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Chromosomal Location of Genes for Gliadin Polypeptides in Durum Wheat *Triticum turgidum* L.

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Summary. The chromosomal location of genes was determined for 19 of 30 gliadin bands extracted from seeds of a set of durum wheat (*Triticum turgidum* L. var. 'durum') aneuploids and durum cultivars. Individual bands were identified by their relative mobility on polyacrylamide gels. The gene(s) for gliadin band 45, which has been associated with strong gluten by several authors, was shown to be controlled by chromosome 1B. A band with similar mobility (band 46) was controlled by 'Chinese Spring' chromosome 1D. Conventional breeding procedures coupled with the use of electrophoresis of gliadin polypeptides should result in rapid conversion of current weaker gluten durum cultivars to strong gluten cultivars.

Key words: Aneuploids – Disomic substitution – Disomic addition – Double ditelosomic – Electrophoresis

Introduction

Durum wheat (*Triticum turgidum* L. var. 'durum') cultivars developed in the United States over the past 20 years have been noted for their desirable high semolina color and good pasta making qualities. However, these cultivars have moderate gluten strength as compared to Canadian and Italian cultivars which possess strong gluten. The USA cultivars have been criticized for their weaker gluten by processors in the European countries that import U.S. durum wheats. Recently, the North Dakota durum breeding program has produced two cultivars which possess strong gluten as well as other good quality characteristics. These cultivars are 'Edmore' (CI 17748) and 'Vic' (CI 17789).

Gluten strength can be measured by several methods such as: mixograph, farinograph, and SDS sedimentation test (Quick and Donnelly 1980). In addition Damidaux et al. (1978, 1980) found a strong relationship between the banding patterns of gliadin polypeptides separated by polyacrylamide gel electrophoresis (PAGE) and the viscoelastic properties of the endosperm proteins of durum wheat. Their conclusions were confirmed by Kosmolak et al. (1980). The PAGE procedure provides a powerful tool for selecting strong gluten genotypes of wheat, because a single kernel of wheat can be analyzed by this procedure. Furthermore, the embryo end of the kernel can be excised and germinated to produce a new plant, and the remaining endosperm can be used for PAGE analysis.

The inheritance and/or chromosomal location of genes for individual gliadin polypeptides in hexaploid wheat have been reported by Wrigley and Shepherd (1973), Kasarda et al. (1976), Sozinov et al. (1979), and Brown and Flavell (1981). These authors indicated that the gliadins were controlled by chromosomes 1A, 1B, 1D, 6A, 6B, and 6D of hexaploid wheat. The chromosomal control of the gliadin polypeptides in durum wheat has not been extensively studied because of the lack of suitable aneuploids in this species. However, Joppa et al. (1978 and unpublished) have recently produced a nearly complete set of durum aneuploids in which the seven D-genome chromosomes were individually substituted for their respective A- or B-genome homoeologues. In addition, chromosome 1B from 'Edmore' was substituted into 'Langdon' durum using the 'Langdon' 1D (1B) disomic-substitution line as the recurrent parent. In this study these and other aneuploids were used to determine the chromosomal location of genes controlling the gliadin polypeptides as determined by PAGE.

Materials and Methods

A set of 'Langdon' D-genome disomic-substitution lines each having a different D-genome chromosome pair substituted for

a homoeologous durum A- or B-genome chromosome pair has been produced. This set represents all possible disomic-nullisomic combinations, except 7D(7A) and 4D(4B) (Joppa et al. 1978 and unpublished). Each of the available disomic-substitutions has been crossed to normal 'Langdon' durum at least six times to eliminate most of the contribution of genes from 'Chinese Spring' wheat, except for genes on substituted D-genome chromosomes.

A few of the D-genome disomic-substitutions must be maintained in the telosomic or monosomic condition by carrying one of the chromosomes being substituted. For example: 3D(3B) is desynaptic and must be maintained as disomic 3D-telosomic 3B (13″ + 1″3D + t′3B). The long arm of 5 B contains a gene which prevents homoeologous pairing. Consequently, the 5D(5B) disomic-substitution is maintained as 13 pairs plus a 5D pair plus a telosomic 5BL (13″ + 1″ + t′5BL). The 6D(6B) disomic-substitution is pistilloid and both female and male sterile. However, it can be maintained as 13″ + 1″6D + t′6BS. The 5D(5A) disomic-substitution is usually male sterile when chromosome 5A is nullisomic, but can be maintained as 13″ + 1″5D + t′5AL. Kernels of the 3D(3B), 5D(5B), 5D(5A), and 6D(6B) lines were cut in half and the embryo half germinated. Kernels nullisomic for 3B, 5B, 5A, or 6B were determined by root-tip analysis of chromosome numbers using the Feulgen staining technique. Only the nullisomics were subjected to electrophoresis of gliadin polypeptides.

The strong gluten cultivar 'Edmore' was crossed with the 'Langdon' 1D(1B) disomic-substitution line and then backcrossed to the 'Langdon' 1D(1B) line five times to substitute chromosome 1B from 'Edmore' into 'Langdon' durum. The double-ditelosomic 1BS-1DS durum line was supplied by S. S. Maan (Maan 1977). Electrophoresis was performed on 'Langdon', 'Edmore', and 'Vic' durum wheats; the 'Langdon' D-genome disomic-substitution lines 1D(1A), 2D(2A), 3D(3A), 4D(4A), 5D(5A), 6D(6A), 1D(1B), 2D(2B), 3D(3B), 5D(5B), 6D(6B), and 7D(7B); the 'Langdon' D-genome disomic-addition lines 1D, 2D, 3D, 4D, 5D, and 6D; the 'Langdon' ('Edmore' 1B) disomic-substitution lines; and double-ditelosomic 1BS-1DS.

The PAGE procedure used in this study was essentially that of Bushuk and Zillman (1978) with some modifications (Khan, unpublished). The modifications included were as follows: the slot former was modified, the acrylamide and bisacrylamide were recrystallized before use, and the gel solution was degassed under vacuum before addition of the polymerization initiator hydrogen peroxide.

Individual bands in the gels were identified by their relative mobility (Rm) with reference to a distinct band which appeared in nearly all of the gels (band 50, Fig. 1). The nomenclature is as suggested by Bushuk and Zillman (1978) except that they refer to our band 50 as band 51. However, this had little effect on Rm calculation for the other bands. The sodium lactate pH 3.1 buffer system recommended by Bushuk et al. (1980) was used in all PAGE separations except where indicated otherwise.

Results and Discussion

The 'Langdon' durum D-genome disomic-substitution lines were subjected to polyacrylamide gel electrophoresis (PAGE) to determine which gliadin bands were controlled by particular chromosomes. Electrophoresis of the various lines produced 30 identifiable bands. Most bands were controlled by genes on the group one chromosomes (Table 1). The absence of chromosome 1B resulted in the loss of bands with relative mobility (Rm) values of 25, 32, 34, 36, 40 (faint), 42, 45, and 64 (Fig. 1). A durum wheat line ditelosomic for chromosome-arms 1BS and 1DS had all of the bands present in Langdon plus those present in the Langdon 1D(1B) disomic substitution line (Fig. 1, pattern 5). Consequently, it is the short arms of 1B and 1D which control these polypeptides. This conclusion agrees with the work of Wrigley (1970) and Sozinov et al. (1979).

'Chinese Spring' chromosome 1D controlled bands 16, 18, 46, and 52 (pattern 4). 'Langdon' chromosome 1A controlled bands 20 and 50 (pattern 3). The group six chromosomes controlled mostly α and β gliadins. Bands 73 and 78 were controlled by chromosome 6A, bands 47 and 48 by 6B, and bands 69, and possibly 75 and 76 by 'Chinese Spring' chromosome 6D (see patterns 7, 8 and 9). These results are in agreement with results from hexaploid wheat reported by Wrigley and Shepherd (1973), Kasarda et al. (1976) and Sozinov et al. (1979).

The chromosome or chromosomes controlling bands 23, 43, 54, 57, 59, 62, 80, 81 and 86 were not identified by the methods used in this study. It is

Table 1. Chromosomal location of genes for gliadin polypeptides and their relative mobility (Rm)

Rm value[a]	Chromosome number[b]					
	1A	1B	1D	6A	6B	6D
16			+			
18			+			
20	+					
25		+				
32		+				
34		+				
36		+				
40		+				
42		+				
45		+				
46			+			
47					+	
48					+	
50	+					
52			+			
64		+				
69						+
73				+		
75						+
76						+
78				+		

[a] Chromosomal location of bands 23, 43, 54, 57, 59, 62, 80, 81 and 86 could not be determined
[b] A plus indicates control by that chromosome

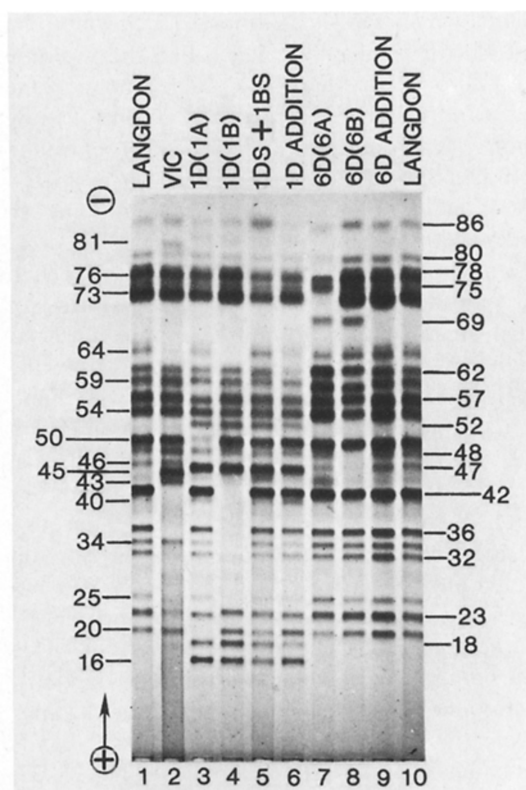

Fig. 1. PAGE of 'Langdon' durum and its group 1 and 6 disomic-substitution and addition lines. The gliadin bands are identified by their relative mobility (Rm) values ranging from 16 to 86 (calculated according to Bushuk and Zillman 1978)

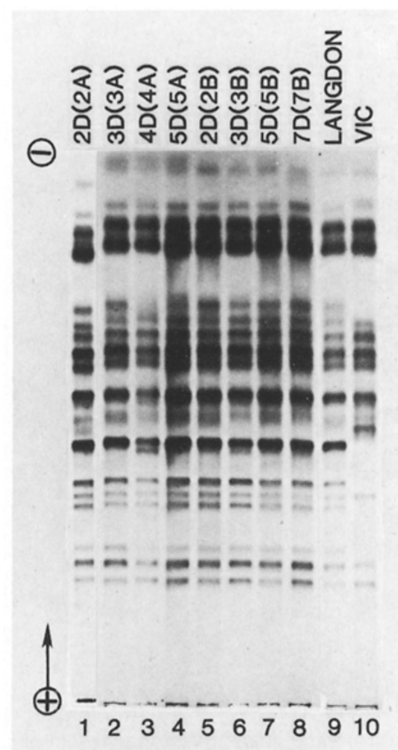

Fig. 2. PAGE of gliadins of 'Langdon' durum D-genome disomic-substitution lines (patterns 1–8); patterns 9 ('Langdon' gliadin) and 10 ('Vic' gliadin) are included for comparison

possible that these are hybrid bands controlled by more than one chromosome and were therefore unresolved by the method used. Isoelectric focusing or two dimensional gel electrophoresis may aid in the resolution of these bands.

The other 'Langdon' disomic-substitution lines (Fig. 2) 2D(2A), 2D(2B), 3D(3A), 3D(3B), 4D(4A), 5D(5A), 5D(5B), and 7D(7B) did not differ appreciably from 'Langdon'.

The 'Langdon' D-genome disomic-addition lines ($2n = 30 = 15''$) did not differ from 'Langdon' except for the expected bands controlled by chromosomes 1D and 6D (Fig. 3). Chromosome ID produced bands at Rm 16, 18, 46, and 52. The chromosome 6D band at Rm 69 was not visible in the 6D disomic-addition line (pattern 5). The absence of band 69 is unexplained. Bands 75 and 76 could not be clearly distinguished in the 6D disomic-addition line. These two bands are probably not clearly resolved due to the strong chromosome 6A bands at Rm 73 and 78.

Damidaux et al. (1978) and Kosmolak et al. (1980) found a strong association between measurements of gluten strength and the presence or absence of gliadin band 42 and 45. In a later study Damidaux et al. (1980) found that the gene controlling band 42 was located on chromosome 1B and that band 45 probably was controlled by an alternative allele at the same, or a closely linked locus. In order to prove that band 45 is controlled by chromosome 1B, the 1B chromosome from the strong gluten cultivar 'Edmore', which contains band 45 but lacks band 42, was substituted into the weak gluten cultivar 'Langdon', which contains band 42 but lacks band 45. 'Edmore' was crossed with the 'Langdon' 1D(1B) disomic-substitution line and the progeny were backcrossed to the disomic-substitution line five times. The 'Edmore' 1B chromosome was always maintained as a monosome in each backcross generation. After the fifth backcross the selected plants, monosomic for 'Edmore' 1B and 'Chinese Spring' 1D, were selfed, and disomic plants were selected from the progeny. Each disomic plant was crossed with the 'Langdon' double-ditelosomic-1B line and also selfed. Cytology indicated that some disomic plants had the 'Edmore' 1B chromosome and some had the 'Chinese Spring' 1D chromosome. One seed from each disomic plant was subjected to PAGE. The electrophoretic patterns (patterns from 5 of the 20 plants examined are shown in Fig. 4) confirmed the results obtained from cytological analysis.

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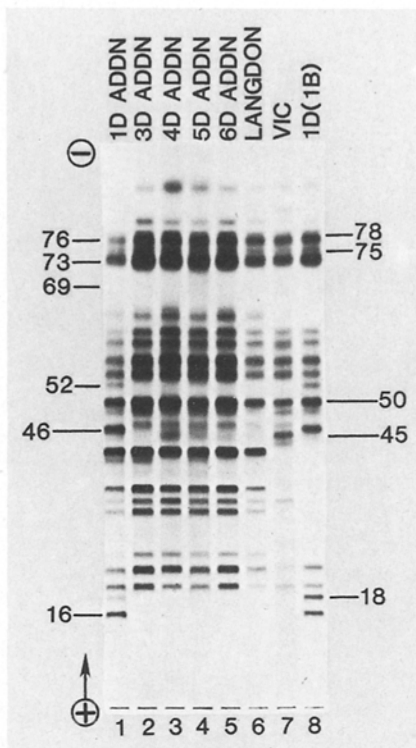

Fig. 3. PAGE of gliadins of 'Langdon' durum D-genome disomic-addition (addn) lines (patterns 1–5). Patterns 6–8 are included for comparison

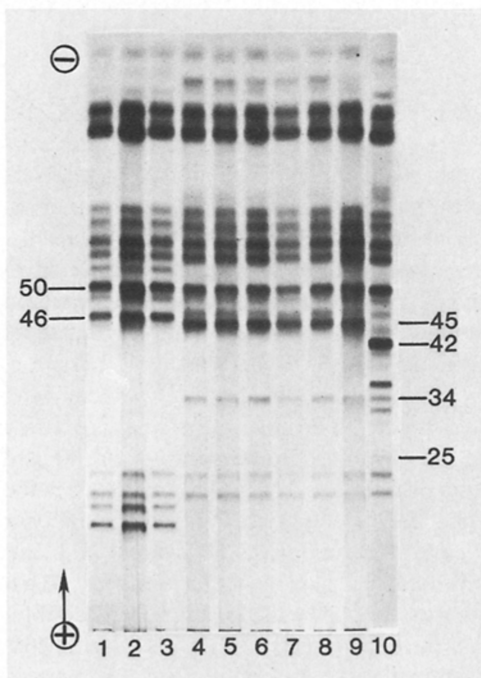

Fig. 4. PAGE of gliadins of 'Langdon' durum disomic-substitution lines containing either a pair of 'Chinese Spring' 1D chromosomes (patterns 1–3) or 'Edmore' durum 1B chromosomes (patterns 4–8) obtained by a series of backcrosses. Patterns 9 ('Edmore' gliadin) and 10 ('Langdon' gliadin) are included for comparison

All 12 plants which had the 'Edmore' 1B chromosome had band 45. All eight plants which had the 'Chinese Spring' 1D chromosome had band 46 and none of the plants had band 42. Therefore, band 45 is controlled by the 'Edmore' 1B chromosome and band 46 is controlled by the 'Chinese Spring' 1D chromosome.

Bands 45 and 46 have almost the same mobility in the gels depending on the buffer system employed for PAGE. Damidaux et al. (1980) appear to have labelled band 46, controlled by chromosome 1D, as band 45 in their Fig. 2 obtained with aluminum lactate buffer at pH 3.1. To resolve this difference the gliadins from the Langdon 1D(1B) disomic-substitution line with band 46 was electrophoresed separately and in combination with that of 'Vic' durum, which has band 45. In aluminum lactate buffer at pH 3.1 the two bands are almost indistinguishable (Fig. 5). However, the two bands clearly have different mobilities in sodium lactate buffer at pH 3.1 (Fig. 5, and also Fig. 1, patterns 2 and 4). These two polypeptides are probably closely related structurally, but may differ by one or more amino acids or in their amino acid sequences.

Chromosome 1B of Langdon differs from chromosome 1B of 'Vic' and 'Edmore' by several other bands as well as bands 42 and 45. 'Langdon' has bands 25, 32, 36, 42, and 64 on chromosome 1B and these bands are absent in the 'Langdon' ('Edmore' 1B) disomic substitu-

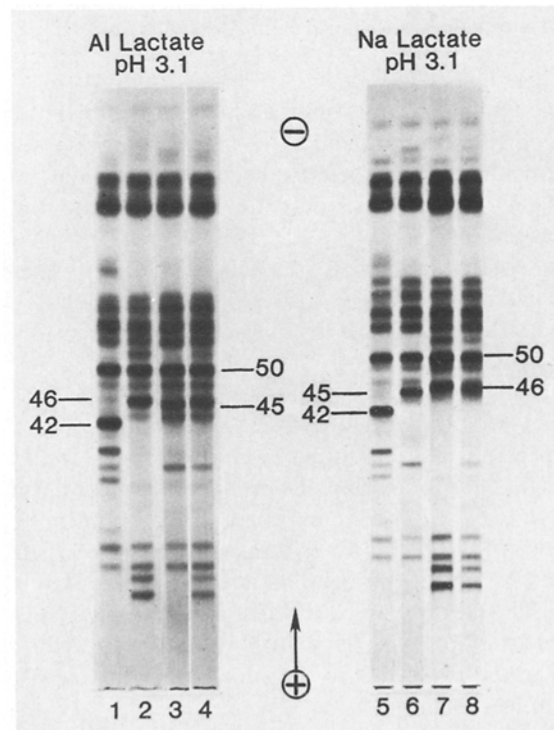

Fig. 5. Influence of buffer system on the Rm of gliadin bands 45 and 46 on PAGE: 1-'Langdon'; 2-1D(1B); 3-mixture of 1D(1B) and 'Vic', 4-'Vic'; 5-'Langdon'; 6-'Vic'; 7-1D(1B); 8-mixture of 1D(1B) and 'Vic'

tion line (compare Figs. 1 and 4). 'Langdon' and the 'Langdon' ('Edmore' 1B) substitution lines both have band 34 (Fig. 4). Most hard red spring wheats appear to have band 45 and band 46 but none have band 42 (for example, see Zillman and Bushuk, 1979). This suggests that the strong gluten determined by band 45 in durum wheat may have originated from the substitution of a chromosome 1B or a segment thereof from hexaploid wheat into durum wheat.

Current weaker gluten durum cultivars can be converted to strong gluten cultivars simply and rapidly by the pedigree or backcrossing method. The PAGE procedure can be used to determine the genotype of plants in segregating generations and therefore materially increase speed and accuracy in the development of strong gluten cultivars of durum wheat.

Acknowledgements

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