

SDS-PAGE OF THE HIGH-MOLECULAR-WEIGHT SUBUNITS OF WHEAT GLUTENIN AND THE CHARACTERIZATION OF 1R(1B) SUBSTITUTION AND 1BL/1RS TRANSLOCATION LINES

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SUMMARY

The high-molecular-weight subunits of glutenin from wheat 1R(1B) substitution and 1BL/1RS translocation lines were fractionated by SDS-PAGE. Two new subunits denoted R₁ and R₂ were characterized in 1R(1B) substitution, but not in 1BL/1RS translocation lines. R₁ and R₂ were proved to be rye proteins by 2d electrophoresis (NEPHGE × SDS-PAGE).

In contrast to literature citations it was demonstrated that the cultivar *Winnetou* is a 1R(1B) substitution line and the cultivars *Clement* and *Mildress* both are 1BL/1RS translocation lines.

INTRODUCTION

In the past wheat breeders have accidentally transferred the rye chromosome 1R into the wheat genome to improve the latter crop. This transfer of rye genetic material into wheat can be recognized by studying disease resistance, morphology of the chromosomes during meiosis and electrophoresis of seed proteins.

The major seed proteins in wheat, glutenin and gliadin, are regulated by genes on the homoeologous chromosomes 1 and 6 (see for a review PAYNE et al., 1982). In regard to the group 1 chromosomes the short arms contain genes for the ω and γ gliadins and the long arms genes for the high-molecular-weight subunits of glutenin. LAWRENCE & SHEPHERD (1981) have shown that rye also possesses on the short and the long arm of the homoeologous chromosome 1R genes that code for secalins and glutelins respectively (The rye proteins secalins and glutelins are comparable to the wheat proteins gliadins and glutenins).

A minor class of wheat proteins, the high sulfur, basic thionins are regulated by genes on the long arm of the homeologous group 1 chromosomes (FERNÁNDEZ DE CALEYA et al., 1976). The 1R(1B) substitutions can be recognized by electrophoretical characterization of gliadins or secalins as a marker for the short arm (ELLIS, 1971) and of wheat thionins or rye thionins as a marker for the long arm (SÁNCHEZ-MONGE et al., 1979).

In this report we demonstrate that fractionation of the high-molecular-weight subunits of wheat glutenin or rye glutelin can be used too as a marker for 1R(1B) substitutions. Besides we show that in the past some apparent misclassification has occurred.

MATERIALS AND METHODS

Wheat samples: Samples of the studied wheat and rye cultivars were kindly given by Cebeco-Handelsraad [Elst (NL)], Koninklijk Kweekbedrijf en Zaadhandel D. J. van der Have [Rilland (NL)], Semundo B.V. [Ulrum (NL)], Foundation for Agricultural Plant Breeding [Wageningen (NL)], Plant Breeding Institute [Cambridge (UK)] and Technische Universität [München (FRG)].

SDS-PAGE: SDS-PAGE was carried out as described before (MOONEN et al., 1982) using the discontinuous buffer system of LAEMMLI (1970) and the 8.33% (w/v) acrylamide, 0.067% (w/v) bisacrylamide separation gel of LAWRENCE & SHEPHERD (1980).

Two-dimensional fractionation: Non-equilibrium pH-gradient electrophoresis (NEPHGE) × SDS-PAGE. NEPHGE × SDS-PAGE was carried out according to HOLT et al. (1981) except that we used PharmalyteTM pH 3–10 as ampholines.

RESULTS

As pointed out by several wheat protein research groups (see PAYNE & LAWRENCE, 1983), SDS-PAGE using the discontinuous buffer system developed by LAEMMLI

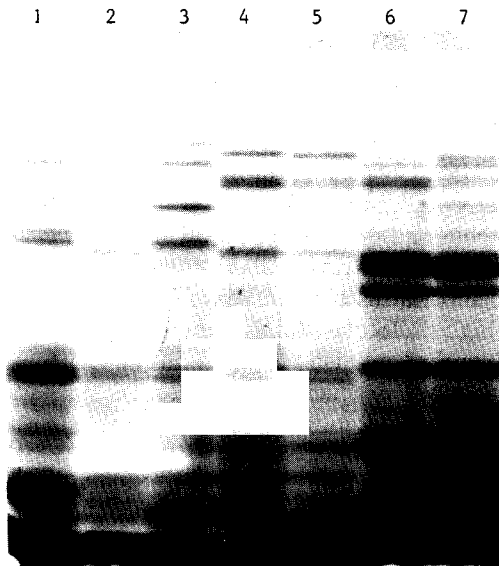


Fig. 1. SDS-PAGE of five wheat and two rye cultivars. Lane 1, Götz; 2, Clement; 3, Disponent; 4, Salz-münder Bartweizen; 5, Riebesel 47–51; 6, Halo; 7, Kustro.

(1970) is the optimal separation method for the fractionation of the high-molecular-weight subunits of glutenin. In Fig. 1 the SDS-PAGE patterns of these proteins from several wheat and rye cultivars are shown. It is depicted in this figure that 'Clement' contains the subunits 2, 4, 8, 11, 'Götz' the subunits 3, 4, 8, 10 and 'Disponent' the subunits 1, 3, 5, 10. Of these glutenin subunits, 4 + 8 and 5 are of importance as they are conditioned by 1/cus Glu-B1 on the long arm of chromosome 1B (1BL). Both the combination 4 + 8 (allele Glu-Bld) and subunit 5 (allele Glu-Bla) are widely distributed in wheat cultivars tested until now (PAYNE & LAWRENCE, 1983; MOONEN, unpublished results).

'Clement' is described as a 1R(1B) substitution line and 'Götz' and 'Disponent' as 1BL/1RS translocation lines (ZELLER et al., 1982). However, we assume that 'Clement' is a translocation line too, because allele Glu-Bld is present in this cultivar.

To test this assumption we fractionated the proteins of earlier released 1R(1B) substitution lines such as 'Salzmünder Bartweizen' and 'Riebesel 47-51'. Both cultivars contain two subunits, denoted by us R_1 and R_2 (see Fig. 1) not earlier described (PAYNE & LAWRENCE, 1983). To indicate that the subunits R_1 and R_2 are rye proteins we fractionated the storage proteins of the rye cultivars Halo and Kustro, genetically related to the rye cultivar Petkus, which was probably used for making the early crosses between wheat and rye by Riebesel (LEIN, 1975). Fig. 1 shows that these rye cultivars

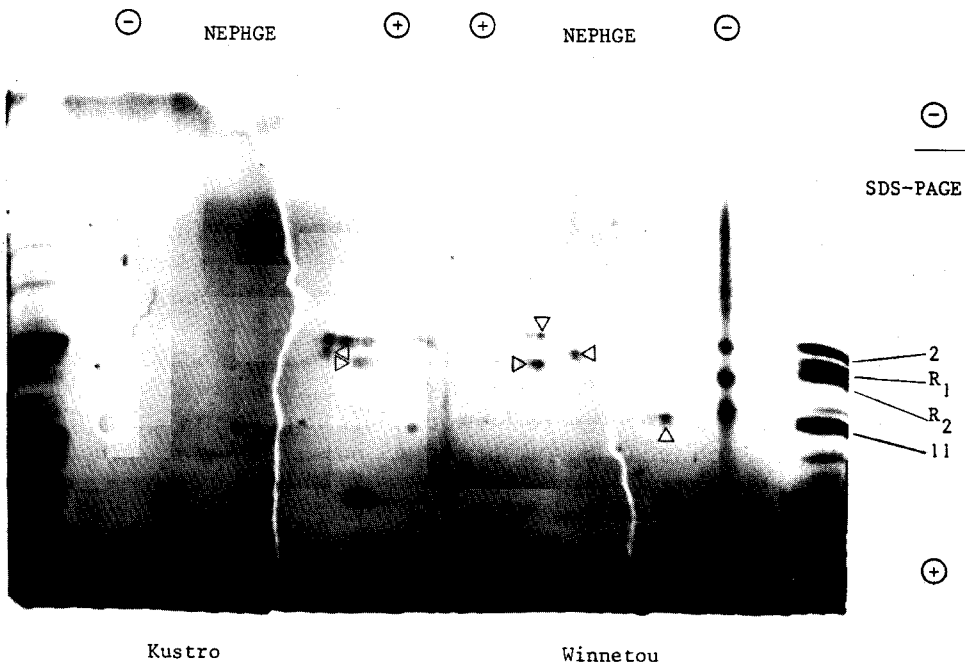


Fig. 2. Two-dimensional fractionation (NEPHGE \times SDS-PAGE) of endosperm proteins of the wheat cultivar Winnetou, 1R(1B) substitution and the rye cultivar Kustro. The proteins 2, R_1 , R_2 and 11 are marked by ∇ , \triangleleft , \triangleright and \triangle respectively. The NEPHGE cylindrical gels used in the first dimension were placed in mirror image on the SDS-PAGE gel used in the second dimension. At the left and right side of the SDS-PAGE gel extracts, respectively, of Kustro and Winnetou were fractionated as standards.

Table 1. High-molecular-weight subunits of glutenin and 1R(1B) or 1BL/1RS classification of some wheat cultivars.

Cultivar	H.M.W. subunits regulated by genes on the long arm of chromosome				Classification
	1A	1B	1R	1D	
Avrora	2*	5		3 + 10	1BL/1RS (1)
Burgas 2	1		R ₁ + R ₂	2 + 11	1R(1B) (2)
Clement	—	4 + 8		2 + 11	1R(1B) (3)
Kavkas	2*	5		3 + 10	1BL/1RS (1)
Mildress	—	4 + 8		2 + 11	1R(1B) (2)
Mironovskaja 10	1		R ₁ + R ₂	3 + 10	
Neuzucht	1		R ₁ + R ₂	2 + 11	1R(1B) (4)
Novi Sad 738	—		R ₁ + R ₂	2 + 11	
Perseus	1	5 + 8		3 + 10	1BL/1RS (4)
Saladin	—		R ₁ + R ₂	3 + 10	1R(1B) (1)
Salzmünder Bartweizen	—		R ₁ + R ₂	2 + 11	1R(1B) (4)
Riebesel 47-51	—		R ₁ + R ₂	2 + 11	
Riebesel 57-41	2*		R ₁ + R ₂	2 + 11	
Weique	—		R ₁ + R ₂	2 + 11	1R(1B) (4)
Winnetou	—		R ₁ + R ₂	2 + 11	1BL/1RS (2)
Zorba	1		R ₁ + R ₂	3 + 10	1R(1B) (4)
Zorba DT 1RL	—		R ₁ + R ₂	3 + 10	(5)

(1) BLÜTHNER & METTIN (1973); (2) BLÜTHNER & METTIN (1977); (3) ZELLER et al. (1982); (4) ZELLER (1973); (5) 2n = 40 + 2t1 1R; ZELLER (personal communication).

contain high-molecular-weight subunits with the same mobilities as the subunits R₁ and R₂. We proved by 2d electrophoresis (NEPHGE × SDS-PAGE) that the subunits R₁ and R₂ are indeed rye proteins (Fig. 2). In respect to this it is worth-while to mention that subunit R₁ and R₂ are more acidic proteins than subunit 11. In contrast, all Glu-B1 subunits are more basic than subunit 11 (HOLT et al., 1981).

In Table 1 we have collected some wheat cultivars screened by us with SDS-PAGE, their glutenin subunits and classification as 1R (1B) substitution or 1BL/1RS translocation as done by others. From these results we conclude that 'Clement' and 'Mildress' cannot be 1R(1B) substitution lines as they do not possess R₁ and R₂ subunits. As they possess genes for the rye marker scalins (ELLIS, 1971; ELLIS & BEMINSTER, 1977) which are located on 1RS we assume that they are 1BL/1RS translocation lines. Concerning 'Clement' this is in agreement with METTIN et al. (1978) who classified this variety as a 1BL/1RS translocation line.

'Winnetou' is classified by BLÜTHNER & METTIN (1977) as a 1BL/1RS translocation line. The presence of the subunits R₁ and R₂ indicated that if this cultivar would be a translocation line the 1R chromosome-segment in this cultivar must be different from that in the other translocation lines. In our opinion 'Winnetou' is not a translocation line but a 1R(1B) substitution line.

DISCUSSION

The results in Fig. 1 and Table 1 demonstrate that SDS-PAGE of wheat endosperm proteins, emphasizing the high-molecular-weight subunits of glutenin, can be used as a technique to recognize the introduction of genes of chromosome 1R into wheat. The rye subunits R_1 and R_2 are only an indication for the introduction of the 1RL segment of the 1R chromosome. Being aware of the introduction of the 1RS segment by electrophoresis of the secalins one can deduce whether a certain cultivar or line is a 1R(1B) substitution or a 1BL/1RS translocation. As an alternative or additional prove for the introduction of the 1RL segment the electrophoretical separation of the thionins as described by SÁNCHEZ-MONGE et al. (1979) can be used.

Recently PAYNE & LAWRENCE (1983) described the alleles for the complex loci Glu-A1, Glu-B1 and Glu-D1, coding for the high-molecular-weight subunits of glutenin. The subunit comparable to the rye proteins R_1 and R_2 could be subunit 21 regulated by allele Glu-B1J. As a variety standard for this allele the authors mentioned the cultivar Dunav. According to ZEVEN & ZEVEN-HISSINK (1976) the genealogy of 'Dunav' is 'Heines VII' \times '129 Genus'. This last variety is a progeny from a wheat \times rye cross. It is conceivable that subunit 21 is identical to the subunits R_1 and R_2 because we noted that these subunits are not separated in the 10% polyacrylamide-gels used by PAYNE & LAWRENCE (1983). This hypothesis was confirmed by Payne (personal communication). As subunit 21 are rye proteins the nomination of allele Glu-B1j for subunit 21 is misleading.

In general it is accepted that the introduction of rye genetic material into wheat lowers bread-making quality. It remains to be investigated, especially in respect to the occurrence of sticky doughs whether the decreased bread-making quality is caused by a different protein composition. PAYNE et al. (1981) and MOONEN et al. (1982, 1983) proved that bread-making quality of wheat is regulated by the high-molecular-weight subunits of glutenin. However as can be seen in Table 1 the glutenin subunits of the 1BL/1RS translocation lines, which produce sticky doughs e.g. 'Clement', are common for hexaploid wheat. In addition some cultivars e.g. Kranich and Maris Huntsman are known which also produce sticky doughs that are no 1R(1B) substitution or 1BL/1RS translocation at all. From these results we have to conclude that the occurrence of sticky doughs with wheat cultivars that are 1BL/1RS translocations are not brought about the introduction of rye high-molecular-weight subunits into wheat glutenin.

In contrast to the 'Riebesel' lines that were bred at Salzmünde, the cultivar Zorba was bred at Weihenstephan. One of Zorba's parents is 'Triticale WRB' (WRB meaning Weizen-Roggen-Bastard = wheat-rye-hybrid), which is very likely the source of the 1R(1B) substitution. That 'Zorba' like the 'Riebesel' lines, also is a 1R(1B) substitution may have its cause in the facts that very likely the same rye variety Petkus was crossed as for 'Riebesel' and that the same selection pressure, stripe rust and leaf rust resistance (ZELLER & FUCHS, 1983) took place. However, there is a another explanation. In 1938 some 50 tons of seeds of various 'Riebesel' lines had been produced. This material was sent to all parts of Germany for experimental growing (LEIN, 1975). It is possible that 'WRB' derives from it. If so, 'Riebesel' 1R(1B) substitution lines and 'WRB' come from the same cross.

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