers following the treatment with 2-mercaptoethanol. This possibility has not been ruled out but it is considered less likely because such physical trapping should not consistently produce the well defined parallel lines. As the polymeric character is taken as the main feature distinguishing glutenins from gliadins, some α-type gliadins, which are coded by group 6 chromosomes and have the capacity to form polymers (Kasarda *et al.*, 1987), can be considered as glutenin subunits. It is possible that these α-type gliadins are infact the C subunits (group 6 chromosome-controlled)

identified in the present study. Further information about their inter-relationship may come from the analysis of their isoelectric points and amino acid sequences.

The genes coding for the LMW subunits on group 1 chromosomes (*Glu-3*) are tightly linked to those coding for ω- and γ-gliadins (*Gli-1*). Based on this linkage, it can be speculated that genes coding for α- and β-gliadins (*Gli-2*) and C subunits on group 6 chromosomes may also be linked. Furthermore, data from aminoacid and nucleotide sequencing have shown sufficient sequence homologies between α- β- and γ-gliadins (Bartels and Thompson, 1983; Okita *et al.*, 1985) and between these and LMW subunit genes (Colot *et al.*, 1989), thus it is almost certain that genes on group 1 and group 6 chromosomes coding for these proteins arose from duplication and divergence of a common ancestral gene. The most obvious hypothesis to account for the spatial separation of these prolamin genes is an ancient translocation between these two groups of chromosomes. However, which chromosome, 1 or 6, carried the ancestral prolamin gene ?

A comparative study of species in the genera *Triticum* (including *Aegilops*), *Elytrigia* (previously *Agropyron*), *Secale* , undertaken in this thesis (Chapters 3, 4) and *Hordeum* (Payne *et al.*, 1982a for a review) showed that group 1 chromosomes in all these species carry genes for LMW subunits or equivalent proteins On the other hand, only in wheat and rye (*Secale montanum*), is the evidence for group 6 chromosomes carrying genes for such proteins. Chromosome 6 in barley does not code for any LMW glutelin subunit nor any other prolamins (Payne *et al.*, 1982a). Thus it can be suggested that genes controlling LMW glutelin subunits were originally present on group 1 chromosome and they later duplicated and translocated onto group 6 chromosomes. Such events appear to have occurred before the divergence of the ancestral diploid into *Secale* and *Triticum* species. The genes on 6R (*Sec-2*) in *S. montanum* might have been translocated again to chromosome arm 2R in *Secale cereale* after speciation because this locus is located on 2RS in cultivated rye (Shewry *et al.*, 1986a). There is no evidence for a similar translocation in *Hordeum* or its progenitor. It is also possible, however, that equivalent LMW glutelin genes on chromosome 6 in barley have been lost, or have become non-functional.

Besides providing information on allelic variation, linkage relationships and the evolution of prolamin (LMW glutelin and monomeric prolamins) genes in the Triticeae, the data presented in this thesis have agronomic significance as they could be used for varietal

identification and in developing strategies for improving the flour quality of wheat. Allelic variation in both LMW and HMW subunits of glutenin are related to variation in physical dough properties (Chapter 5). The association between HMW subunits of glutenin and dough parameters namely, resistance and extensibility, can be attributed to the bands themselves whereas the LMW subunits can be reliably associated with dough resistance only. Because of the linkage between LMW subunits and gliadins, dough extensibility can be associated with one or both of these proteins (as discussed in Chapter 5).

The question arises, whether these differences in dough quality were due to quantitative or qualitative differences between the glutenin/gliadin components. Where comparisons are made between two alleles, one coding for a detectable band and the other having a null phenotype (Gupta *et al.*, 1989; Payne *et al.*, 1987a), differences in the amount of glutenin may account for the observed dough quality differences. However, the structural or qualitative advantages of the detectable band cannot be ruled out. In other cases, where both alleles code for detectable bands with similar staining intensity, quantitative differences may not be obvious and thus structural differences have been assumed to be the main explanation for observed differences in flour quality (Gupta and Shepherd, 1988; Gupta *et al.*, 1989). For example, HMW subunits 5+10 and 2+12, although having similar staining intensity, are related to major flour quality differences (Payne *et al.*, 1981b; Moonen and Zeven, 1985; Lawrence *et al.*, 1987). Structural differences (number and position of cysteine residues) have been found between these HMW subunits (Moonen *et al.*, 1985; Goldsbrough *et al.*, 1988; Greene *et al.*, 1988) and it has been suggested that these are responsible for the observed flour quality differences. However, it is still not understood completely how such qualitative differences can eventually lead to differences in dough visco-elasticity. It is believed that cysteine residues in terminal locations could lead to the formation of linear polymers (Graveland *et al.*, 1985) and those in the middle and repetitive domains would allow for branching and cross-linking of polymers and interaction with LMW subunits of glutenin (Shewry *et al.*, 1984c). Thus, changes in the position and number of cysteine residues will affect the rheological properties of gluten or dough.

The HMW subunits have been considered to be the main elastic component of gluten because of their large size and the similarity of their molecular organization to that of elastin. Both of these proteins possess a β-spiral configuration which is believed to confer on them

intrinsic elasticity (Shewry *et al.*, 1984c; Tatham *et al.*, 1985, 1987). Since elasticity also needs some extensibility and is not mutually exclusive to it, increase in the former due to HMW subunits of glutenin may also influence the latter positively, as observed in the present study. The LMW subunits are thought to be mainly involved in branching with HMW subunits or with themselves and the gliadins provide molecular slippage between the polymers as well as their subunits (Shewry *et al.*, 1984c). Since it is not yet known whether the molecular organization of the LMW subunits is similar to elastin, the presumed secondary role of LMW subunits of glutenin in dough elasticity may be an underestimation of their significance. For example, the difference in dough elasticity associated with the LMW subunit Glu-A3c was significantly greater than that with HMW subunit Glu-A1b (Chapter 5). Since both bands showed similar staining intensity, structural differences seem to be the most likely explanation. From these data, it does not appear that size differences between LMW and HMW subunits are always critical, so their structural differences could be a major qualitative factor for the variation in dough quality.

On the basis of quantity, LMW subunits will be more important than HMW subunits as the former contribute about 30% of the total seed proteins whereas the latter make up only about 10% (Payne *et al.*, 1982a). Definite evidence on their relative importance is expected to come from analysing the dough properties of the 'Triple translocation stock' lacking most of the LMW subunits (Chapter 4) and the 'Triple null stock' lacking all HMW subunits of glutenin (Lawrence *et al.*, 1988). This work is currently in progress in collaboration with Dr. F. MacRitchie, CSIRO-Sydney.

The most important finding from the breeder's point of view is that the bread-making quality of wheat can be improved by selecting for the favourable glutenin bands (both LMW and HMW subunits), without increasing the protein concentration in the flour. This is of practical importance because total grain protein content is usually negatively correlated with grain yield. This hypothesis is based on the data (Chapter 5; Gupta *et al.*, 1989) that presence of certain HMW and LMW subunits had significant positive effects on physical dough properties at two constant levels of flour protein. Data have also revealed that incorporation of different LMW and HMW subunits in the same genotype has additive effects (Payne *et al.*, 1981b, 1987a; Moonen *et al.*, 1983; Gupta *et al.*, 1989).

It is expected that once sufficient data on the LMW subunits of glutenin have been obtained, they can be combined with those on HMW subunits to formulate a joint glutenin score for predicting the quality of segregating progeny in early generations. The joint score will be a more reliable criterion than only HMW or LMW glutenin score alone. For instance, some varieties such as Halberd, Heron, Insignia, Summit, Tincurrin and Egret in which HMW glutenin score is high i.e. 7 to 9 (according to Payne *et al.*, 1987a), nevertheless have poor dough resistance and bread-making quality. Similarly, some 1RS rye translocation lines present in Oxley, Cook and Egret wheat background with very high HMW glutenin score, also possess poor dough quality (Dhaliwal *et al.*, 1987). The most obvious reason for these discrepancies is that these cultivars or lines are deficient for LMW subunits of glutenin (see Appendix table and discussion section in Chapter 5). Moreover, dough quality of genotypes with the same HMW glutenin score but with different LMW subunit composition can differ (Chapter 5; Gupta and Shepherd, 1988). Thus, all these data suggest that both LMW and HMW subunits are important in determining the suitability of flour for bread-making. Hence, a new strategy for wheat breeders will be to cross varieties that have complementary, favourable quality glutenin subunits and to screen for the progeny which have the best combination of the LMW and HMW subunits i.e. the highest glutenin score.

Appendix A. Classification of 222 bread wheat cultivars/breeding lines with respect to Low-molecular-weight glutenin subunit composition

Cultivar/breeding line	Collection number	Country of Origin	Seeds tested	LMW glutenin band pattern Glu-A3	Glu-B3	Glu-D3
Almargarit	AUS 22253	USSR	4	b	d	a
Aotea	WRU 2481	New Zealand	5	e	b	b
Aranda	AUS 22506	France	3	e	g	b
Arawa	WRU 2482	New Zealand	3	d	h	b
Arcane	AUS 22507	France	3	c	c	a
Ariana–66	ACQ 11039	Tunisia	3	b	b	b
Aroona	ACQ 11166/11167	Australia	38	c	b	b
Arzu	AUS 22218	Austria	3	c	d	a
Azerbajzanskaja–1	AUS 22211	USSR	3	a	a	a
Bacvanka	AUS 22540	Yugoslavia	3	c	b	c
Bajio	ACQ 11040	Mexico	11	e	h	a
Barkai	AUS 22508	Yugoslavia	3	e	b	b
Bayonet	ACQ 11117–11120	Australia	9	c/e	b	b
BB-Inia	ACQ 11041	Mexico	6	e	h	c
Becejka	AUS 22548	Yugoslavia	4	e	e	c
Bencubbin	ACQ 11110	Australia	6	e	g	b
Bindawarra	ACQ 11121–11124	Australia	8	b	b	b
Bokal	WRU 923	Australia	6	e	b	b
Brimstone	AUS 22199	UK	4	c	f	c
Brock	AUS 22198	UK	3	e	g	c
BT–2288	AUS 12047	Tunisia	17	f/e	i	a
BT–2296	ACQ 11042	Tunisia	4	d	i	a
Bungulla	WRU 924	Australia	6	e	g	b
C–271	ACQ 11044	India	6	c	b	b
C–273	ACQ 11045	India	9	e	i	b
C–306	ACQ 11046	India	9	c	i	a
C–518	ACQ 11047	India	6	c	h	d
C–591	ACQ 7163	India	3	c	h	a
Calidad	ACQ 11048	Mexico	4	e	i	e
Cappelle-Desprez	ACQ 10861	France	6	d	g	c
Carazinho	ACQ 11049	Brazil	4	c	g	e
Cebecco–97 (1RS)	AUS 22212	Holland	4	d	–	c
Chanab–70	ACQ 11050	Pakistan	10	d	g	b
Cheyenne	ACQ 7712	USA	18	c	e	f?
Chhoti Lerma	ACQ 11051	India	3	d	h	a
Chinese Spring	ACQ 6739	China	100	a	a	a
Chris	ACQ 11052	USA	3	d	h	c
Ciano (S)	ACQ 11053	Mexico	2	c	g	c
Compton	AUS 22260	USA	6	e	g	a
Condor	ACQ 11125–11128	Australia	46	b/c	b	b
Cook	WRU 3876	Australia	16	b	b	b
Crespo	ACQ 11054	Colombia	3	b	c	a
Crim	ACQ 11055	USA	2	e	b	a
CSP–44	WRU 4121	Australia	6	b	b	b
Currawa	ACQ 11056	Australia	2	e	h	c
Darkan	AUS 99004	Australia	6	b	b	a
Dirk	WRU 926	Australia	6	e	b	b
Dugoklasa	AUS 22527	Yugoslavia	3	e	g	b
Dunav	AUS 10467	Yugoslavia	3	e	e	a
Eagle	WRU 3861/3862	Australia	8	e	b	b
Egret	WRU 3858	Australia	6	c	b	b
Emblem	WRU 930	Australia	7	e	b	a

Cultivar/breeding line	Collection number	Country of Origin	Seeds tested	LMW glutenin band pattern Glu-A3	Glu-B3	Glu-D3
Era	ACQ 11057	USA	3	c	b	c
Erenaceum–36	AUS 22269	USSR	2	c	f	a
Falcon	WRU 931	Australia	6	e	b	b
Federation	AUS 218	Australia	6	e	c	c
Festiguay	WRU 932	Austarlia	4	e	b	b
Festin	AUS 22510	France	3	e	b	b
Festival	AUS 2406	Australia	6	c	b	b
Fillmore	AUS 22259	USA	9	c	b	a
Fleuron	AUS 22155	Belgium	2	c	g	a
Free Gallipoli	ACQ 11058	Australia	3	e	c	c
Gabo	ACQ 1910	Australia	50	b	b	b
Gaboto	ACQ 11059	Argentina	3	c	c	a
Gala	WRU 934	Australia	6	e	b	b
Gambee	WRU 4069	Australia	6	b	b	b
Gamenya	WRU 3863/3864	Australia	29	b/c	b	b
Gamut	WRU 936	Australia	6	b	b	b
Gatcher	WRU 1444	Australia	6	e	b	b
Gawain	AUS 22196	UK	5	c	f	c
Gibrid–115	AUS 22213	USSR	5	c	b	a
Giza–150	ACQ 11060	Egypt	7	e	d	a
Glaive	WRU 938	Australia	4	b	b	b
Glenwari	WRU 1448	Australia	7	e	b	b
Gluclub	WRU 2941	Australia	5	e	d	a
Gluyas	ACQ 11112	Australia	4	e	d	c
Halberd	ACQ 11129–11132	Australia	30	e	c	c
Haruhikari	ACQ 11061	Japan	3	c	b	b
Heron	WRU 4070	Australia	6	e	c	c
Hezera–2152	ACQ 11062	Israel	3	c	g	c
Hira	ACQ 11063	India	10	d	h	b
Holdfast	ACQ 6796	UK	10	e	b	c
Hope	ACQ 6202	USA	14	e	b	a
Hopps	WRU 941	Australia	7	c	b	a
Huelquen	ACQ 11064	Chile	16	c/b	g	c
Illana	AUS 22219	Romania	10	c	f	a
India–115	ACQ 11111	India	8	c	c	?
Inia–66	ACQ 11065	Mexico	12	e	h	c
Insignia	WRU 4071	Australia	20	e	c	c
Isis	WRU 943	Australia	6	e	f	a
Jabiru	ACQ 11133–11136	Australia	12	c	c	c
Jaral*S*/Lee-SK-Mania	ACQ 11066	Zimbabwe	2	b	b	c
Jarka	AUS 22526	Yugoslavia	2	e	b	b
Jufy–1	ACQ 11067	Belgium	13	e	i	d
Justin	ACQ 11068	USA	3	e	b	a
Kalkee	WRU 2946	Australia	6	e	c	a
Kalyansona–227	ACQ 11069	India	8	c	g	a
Karamu	WRU 2485	New Zealand	5	c	b	b
Kenya Farmer	ACQ 6225	USA	5	b	b	a
Kenya Leopard	ACQ 11070	Kenya	3	e	b	b
Kewell	ACQ 11137–11144	Australia	12	c	g	b
Kharkov	ACQ 7486	USSR	3	c	g	a
Kite	ACQ 11114	Australia	20	c	b	b
Klein-Petiso RAF	ACQ 11071	Argentina	1	e	h	c
Koga	ACQ 11072	Belgium	3	e	g	a
Kolubara	AUS 22541	Yugoslavia	6	c	b	c
Kondut	WRU 944	Australia	6	e	a	a

Cultivar/breeding line	Collection number	Country of Origin	Seeds tested	LMW glutenin band pattern Glu-A3	Glu-B3	Glu-D3
Kopara	WRU 2486	New Zealand	6	b	h	a
Kosava	AUS 22542	Yugoslavia	2	c	e	b
Kozara (1RS)	AUS 22537	Yugoslavia	3	c	–	c
Lance	ACQ 11145–11153	Australia	12	c	b	b
La Prevision W 1636	ACQ 11038	France	6	e	h	b
Lelija	AUS 22529	Yugoslavia	4	e	b	b
Lerma–rojo 64A	ACQ 11073	Mexico	3	d	d	c
Licanka (1RS)	AUS 22547	Yugoslavia	3	c	–	a
Lovrin–13 (1RS)	AUS 22518	China	6	c	–	c
Lundi	ACQ 11074	Zimbabwe	3	c	f	a
Lutescens–505	AUS 22588	GDR	4	c	c	a
Macquarie	WRU 945	Australia	6	e	f	a
Macvanka–2 (1RS)	AUS 22544	Yugoslavia	3	c	–	c
Madden	WRU 4072	Australia	6	c	b	b
Magnif	ACQ 11075	Argentina	2	e	a	a
Manitou (Insen)	ACQ 11076	Canada	1	e	d	c
Mara-sepremo-mentana-mcm	ACQ 11077	Chile	1	c	f	b
Marquis	ACQ 11078	Canada	8	e	b	a
Mendos	WRU 946	Australia	6	b	b	b
Mengavi	WRU 947	Australia	6	b	b	b
Mersey	WRU 948	Australia	6	e	c	c
Mexicana–1481	ACQ 11079	Portugal	1	c	g	b
(MH 3*Warimba)MH9	ACQ 11080	Australia	3	c	b	b
(MH 3*Warimba)MH11	ACQ 11081	Australia	8	c	b	b
MKR (111/8)	ACQ 11113	Australia	40	c	b	b
Nabawa	ACQ 11082	Australia	2	e	g	a
Nainori–60	ACQ 11083	Mexico	13	b	b	b
Nanbukomugi	AUS 22504	Japan	5	d	c	c
Naphal	ACQ 7682	Nepal	5	c	d	b
Nayab–70	ACQ 11084	Pakistan	4	e	b	b
Neretva	AUS 22530	Yugoslavia	4	c	b	a
Neuzucht (1B/1R)	AUS 22520	GDR	3	e	–	c
Newbury	AUS 22556	UK	4	c	c	c
Norin–61	AUS 724	Japan	27	e/d	i	d/e
Nova Banatka	AUS 19797	Yugoslavia	3	c	e	a
Okukomugi	AUS 22505	Japan	17	d/e	d	e
Olympic	WRU 3860	Australia	6	b	b	a
Opal	ACQ 11085	Holland	6	e	g/b	a
Orca	ACQ 11086	Holland	18	d	d	e
Oxley	WRU 4073	Australia	8	b	b	b
Partizanka	AUS 20584	Yugoslavia	2	c	e	c
Pato Argentino	ACQ 11087	Argentina	3	d	i	e
Persang	AUS 22511	France	2	e	c	c
Pinnacle	WRU 950	Australia	6	e	c	c
Podunavka	AUS 22546	Yugoslavia	3	c	e	c
Posavka–1 (1RS)	AUS 22543	Yugoslavia	2	c	–	c
Potam–70	ACQ 11088	Mexico	18	c	g/i	a
Radja	AUS 22512	France	2	c	f	c
Raduse	AUS 22528	Yugoslavia	2	a	a	a
Rance	ACQ 11089	Australia	4	e	c	c
Raven	WRU 951	Australia	6	c	b	b
Red Egyptian	ACQ 11090	USA	10	e	g	a
Renard	AUS 22197	UK	4	e	c	c
Rescue	ACQ 4734	Canada	10	f	h	a
Roazon	AUS 22257	France	3	e	b	c

Cultivar/breeding line	Collection number	Country of Origin	Seeds tested	LMW glutenin band pattern Glu-A3	Glu-B3	Glu-D3
Robin	WRU 952	Australia	6	e	c	c
Romanija	AUS 22531	Yugoslavia	3	c	b	b
Roque–66	ACQ 11091	Mexico	1	e	b	b
Sabre	WRU 953	Australia	6	e	b	b
Saladin (1B/1R)	AUS 22173	GDR	5	c	–	c
Saturn	AUS 22214	FRG	3	d	g	c
Selkirk	ACQ 11092	Canada	4	e	g	a
Sherpa	WRU 954	Australia	6	c	b	b
Shortim	WRU 4149	Australia	6	b	b	b
Sidjanka	AUS 22533	Yugoslavia	4	e	b	b
Silvana	AUS 22220	Romania	3	d	h	c
Solitaire	AUS 22555	UK	5	c	g	c
Son–64*KL Rend	ACQ 11093	Argentina	1	e	h	b
Sonalika	ACQ 11094	India	12	e	h	c
Songlen	ACQ 11154–11157	Australia	9	a	b	b
Sonora–64A	ACQ 11095	Mexico	6	e	h	a
Spica	WRU 955	Australia	6	b	b	c
Summit	ACQ 11158–11161	Australia	11	e	c	c
Sun–92A	ACQ 10908	Australia	5	c	b	a
Sunkota	ACQ 11162–11165	Australia	7	b	b	a
Sutjeska	AUS 20081	Yugoslavia	3	c	–	c
Suwon–11	AUS 22519	North Korea	6	c	f	a
T–64–2W	ACQ 11096	Tunisia	2	d	b	c
Tainui	ACQ 11115	New Zealand	3	c	c	a
Takahe	WRU 2488	New Zealand	6	c	b	b
Tammi	AUS 7454	Finland	3	e	b	b
Tanori–71	ACQ 11097	Mexico	6	e	h	b
Tarsa	WRU 979	Australia	6	b	b	b
Teal	WRU 3874	Australia	6	e	g	b
Thatcher	ACQ 11098	Canada	12	e	h	e
Timgalen	WRU 3873	Australia	6	b	b	b
Timson	WRU 4151	Australia	6	c	b	b
Timstein	AUS 1393	USA	16	c/e	b	b
Tincurrin	WRU 3185	Australia	6	e	d	a
Tobari–66	ACQ 11099	Mexico	6	e	i	e
Toquifen*S*	ACQ 11100/11200	Chile	6	e	b/h	c/e
Turbo	AUS 22598	USA	3	c	g	c
Turpin–7	ACQ 11101	South Africa	6	b	b	b
Una	AUS 22545	Yugoslavia	3	c	g	b
UP–301	ACQ 11102	India	5	d	h	c
Ushiokomugi	AUS 22501	Japan	4	d	d	c
Vicam–71	ACQ11103	Mexico	6	e	g	a
Victor–1	ACQ 11104	Italy	6	c	c	b
Wards Prolific	ACQ 11105	Australia	2	e	g	a
Warigal	ACQ 11116	Australia	15	c	b	a
Warimba	WRU 2721	Australia	6	c	b	b
Wembley (1RS)	AUS 22554	UK	6	d	–	e
Windebri	WRU 958	Australia	6	e	c	a
Winglen	WRU 959	Australia	6	e	b	b
Wongoondy	WRU 960	Australia	6	e	g	b
Wren	WRU 1486	Australia	6	e	g	b
Xelaja–66	ACQ 11106	Guatemala	2		a	a
Yantar	AUS 22599	USA	2	e	b	c
Yolo	AUS 22265	USA	3	c	h	a

Cultivar/breeding line	Collection number	Country of Origin	Seeds tested	LMW glutenin band pattern Glu-A3	Glu-B3	Glu-D3
Zambezi	ACQ 11107	Zimbabwe	2	b	f	a
Zelengora (1RS)	AUS 22539	Yugoslavia	3	c	–	c
Zenith	WRU 2848	Australia	6	e	c	c
Zipa–68	ACQ 11108	Colombia	6	e	g	a
Zitnica	AUS 22536	Yugoslavia	4	a	a	a
Zrenjaninka	AUS 22534	Yugoslavia	3	c	g	a
8156 (White Grain)	ACQ 11109	Mexico	4	c/b	g	b

Notes; 1- India-115 lacked some C subunits controlled by *Glu-D3* locus. B subunits were Gabo type.
2- *Glu-D3* locus in Cheyenne probably codes for a pattern f, not detected in any other cultivar.
3- Presence of rye chromosome 1R or arm 1RS in cultivars is indicated in parenthesis. These cultivars did not carry any Glu-B3 pattern, as indicated by '–'.

Appendix B. Cultivars recommended as standards for each pattern controlled by *Glu-A3*, *Glu-B3* and *Glu-D3* loci

Locus	Patterns	Standard
Glu-A3	a	Chinese Spring
	b	Gabo
	c	Cheyenne
	d	Orca, Capelle-Desprez
	e	Hope, Insignia
	f	Rescue
Glu-B3	a	Chinese Spring
	b	Gabo, Timstein, Hope
	c	Insignia, Halberd
	d	Orca,
	e	Cheyenne
	f	Radja
	g	Kharkov, Bungulla
	h	Thatcher, Rescue
	i	Norin-61
Glu-D3	a	Chinese Spring
	b	Gabo
	c	Insignia, Capelle-Desprez
	d	Norin-61A
	e	Orca, Thatcher

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