P L A N T B R E E D I N G A N D S E E D S C I E N C E
Volume 52 2005

Maria Chrząstek

Institute of Genetics and Plant Breeding, Agricultural University, ul. Akademicka 15, 20-934 Lublin, Poland

IDENTIFICATION OF GLUTENIN AND SECALIN SUBUNITS IN ADDITION AND SUBSTITUTION LINES OF WHEAT (*TRITICUM AESTIVUM* L.) USING BIOCHEMICAL MARKERS

ABSTRACT

The aim of paper was evaluation of composition of high molecular weight (HMW) and low molecular weight (LMW) glutenin subunits in wheat-rye addition lines cv. Grana - cv. Dańkowskie Złote, substitution line 1B/1R and initial forms, as well as, localization of *Sec* genes on chromosomes 'Dańkowskie Złote' rye. Analysis were performed using SDS-PAGE electrophoresis.The composition of HMW glutenin for all addition lines and 'Grana' wheat was: N - 6+8 - 2+12. Composition of LMW glutenin was estimated only for 2R and 6RL addition lines, wheat, and octoploid triticale. In both addition lines the subunits N-6+9-15 were identified, in wheat cv. Grana subunits 1-6+9-15 were present, whereas in octoploid triticale polymorphism was observed (1-6+9-15 or 1-11+13-N). Rye storage proteins (secalins) was found in addition lines: 1R, 2R and substitution line 1B/1R. It can be concluded that genes responsible for secalin biosynthesis in rye cv. Dańkowskie Złote are localized on 1R and 2R chromosomes.

Key words: addition lines, HMW glutenins, LMW glutenins, 1B/1R substitution line, SDS-PAGE electrophoresis, secalins

INTRODUCTION

Baking value of wheat flour is determined mainly by glutenins (Payne *et al.* 1987, Verbruggen *et al.* 2001), and therefore identification of genes responsible for these proteins synthesis is very important from practical point of view.

On the base of numerous studies, it was found that complex loci Glu A1, Glu B1, Glu D1 localized on long arms of chromosomes 1A, 1B and 1D respectively are responsible for wheat high-molecular weight glutenin biosynthesis (Lawrence and Shepherd 1981, Payne *et al.* 1982). New techniques for DNA molecular weight markers identification made possible to separate high-molecular weight glutenins into "x" type (fractions of lower mobility) and "y" type (fractions of higher mobility) (Lafiandra *et al.* 1997). Rogers *et al.* (2001) predicted the consequences of the lack of some "x" or "y" subunits in almost isogenic lines of wheat cv. Sicco. In general, the absence of those subunits negatively affected the elasticity and extensibility of gluten and worsened the

Communicated by Danuta Miazga

physicochemical properties of the dough. High-molecular weight glutenins encoded by Glu D1 locus (Nieto *et al.* 1998), especially 5+10 subunits, have the major influence on grain quality of bread wheat (Payne *et al.* 1987, D'Ovidio *et al.* 1994, Lafferty and Lelley 2001).

Robin *et al.* (1998) observed the evolution of loci determining biosynthesis of high-molecular weight glutenins in genomes A, B, D and G of wheat. Applying DNA molecular markers, the authors found the philogenetic associations among *Triticum aestivum*, *Triticum monococcum*, *Triticum timopheevi*, *Triticum turgidum*, *Triticum urartu* and *Aegilops squarrosa* and they predicted probable forming time for particular genomes and loci encoding glutenins.

While the influence of HMW glutenins on flour properties is established, the assotiation between LMW glutenin subunits and breadmaking quality is still researched. Among others LMW glutenins affect on dough extensibility (Verbruggen *et al.* 2001) and pasta-making quality of durum wheat (Liu and Shepherd 1996).

Low-molecular weight glutenins are encoded by Glu A3, Glu B3 and Glu D3 genes that are localized on short arms of chromosomes from the first homoeologous group (1A, 1B, 1D) (Van Campenhout *et al.* 1995).

Genes responsible for secalin biosynthesis were localized on 1R and 2R chromosomes (Sybenga *et al.* 1990, 1991, Orellana *et al.* 1993). Secalins negatively affected the technological parameters of wheat and triticale flour. Substitution 1B/1R and translocations 1BL.1RS and 1AL.1RS cause decrease in flour baking quality (Lee *et al.* 1995, Graybosch 2001). Comprehensive studies upon elimination or reducing this unfavorable effect mainly by directed translocations and substitution of chromosomes are conducted (Kumlay *et al.* 2003).

The aim of present paper was to evaluate the composition of HMW and LMW glutenin subunits in wheat-rye addition lines cv. Grana - cv. Dańkowskie Złote and substitution line 1B/1R as well as localization of *Sec* genes in 'Dańkowskie Złote' rye using biochemical markers (SDS-PAGE electrophoresis).

MATERIAL AND METHODS

Series of lines of hexaploid wheat (*Triticum aestivum* L.) cv. Grana with added pairs of complete or telocentric chromosomes of rye (*Secale cereale* L.) cv. Dańkowskie Złote (1R, 2R, 3R, 3RS, 4R, 5R, 6R, 6RL, 7R) as well as substitution line 1B/1R of cv. Grana / cv. Dańkowskie Złote were the subject of studies. All lines were obtained at Institute of Genetic and Plant Breeding of Agricultural University in Lublin (Miazga and Chrząstek 1987, Chrząstek and Miazga 1988). At the same time, initial forms; wheat cv. Grana, rye cv. Dańkowskie Złote and the octoploid triticale obtained crossing them, were studied.

All materials were previously cytologically tested in order to choose stable forms. Number of chromosomes in somatic cells and chromosome configurations in meiosis were studied.

The composition of high molecular weight glutenin (HMW) subunits and low molecular weight glutenin (LMW) subunits, as well as presence of secalins were determined using electrophoresis technique on polyacrylamide gel in presence of sodium dodecyl sulfate (SDS-PAGE) according to Laemmli (1970)

method. Analysis were performed in WIBEX electrophoresis laboratory in Poznań. Identification of glutenin subunits was performed on the basis of Payne and Lawrence (1983) catalogue. Ten kernels from every line and initial form were analyzed. In order to simplify high-molecular weight glutenin subunits identification, standard varieties of hexaploid wheat Jubilatka and Begra were applied. Loci of allels encoding low-molecular weight glutenin subunits were found using aneuploid lines of 'Chinese Spring' and 'Pavon' wheat.

RESULTS AND DISCUSSION

Usually from three to five high-molecular weight glutenin subunits occur in hexaploid wheat varieties. Locus *Glu A1* is responsible for one subunit, locus Glu B1 for one or two subunits and locus *Glu D1* encodes usually two subunits. In literature data, one can find reports on the discovery of new allels of particular loci encoding unidentified high-molecular weight glutenin subunits (Tahir *et al.* 1986).

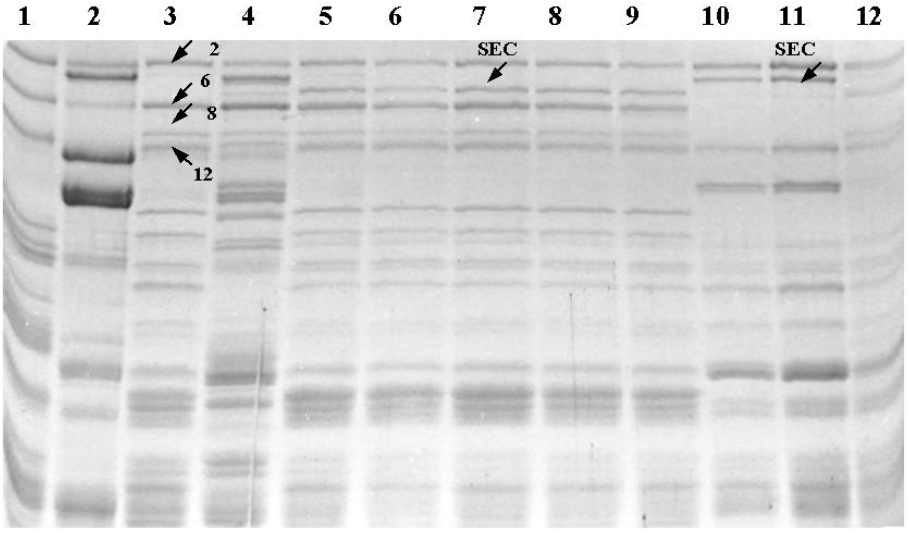

Fig. 1. Electrophoretic separation of high molecular weight glutenins and secalins in addition line 1R, substitution line 1B/1R and initial forms. Lanes: **1** - standard, **2** - 'Dańkowskie Złote' rye, **3** - 'Grana' wheat, **4** - octoploid triticale 'Grana' × 'Dańkowskie Złote', **5-9** addition line 1R, **10-11** substitution line 1B/1R, **12** - standard

Analysis of high-molecular weight glutenins in studied cv. Grana wheat lines with added or substituted chromosomes of rye cv. Dańkowskie Złote revealed that locus *Glu A1* determined block N (Null) in all lines and in wheat, which means the lack of glutenin subunits (Table 1). Locus *Glu D1* encoded subunits 2+12 in all studied lines and wheat. Presence of 6+8 subunits encoded by *Glu B1* locus was found in tested addition lines and 'Grana' wheat. Received results are agreed with these obtained previously by Brzeziński (1993). In substitution line 1B/1R no subunits encoded by *Glu B1* locus was observed. However, the band characteristic for rye proteins, i.e. secalins, was clearly visible on electrophoregram (Fig. 1). This band was situated in place respective for 'Dańkowskie Złote' rye. All subunits present in 'Grana' wheat, i.e. N-6+8-2+12

and one secalin band characteristic for rye, were observed in addition line 1R. Also on electrophoregram of addition line 2R, besides glutenin subunits respective for 'Grana' wheat, two bands confirming the presence of rye proteins were found (Fig. 2). No secalin bands were found in other lines. From analyses it follows that genes controlling secalin synthesis occurred on 1R and 2R chromosomes of 'Dańkowskie Złote' rye. Many authors found that 1R chromosome had three loci encoding rye storage proteins. Locus *Sec 3* localized on long arm of this chromosome is responsible for high-molecular weight secalin synthesis homologous with wheat high-molecular weight glutenins (Orellana *et al.* 1993). Complex locus *Sec 1* determining synthesis of type and 40 K secalins occurred on short arm of 1R chromosome in a region of satellite (Sybenga *et al.* 1990). Proteins encoded by *Sec 1* are homologous with wheat gliadins. Linkage between loci *Sec 3* and *Sec 1* are well known (Carillo *et al.* 1990, Sybenga *et al.* 1990). The third locus (*Sec 4*) is situated between *Sec 1* and *Sec 3*, on short arm of 1R chromosome (Benito *et al.* 1990) and is responsible for -secalin synthesis.

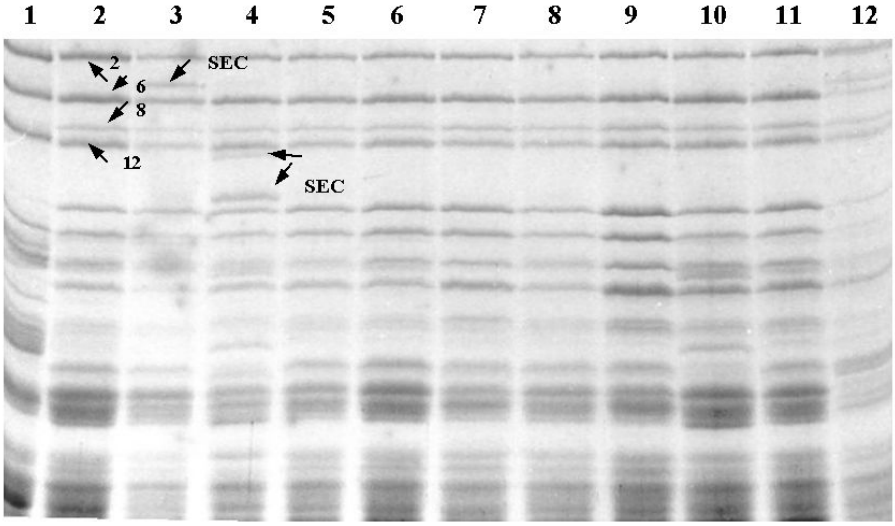

Fig. 2. Electrophoretic separation of high molecular weight glutenins and secalins in wheat and addition lines. Lanes: **1** – standard, **2** – 'Grana' wheat, **3** -1R, **4** - 2R, **5** - 3R, **6** - 3RS, **7** - 4R, **8** - 5R, **9** - 6R, **10** - 6RL, **11** - 7R, **12** - standard

Gene *Sec 2* that is responsible for synthesis of type 75 K secalins having no analogues in other cereals was identified on 2R chromosome (Sybenga *et al.* 1991, Murray *et al.* 2001). The latest studies (Malyshev *et al.* 1998) revealed the presence of another gene (*Sec 5*) determining secalin synthesis that is also localized on short arm of chromosome 2R. Genes responsible for synthesis of 75 K secalin type were also found on 6R chromosome in *Secale montanum* (Shevry *et al.* 1985). Probably, during evolution process of *Secale cereale* as a consequence of translocation, genes encoding secalins were transferred from chromosome 6R *Secale montanum* into chromosome 2R *Secale cereale*. New alleles of previously reported genes are isolated and characterised (De Bustos and Jouve 2003).

Table 1

Composition of high molecular weight glutenin subunits in addition lines 'Grana' - 'Dańkowskie Złote', 1B/1R substitution line, initial form and standard wheat varieties

Chromosomes and varieties	Composition of subunits controlled by loci			Presence of secalin
	Glu-A1	*Glu-B1*	*Glu-D1*	
1R	N	6+8	2+12	+
2R	N	6+8	2+12	+
3R	N	6+8	2+12	
3RS	N	6+8	2+12	
4R	N	6+8	2+12	
5R	N	6+8	2+12	
6R	N	6+8	2+12	
6RL	N	6+8	2+12	
7R	N	6+8	2+12	
1B/1R	N	-	2+12	+
Grana	N	6+8	2+12	
Jubilatka	2*	6+8	2+12	
Begra	N	7+9	5+10	

Within studied addition lines 'Grana' – 'Dańkowskie Złote' and substitution line 1B/1R, no polymorphism referring to the composition of high-molecular weight glutenin subunits was observed.

While genes encoding high-molecular weight glutenins are relatively easy to identify, localization genes controlling low-molecular weight glutenin synthesis is difficult, because of the fact that they are strongly linked with genes controlling biosynthesis of gliadins situated on the same chromosomes (Dubcovsky *et al.* 1997). Further difficulties result that the low-molecular weight glutenins are close to some gliadins, albumins and globulins, which makes electrograms difficult to interpret. Even hypotheses that some low-molecular weight glutenins are modified gliadins encoded by Gli genes, have arisen (Metakovsky *et al.* 1997). According to Payne and Corfield (1979), low-molecular weight glutenin subunits B and C are similar to type gliadins in respect to molecular weight, and D subunit to type gliadins. Despite of these difficulties, numerous studies are carried out aiming to learn genetic conditions of low-molecular weight glutenin synthesis. Application of the newest techniques for DNA molecular weight markers identification made possible to localize genes encoding low-molecular weight glutenins and polymorphism occurring among them (D'Ovidio 1992, Van Campenhout *et al.* 1995). Low-molecular weight glutenins are encoded by genes *Glu A3*, *Glu B3* and *Glu D3* localized on short arms of the first homoeologous group chromosomes (1A, 1B, 1D). Sreeramulu *et al.* (1997) reported the identification of new subunits of low-molecular weight glutenins being under control of *Glu D4* and *Glu D5* genes that are found on 1D and 7D chromosomes in hexaploid Indian wheat varieties.

The attempt to preliminary evaluation of the 'Grana' wheat lines with added and substituted 'Dańkowskie Złote' rye chromosomes with respect to low-molecular weight glutenin composition applying aneuploid wheat lines ('Chinese Spring' and

'Pavon'), was undertaken in present study. Analysis of electrophoregrams revealed that Glu A3 locus localized on 1AS chromosome is responsible for synthesis of N-1-2 subunits, Glu B3 gene on 1BS chromosome encodes 6-13 subunits, and Glu D3 occurring on short arm of 1D chromosome encodes subunit 15 (Table 2). However, electrophoregrams were unclear and difficult for interpretation. It was only possible to assess the composition of subunits for wheat, triticale and two addition lines. In 'Grana' wheat, low-molecular weight glutenin fraction consisted of subunits 1-6+9-15, and in addition lines 2R and 6RL subunits N-6+9-15 were included (Table 2). Octoploid triticale 'Grana' × 'Dańkowskie Złote' was not uniform regarding to the composition of low-molecular weight glutenin fraction. Composition of 1-6+9-15 was observed in one case, 1-11+13-N in another. On a basis of results achieved and poor data in available literature, it is hard to univocally account for the reason of such polymorphism. According to Rozynek *et al.* (1998) who analyzed electrophoretic separation of gliadins and secalins in primary forms of octoploid triticale, differentiation of gene expression encoding those proteins can result from the interaction between parental rye and wheat genomes, effect of foreign cytoplasm as well as high level of aneuploidy.

Table 2

Composition of low molecular weight glutenin subunits in addition lines 'Grana' - 'Dańkowskie Złote', initial forms and standard wheat variety

Chromosomes and varieties	Composition of subunits controlled by loci on chromosomes		
	1A	1B	1D
2R	N	6+9	15
6RL	N	6+9	15
Grana	1	6+9	15
Grana × Dańkowskie Złote (2n=8x=56)	1	6+9	15
	1	11+13	N
Begra	2	6+9	15

CONCLUSIONS

Addition of rye chromosomes to wheat had no effect on high-molecular weight glutenin composition. In 'Grana' wheat and all addition lines, the same composition of glutenin fraction (N-6+8-2+12) was observed. In analyzed addition lines, no polymorphism referring to high-molecular weight glutenin composition was found. Rye storage proteins (secalins) were identified in 1R and 2R addition lines and substitution line 1B/1R. Results achieved allow concluding that in rye cv. Dańkowskie Złote *Sec* genes responsible for secalin synthesis are localized on 1R and 2R chromosomes.

REFERENCES

Benito C., Frade J.M., Orellana J., Carrillo J.M. (1990). Linkage and cytogenetic maps of genes controlling endosperm storage proteins and isozymes in rye (*Secale cereale* L.). Theor. Appl. Genet. 79: 347-352.

Brzeziński W. (1993). Katalog elektroforetyczny pszenic polskich, COBORU, Słupia Wielka, Wiadomości Odmianoznawcze 54, 1-74,1993

Bustos de A., Jouve N. (2003). Characterisation and analysis of new HMW-glutenin alleles encoded by the Glu-R1 locus of Secale cereale. Theor. Appl. Genet. 107: 74-83

Carrillo J.M., Vazquez J.F., Orellana J. (1990). Linkage relationships between the loci *Sec* 1 and *Sec* 3 in rye (*Secale cereale* L.). Heredity 64: 125-130.

Chrząstek M., Miazga D. (1988). Studies on the cv. Grana wheat (*Triticum aestivum* L.) lines with the cv. Dańkowskie Złote rye (*Secale cereale* L.) chromosome addition. Part II. An estimate of important quantitative traits. Genetica Polonica 29(1), 21 – 26.

D'Ovidio R., Tanzarella O.A., Masci S., Lafiandra D., Parceddu E. (1992). RFLP and PCR analyses at Gli-1, Gli-2, Glu-1 and Glu-3 in cultivated and wild wheats. Hereditas 116: 79-85.

D'Ovidio R., Parceddu E., Lafiandra D. (1994). PCR analysis of genes encoding allelic variants of high-molecular-weight glutenin subunits at the Glu-D1 locus. Theor. Appl. Genet. 88: 175-180.

Dubcovsky J., Echaide M., Giancola S. (1997). Seed-storage-protein loci in RFLP maps of diploid, tetraploid and heksaploid wheat. Theor. Appl. Genet. 95: 1169-1180.

Graybosch R.A. (2001). Uneasy unions: Quality effects of rye chromatin transfers to wheat. J. Cereal Sci. 33: 3-16.

Kumlay A.M., Baenziger P.S., Gill K.S., Shelton D.R., Graybosch R.A., Lukaszewski A.J., Wesenberg D.M. (2003). Understanding the effect of rye chromatin in bread wheat. Crop Science 43: 1643-1651.

Laemmli U.K. (1970). Cleavage of structural proteins during the assembly of the head of bacteriophage T_4. Nature 4(227): 680-685.

Lafferty J., Lelley T. (2001). Introduction of high molecular weight glutenin subunits 5+10 for the improvement of the bread-making quality of hexaploid triticale. Plant Breeding 120: 33-37.

Lafiandra D., Tucci G.F., Pavoni A., Turchetta T., Margiotta B. (1997). PCR analysis of x- and y- type genes present at the complex Glu-A1 locus in durum and bread wheat. Theor. Appl. Genet. 94: 235-240.

Lawrence G.J., Shepherd K.W. (1981). Inheritance of glutenin protein subunits of wheat. Theor. Appl. Genet. 60: 333-337.

Lee Y.H., Graybosch R.A., Peterson C.J. (1995). Quality and biochemial effects of a 1BL/1RS wheat-rye translocation in wheat. Theor. Appl. Genet. 90: 105-12.

Liu C., Shepherd K.W. (1996). Variation of B subunits of glutenin in durum, wild and less-widely cultivated tetraploid wheats. Plant Breeding 115: 172-178

Malyshev S.V., Khmyl T.O., Zabenkova K.I., Voylokov A.V., Korzun V.N., Kartel N.A. (1998). RFLP-based mapping of the *Sec-2* and *Sec-5* loci encoding 75K -secalins of rye. Plant Breeding 117: 329-333.

Metakovsky E.V., Branlard G., Chernakov V.M., Upelniek V.P., Redaelli R., PognaN.E. (1997). Recombination mapping of some chromosome 1A-1B-1D and 6B-controlled gliadins and low-molecular-weight glutenin subunits in common wheat. Theor. Appl. Genet. 94: 788-795.

Miazga D., Chrząstek M. (1987). Studies on the cv. Grana wheat (*Triticum aestivum* L.) lines with the cv. Dańkowskie Złote rye (*Secale cereale* L.) chromosome addition. Part I. Chromosome configurations at metaphase I. Genetica Polonica 28(4), 327-331.

Murray F.R., Skerritt J.H., Appels R. (2001). A gene from the *Sec2* (Gli-R2) locus of a wheat 2RS.2BL chromosomal translocation line. Theor. Appl. Genet. 102: 431-439.

Nieto-Toladriz M.T., Rodriguez-Quijano M., Carrillo J.M. (1998). Biochemical and genetic characterisation of a D glutenin subunit encoded at the Glu-B3 locus. Genome 41: 215-220.

Orellana J., Fernandez-Calvin B., Vazquez J.F., Carrillo J.M. (1993). Mapping of genes controlling seed storage-proteins and cytological markers on chromosome 1R of rye. Theor. Appl. Genet. 85: 639-643.

Payne P.I., 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., Hold L.M., Worland A.J., Law C.N. (1982). Structural and genetical studies on the high-molecular weight subunits of wheat glutenin. Theor. Appl. Genet. 63: 129-138.

Payne P.I., Lawrence G. (1983). Catalogue of alleles for the complex gene loci Glu-A1, Glu-B1 and Glu-D1 which code high-molecular-weight subunits of glutenins in hexaploid wheat. Cereal Res. Comm. 11: 29-35.

Payne P.I., Nightingale M.A., Krattiger A.F., HOLD L.M. (1987). The relationship between HMW glutenin subunit composition and the bread-making quality of british-grown wheat varieties. J. Sci. Food Agric. 40: 51-65.

Robin G., Banerjee M., Brown T.A. (1998). Evolution of the high molecular weight glutenin loci of the A, B, D and G genomes of wheat. Genome 42: 296-307.

Rogers W.J., Sayers E.J., Ru K.L. (2001). Deficiency of individual high molecular weight glutenin subunits affords flexibility in breeding strategies for bread-making quality in wheat *Triticum aestivum* L. Euphytica 117: 99-109.

Rozynek B., Günther T., Hesemann C.U. (1998). Gel electrophoretic investigations of prolamins in eu- and alloplasmatic octoploid primary triticale forms. Theor. Appl. Genet. 96: 46-51.

Shewry P. R., Parmar S., Miller T. E. (1985). Chromosomal location of the genes for the Mr 75000 □-secalins in *Secale montanum* Guss: Evidence for a translocation involving chromosomes 2R and 6R in cultivated rye (*Secale cereale* L.). Heredity 54: 381-383.

Sreermulu G., Singh N.K. (1997). Genetic and biochemical characterization of novel low molecular weight glutenin subunits in wheat (*Triticum aestivum* L.). Genome 40: 41-48.
Sybenga J., Parmar S., Eden von J., Shewry P. (1990). Mapping seed storge protein loci *Sec-1* and *Sec-3* in relation to five chromosomes rearrangements in rye (*Secale cereale* L.) Theor. Appl. Genet. 79: 201-208.
Sybenga J., Parmar S., Eden von J., Shewry P. (1991). Mapping of the seed storage protein locus *Sec-2* in rye. Genetica 84: 101-105.
Tahir M., Pavoni A., Tucci G. F., Turchetta T., Lafiandra D. (1996). Detection and characterization of a glutenin subunit with unusual high Mr at the Glu-A1 locus in hexaploid wheat. Theor. Appl. Genet. 92: 654-659.
Campenhout van S., Stappen J.V., Sagi L., Volckaert G. (1995). Locus specific primers for LMW glutenin genes on each of the group 1 chromosomes of hexaploid wheat. Theor. Appl. Genet. 91: 313-319.
Verbruggen I.M., Veraverbeke W.S., Delcour J.A. (2001). Significance of LMW-GS and HMW-GS for dough extensibility: 'addition' versus 'incorporation' protocols. J. Cereal Sci. 33: 253-260.