*Hereditas 116: 233–238 (1992)*

# Relationship between the D genome of hexaploid wheats (AABBDD) and *Ae. squarrosa* as deduced by seed storage proteins and molecular marker analyses

D. LAFIANDRA[1], S. MASCI[1], R. D'OVIDIO[1], O. A. TANZARELLA[1], E. PORCEDDU[1] and B. MARGIOTTA[2]

[1] *Department of Agrobiology and Agrochemistry, University of Tuscia, Viterbo, Italy*
[2] *Germplasm Institute, C.N.R., Bari, Italy*

LAFIANDRA, D., MASCI, S., D'OVIDIO, R., TANZARELLA, O. A., PORCEDDU, E. and MARGIOTTA, B. 1992. Relationship between the D genome of hexaploid wheats (AABBDD) and *Ae. squarrosa* as deduced by seed storage proteins and molecular marker analyses. — *Hereditas 116*: 233–238. Lund, Sweden. ISSN 0018-0661. Received August 2, 1991. Accepted December, 19, 1991

The electrophoretical analyses of seed storage protein components from the gliadin and glutenin fractions in *T. aestivum* ssp. *vulgare*, *compactum*, *sphaerococcum*, *macha*, *vavilovii*, and *spelta* have revealed limited variation at the tightly linked coding loci *Gli-D1*/*Glu-D3*, and *Glu-D1*, located respectively on the short and long arm of chromosome 1D, and at the *Gli-D2* locus, positioned on the short arm of chromosome 6D. Much higher variation was observed, for the same protein components, in the wild diploid *Ae. squarrosa*, the D genome donor of the *aestivum* group. Genetic variation in the same wheat subspecies and in *Ae. squarrosa* has also been evaluated by Southern hybridization of genomic DNAs, which were digested with several restriction enzymes, and hybridized with cloned sequences of genes coding for seed storage proteins. The much higher degree of variation observed for the seed storage protein genes of *Ae. squarrosa*, in comparison with the variation exhibited by the proteins encoded by the D genome chromosomes of hexaploid wheats, supports the hypothesis that a limited number of crosses gave rise to hexaploid wheats of the *aestivum* group.

*D. Lafiandra, Department of Agrobiology and Agrochemistry, University of Tuscia, Via S. Camillo de Lellis, Blocco B, 01100 Viterbo, Italy*

*Ae. squarrosa* L. is recognized as the donor of the D genome of the *Triticum aestivum* (L.) Thell. group (MCFADDEN and SEARS 1946), which is composed of several subspecies, namely: *vulgare* (Vill.) MK., *compactum* (Host.) MK., *sphaerococcum* (Perc.) MK., *macha* (Dek. et Men.) MK., *vavilovii* (Tum.) Sears., and *spelta* (L.) Thell. In order to explain the large diversity for morphological features, which characterizes the entire hexaploid group, KUCKUCK (1964) postulated a polyphyletic origin: different amphiploid combinations, involving different *Ae. squarrosa* and cultivated as well as wild tetraploids, would have occurred. Using protein electrophoretic patterns of the non-gliadin fraction, JOHNSON (1972) showed that a great variety of protein patterns could be produced by combining *Ae. squarrosa* and *T. dicoccum* Schubl. proteins; however, he observed such a strikingly uniform pattern in the *aestivum* wheats that he hypothesized their monophyletic origin.

The matter has been debated several times, and it remains a subject of considerable interest not only from an evolutionary point of view, but also in relation to breeding strategies. Aspects like the transfer of genes for resistance to biotic and abiotic stresses, nutritional and technological properties are becoming increasingly relevant.

Among the different approaches for establishing phyletic relationships, biochemical and molecular markers, including storage proteins, have been used. Wheat storage proteins include gliadins and glutenins, the former being monomers and the latter, polymers. Gliadins are subdivided into alpha, beta, gamma, and omega according to their decreasing electrophoretic mobility in acid polyacrylamide gels (WOYCHIK et al. 1961); the former two groups are encoded for by *Gli-2* loci, located on the short arm of chromosomes of the homoeologous group 6, while most gamma and omega gliadins are encoded for by *Gli-1* loci which are present on the short arm of group 1 chromosomes. Glutenins include the so called A, B, C and D subunits. The A group includes the high molecular weight glutenin subunits, whereas low molecular

weight glutenin subunits form collectively the B, C and D groups (PAYNE and CORFIELD 1979; JACKSON et al. 1985). The former are encoded by the *Glu-1* loci present on the long arm of group 1 chromosomes and the latter by the *Glu-3* loci present on the short arm of the same chromosomes and linked to the *Gli-1* loci. Both classes of proteins show large intraspecific variation resulting from the existence of multiple allelism at each complex locus. This has been advantageous for wheat cultivar identification, and their use has also proved valuable in establishing phylogenetic relationships (LAFIANDRA et al. 1990). These aspects brought us to undertake a study aimed at investigating the relationships between the *aestivum* group and *Ae. squarrosa* by using the D-genome encoded storage proteins and related genes at the four different loci.

## Materials and methods

Several accessions of hexaploid wheats, belonging to all the subspecies included in the *aestivum* group, were electrophoretically analysed along with several accessions of *Ae. squarrosa*. Gliadins were extracted and analysed by one- and two-dimensional techniques according to LAFIANDRA and KASARDA (1985); high molecular weight (HMW) glutenin subunits were analysed by SDS-PAGE according to PAYNE et al. (1980), while low molecular weight (LMW) glutenin subunits were separated as reported by MASCI et al. (1991a). Nuclear DNA was extracted from green leaves according to the procedure of DVORAK et al. (1988). 10 µg of genomic DNA was digested with Hind III and Bam HI, fractionated on a 1 % agarose gel and transferred to a nylon membrane (Hybond-N, Amersham). Hybridization reactions for gamma-gliadin genes were carried out using the $^{32}$P-labelled pTDA16 clone (D'OVIDIO et al. 1991); HMW glutenin sequences were revealed using the $^{32}$P-labelled pAS2 clone (D'OVIDIO et al., in preparation). Post-hybridization washes were performed at high stringency conditions. Filters were exposed to X-ray for 48 hours.

## Results

### Variation at the *Gli-D1* and *Gli-D2* loci

Two-dimensional separations of gliadins extracted from different subspecies of the *aestivum* group are reported in Fig. 1. Components encoded at the *Gli-D1* and *Gli-D2* loci are indicated. As regards the former locus, our analyses have shown that all the allelic variants (LAFIANDRA et al. 1984, 1989; METAKOVSKY et al. 1984) can actually be subdivided into two main families (MASCI et al. 1991b). The first family has *Gli-D1* encoded components similar to those present in the bread cultivar Chinese Spring (CS), while the second family is represented by the alleles with components similar to those present in the cultivar Cheyenne (CNN). For this reason, we shall refer to the two alleles as 'CS-type' and 'CNN-type', respectively (Fig. 1a and b). These two allelic clusters of gliadin components differ mainly in number and position of their slow omega components; however, both types have an identical major gamma component. Results show that the other subspecies of the *aestivum* group have similar allelic types to those observed for ssp. *vulgare*. As far as components encoded by the *Gli-D2* locus are concerned, several allelic variants have been identified in all six subspecies, but unlike the situation observed at the *Gli-D1* locus, all these different variants are related to each other.

### Variation at the *Glu-D3* locus

1D encoded D subunits consist of two acidic bands which are peculiar to *T. aestivum* with 'CS-type' omega gliadins, and no electrophoretic variant was found in the plant material analysed here. As regards B subunits, four bands were found to be encoded at the *Glu-D3* locus in CS and CNN. These two cultivars differ from one another only for the slowest moving band. Among the 'CS-type' material of ssp. *vulgare* analysed here, the only striking exception was represented by Capelle Desprez, which presented only one basic band encoded by the 1D chromosome. The accession of ssp. *sphaerococcum* analysed showed a distinct pattern from the other subspecies. The remaining 'CS-type' wheats present *Glu-D3* encoded bands identical or similar to those found in CS itself. As regards 'CNN-type' wheats, most of bread wheats possessed three *Glu-D3* encoded bands, two of which were common to CS and CNN, while one differed (Fig. 2).

### Variation at the *Glu-D1* locus

Usually, the *Glu-D1* locus encodes two subunits designated x and y according to their electrophoretic mobilities (PAYNE et al. 1981). The

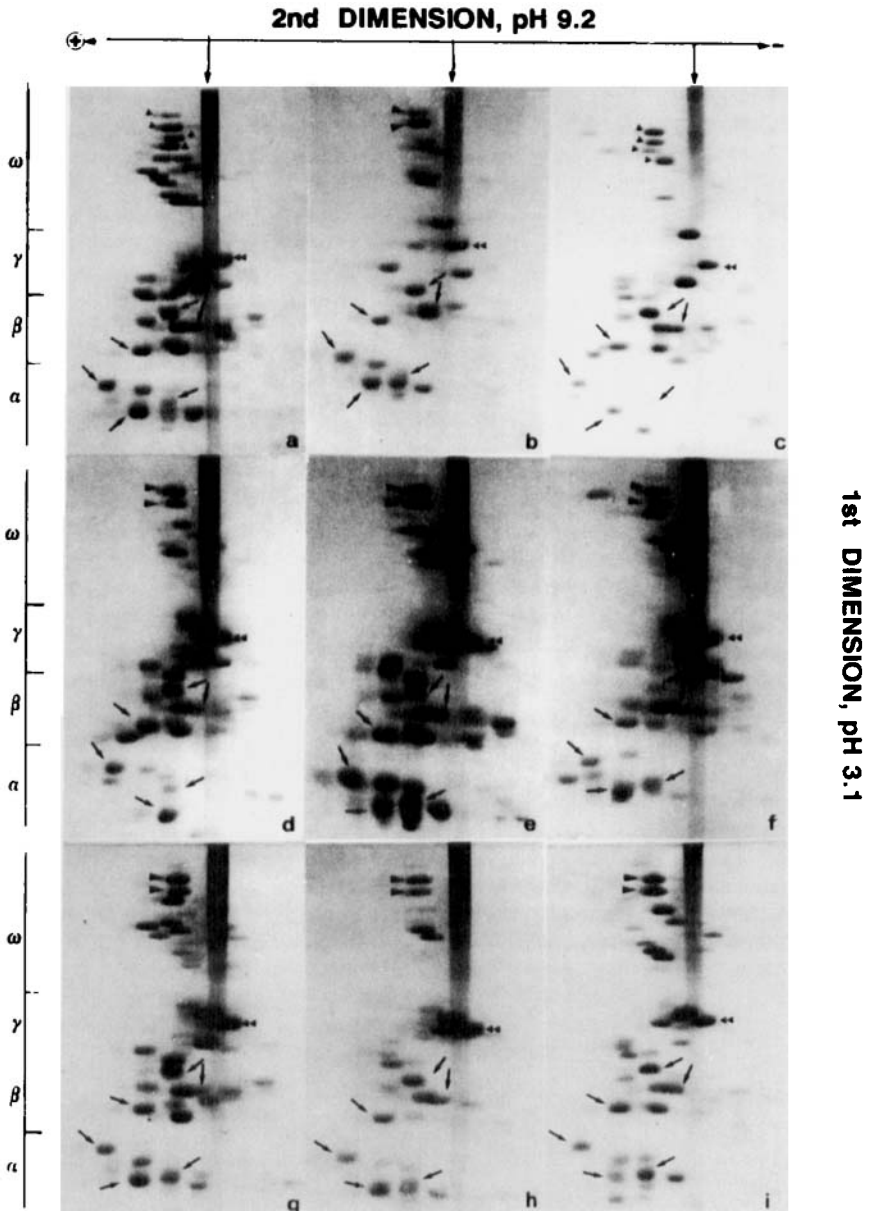


**Fig. 1a–i.** Two-dimensional electrophoretical separation of gliadins from different *T. aestivum* subspecies. **a and b** *vulgare*; **c and d** *compactum*; **e** *sphaerococcum*; **f and h** *spelta*; **g** *macha*; **i** *vavilovii*. Arrowheads indicate *Gli-D1* encoded components; small arrowheads indicate 'CNN-type' allele, large ones indicate 'CS-type' allele. Double arrowheads used to indicate the gamma components common to the two different allelic types. Gliadins encoded at the *Gli-D2* locus are indicated by arrows.

two alternative (allelic) pairs at the *Glu-D1* locus known as 2 + 12 and 5 + 10 are detected in most bread wheat cultivars and in the subspecies of the *aestivum* group along with the pairs 3 + 12, 4 + 12, 2 + 10 and 2.2 + 12. This last group of alleles are all clearly related to the first two, being the direct result of recombination between 2 + 12 and 5 + 10 or the result of mutations, which most probably

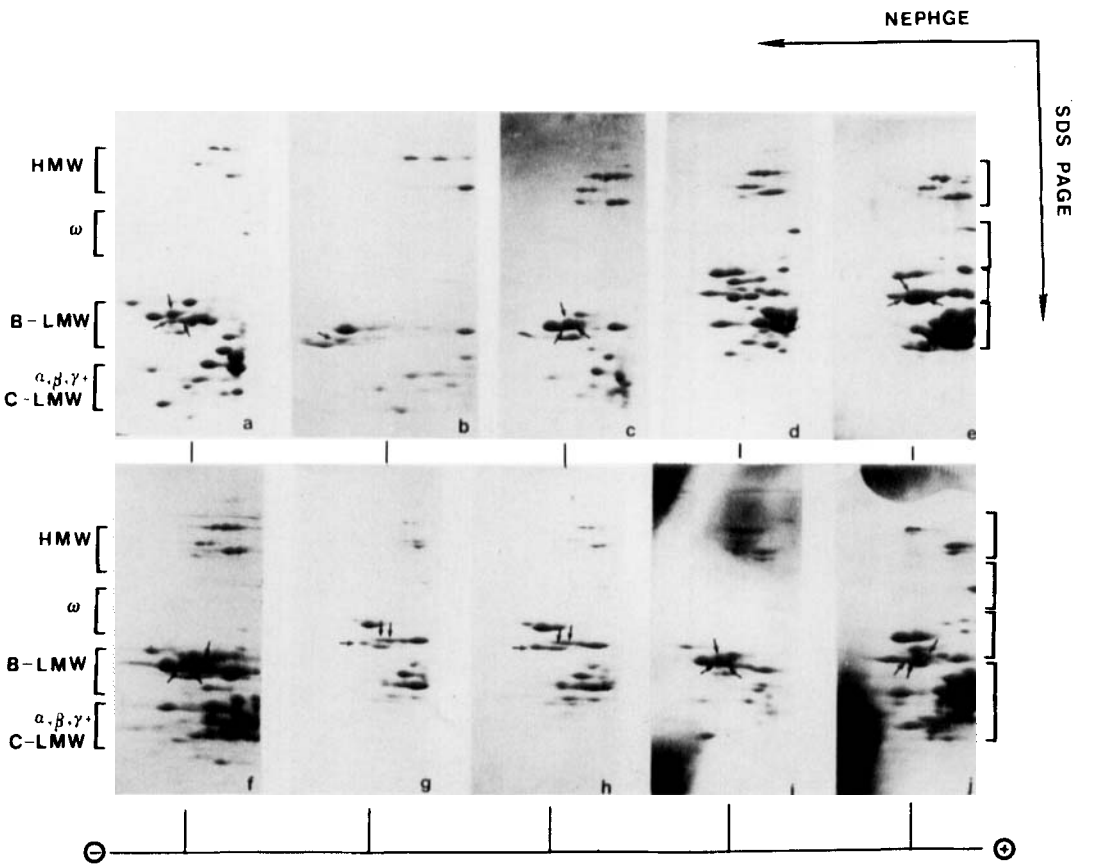


**Fig. 2a–j.** Two-dimensional electrophoretic separation of low molecular weight glutenin subunits of *T. aestivum* subspecies *vulgare*. **a** cv. 'Chinese Spring'; **b** cv. 'Cappelle Desprez'; **c** ssp. *vulgare*; **f** cv. 'Cheyenne'; **g** cv. 'Thatcher'; **h** cv. 'Katepwa'; **d** ssp. *sphaerococcum* G 515; **e** ssp. *spelta* G 1566; **i, j** ssp. *macha* G 532, G 535. Arrows indicate the 1D encoded bands.

occurred after hexaploid formation, affecting protein charge and/or size. Two accessions were identified, one in ssp. *vulgare* and the other in ssp. *compactum*, possessing a 1Dx subunit with a much higher molecular weight, similar to the subunit 2.2 described by PAYNE et al. (1983).

### Molecular analyses

Genetic variation at gliadin and glutenin loci was also investigated by RFLP analysis. Filters with genomic DNAs from *T. aestivum* ssp. *vulgare*, *compactum*, *sphaerococcum*, *macha*, *vavilovii*, and *spelta* were hybridized with labelled probes corresponding to the coding sequences of gamma gliadins and HMW glutenin components. Hind III, Bam HI, and Eco RI digestions, as expected on the basis of protein data, revealed variation between the analysed accessions of *Ae. squarrosa*. The same restriction enzymes were also used to detect polymorphism at the *Gli-D1* and *Glu-D1* loci of the different hexaploid subspecies. The *Gli-D1* locus did not show any variation, whereas digestion of the same genomic DNAs with both Hind III and Bam HI detected polymorphism at the *Glu-D1* locus in the accession of the subspecies *compactum*, previously described, which possessed a 1Dx subunit of higher molecular weight. As shown in Fig. 3, in fact, this subspecies possesses the Hind III fragment, corresponding to the sequence coding for the slow subunit of the *Glu-D1* locus, which is 3.2 kb, whereas in all the other materials analysed it was 2.5 kb. This polymorphism was also revealed by Bam HI digestion (Fig. 3); the corresponding fragment cut by Bam HI was also longer in this particular accession of the subspecies.

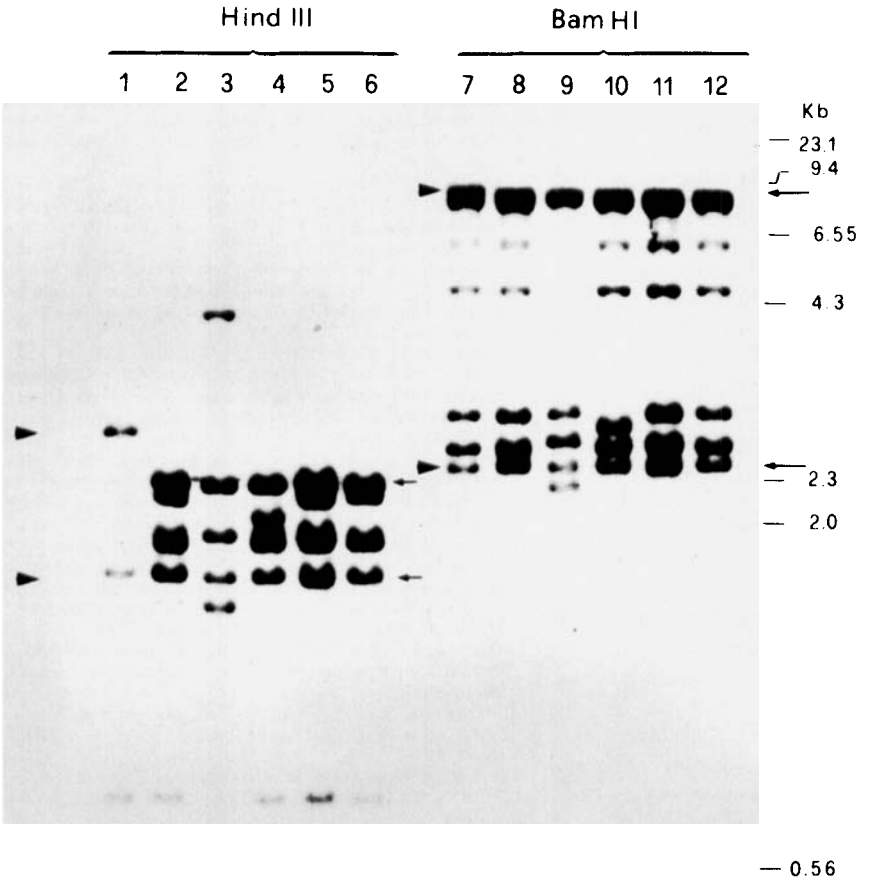


**Fig. 3.** Southern blot of genomic DNA digested with Hind III and Bam HI and hybridized with $^{32}$P-labelled pAS2 clone. 1, 7) *compactum*; 2, 8) *macha*; 3, 9) *spelta*; 4, 10) *sphaerococcum*; 5, 11) *vavilovii*; 6, 12) *vulgare* cv. 'Chinese Spring'. Arrows indicate Hind III and Bam HI fragments of corresponding to *Glu-D1* genes in 'Chinese Spring'; arrowheads indicate similar fragments in *compactum*. Molecular weights were determined using DNA digested with Hind III as marker.

## Discussion and conclusions

Electrophoretical analyses of both gliadins and glutenins show large variation in *Ae. squarrosa*, as also reported by LAGUDAH and HALLORAN (1988a,b). One or two-dimensional patterns of glutenins and gliadins of some *Ae. squarrosa* accessions identical to D-genome encoded proteins present in hexaploid wheats have been found (PORCEDDU and LAFIANDRA 1986).

In contrast with the wide variation found in the wild progenitor of the D genome, our analyses have shown that genes at either *Gli-D1*, *Glu-D1*, and *Glu-D3* loci code for proteins which can be clustered into two groups. In all the plant material here analyzed, the *Gli-D1* and *Glu-D3* encoded proteins were always both of the CS-type or CNN-type, as expected on the basis of their tight genetic linkage. As regards the *Glu-D1* locus, limited variation is present in all the subspecies analyzed; the 1Dx encoded subunits, which seem much unrelated to the other 1Dx subunits because of their higher molecular weight, could have arisen through a rare event of unequal crossing over after hexaploid wheat formation, as already postulated by PAYNE et al. (1983).

Two subspecies of *Ae. squarrosa* can be recognised, namely ssp. *eusquarrosa* Eig. and *strangulata* Eig. The former subspecies incorporates three varieties: *anathera*, *typica* and *meyeri*, while ssp. *strangulata* has the single variety *strangulata* (EIG 1929). Isozyme and cytological studies have shown

that ssp. *strangulata* is most likely to have donated the D genome to *aestivum* wheats, and these findings have been confirmed by analyses of the gliadin fraction (KONAREV et al. 1979; KASARDA et al. 1984).

The discrepancy between the wild progenitor and the different subspecies of the *aestivum* group on the basis of electrophoretic variation of their storage proteins could be explained assuming that hexaploid wheats originated from only few crosses, as already hypothesized by LAWRENCE and SHEPHERD (1980), between a tetraploid wheat and *Ae. squarrosa*. The presence of apparently related alleles in all hexaploid subspecies supports the monophyletic origin of this group. The initial amphiploid combination gave rise, through recombination, translocation and mutations, to a series of recombinants and, consequently, to a wide range of variability, as seen in today's hexaploid wheats, which guaranteed the flexibility required to colonize new environments, whereas self-pollination ensured that the most advantageous combinations would be maintained. Since the D genome has greatly improved breadmaking capabilities of *aestivum* wheats, the large variation existing in the gene pool of *Ae. squarrosa*, among other interesting possibilities, offers an attractive way to improve this characteristic.

## References


D'OVIDIO, R., TANZARELLA, O. A. and PORCEDDU, E. 1991. Cloning and sequencing of PCR amplified γ-gliadin. — *Plant Sci. 75*: 229–236

DVORAK, J., MCGUIRE, P. E. and CASSIDY, B. 1988. Apparent sources of the A genome of wheats inferred from polymorphism in abundance and restriction for length of repeated fragments in nucleotide sequences. — *Genome 30*: 680–689

EIG, A., 1929. Monographisch-kritische Übersicht der Gattung *Aegilops*. — *Rep. Spec. Nov. Reg. Veg., Behi. 55*: 1–228

JACKSON, A. J., HOLT, L. M. and PAYNE, P. I. 1985. *Glu-B2*, a storage protein locus controlling the D group of LMW glutenin subunits in bread wheat (*Triticum aestivum*). — *Genet. Res. (Cambridge) 46*: 11–17

JOHNSON, B. L. 1972. Seed protein profiles and the origin of the hexaploid wheats. — *Am. J. Bot. 59*: 952–960

KASARDA, D. D., LAFIANDRA, D., MORRIS, R. and SHEWRY, P. R. 1984. Genetic relationships of wheat gliadin proteins. — *Kulturpflanze 32*: 41–60

KONAREV, V. G., GAVRILYUK, I. P., GUBAREVA, N. K. and PENEVA, T. I. 1979. Seed proteins in genome analysis, cultivar identification, and documentation of cereal genetic resources: a review. — *Cereal Chem. 56*: 272–278

KUCKUCK, H. 1964. Experimentelle Untersuchungen zur Entstehung der hexaploiden Kulturweizen II. — *Z. Pflanzenzücht. 51*: 215–228

LAFIANDRA, D. and KASARDA, D. D. 1985. One- and two-dimensional (Two-pH) polyacrylamide gel electrophoresis in a single gel: separation of wheat proteins. — *Cereal Chem. 62*: 314–319

LAFIANDRA, D., KASARDA, D. D. and MORRIS, R. 1984. Chromosomal assignment of genes coding for the wheat gliadin protein components of the cultivars Cheyenne and Chinese Spring by two-dimensional electrophoresis. — *Theor. Appl. Genet. 68*: 531–539

LAFIANDRA, D., COLAPRICO, G., KASARDA, D. D. and PORCEDDU, E. 1987. Null alleles for gliadin blocks in bread and durum wheat cultivars. — *Theor. Appl. Genet. 74*: 610–616

LAFIANDRA, D., BENEDETTELLI, S., MARGIOTTA, B. and PORCEDDU, E. 1989. Chromosomal location of gliadin coding genes in *T. aestivum* ssp. *spelta* and evidence on the lack of components controlled by *Gli-2* loci in wheat aneuploids. — *Theor. Appl. Genet. 78*: 177–183

LAFIANDRA, D., BENEDETTELLI, S., MARGIOTTA, B., SPAGNOLETTI-ZEULI, P. L. and PORCEDDU, E. 1990. Seed storage proteins and wheat genetic resources. — In: *Wheat Genetic Resources: Meeting Diverse Needs* (*eds* J. P. SRIVASTAVA *and* A. B. DAMANIA), *John Wiley & Sons, England*, p. 73–87

LAGUDAH, E. S. and HALLORAN, G. M. 1988a. Phylogenetic relationships of *Triticum tauschii*, the D genome donor to hexaploid wheats. 1. Variation in HMW subunits of glutenin and gliadins. — *Theor. Appl. Genet. 75*: 592–598

LAGUDAH, E. S. and HALLORAN, G. M. 1988b. Phylogenetic relationships of *Triticum tauschii*, the D genome donor to hexaploid wheats. 2. Inheritance and chromosomal mapping of the HMW subunits of glutenin and gliadin gene loci of *T. tauschii*. — *Theor. Appl. Genet. 75*: 599–605

LAWRENCE, G. J. and SHEPHERD, K. W. 1980. Variation in glutenin protein subunits of wheat. — *Aust. J. Biol. Sci. 33*: 221–233

MCFADDEN, E. S. and SEARS, E. R. 1946. The origin of *Triticum spelta* and its free-threshing hexaploid relatives. — *J. Hered. 37*: 81–89; 107–116

MASCI, S. M., PORCEDDU, E., COLAPRICO, G. and LAFIANDRA, D. 1991a. Comparison of the B and D subunits of glutenin encoded at the *Glu-D3* locus in two biotypes of the common wheat cultivar Newton with different technological characteristics. — *J. Cereal Sci. 14*: 35–46

MASCI, S. M., PORCEDDU, E. and LAFIANDRA, D. 1991b. Two-dimensional electrophoresis of 1D encoded B and D glutenin subunits in common wheats with similar omega gliadins. — *Biochem. Genet. 29*: 403–413

METAKOVSKY, E. V., NOVOSELSKAYA, A. YU, KOPUS, M. M., SOBKO, T. A. and SOZINOV, A. A. 1984. Blocks of gliadin components in winter wheat detected by one-dimensional polyacrylamide gel electrophoresis. — *Theor. Appl. Genet. 67*: 559–568

PAYNE, P. I. and CORFIELD, K. G. 1979. Subunit composition of wheat glutenin proteins, isolated by gel filtration in a dissociating medium. — *Planta 145*: 83–88

PAYNE, P. I., LAW, C. N. and MUDD, E. E. 1980. Control by homoeologous group 1 chromosomes of the high-molecular-weight subunits of glutenin, a major protein of wheat endosperm. — *Theor. Appl. Genet. 58*: 113–120

PAYNE, P. I., HOLT, L. M. and LAW, C. N. 1981. Structural and genetic studies on the High-Molecular-Weight subunits of wheat glutenin. Part 1: Allelic variation in subunits amongst varieties of wheat (*Triticum aestivum*). — *Theor. Appl. Genet. 60*: 229–236

PAYNE, P. I., HOLT, L. M. and LAWRENCE, G. J. 1983. Detection of a novel high molecular weight subunit of glutenin in some Japanese hexaploid wheats. — *J. Cereal Sci. 1*: 3–8

PORCEDDU, E. and LAFIANDRA, D. 1986. Origin and evolution of wheats. — In: *The Origin and Domestication of Cultivated Plants* (*ed* C. BARIGOZZI), *Elsevier, Amsterdam*, p. 143–178

WOYCHIK, J. H., BOUNDY, J. A. and DIMLER, R. J. 1961. Starch gel electrophoresis of wheat gluten proteins with concentrated urea. — *Arch. Biochem. Biophys. 94*: 477–482