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Electrophoretic and Immunological Evaluation of Secalin in Rye, Triticale, and Wheat-Rye Translocation Wheat

Yong Weon Seo*† and Byung Hee Hong*

ABSTRACT

Seed storage proteins have been used for studying biochemical genetics and end-use quality aspects. We conducted enzyme-linked immunosorbent assay (ELISA) and one-dimensional SDS-PAGE (1D SDS-PAGE) to evaluate different cereal crop species and Korean wheat lines for rye secalin proteins. The antisecalin antibody showed consistent specificity for rye secalin with little cross-reactivity to gliadins. Immunological cross-reactivities measured by the ELISA technique using competition assay showed significant differences of absorbance among rye, triticale, wheat-rye translocated wheat and non-translocated wheat. The absorbance values were lowest in rye followed by triticale, translocated wheat and non-translocated wheat. The ELISA for discrimination of wheat-rye translocation on the basis of antigen-antibody reactivity showed that none of the Korean wheat lines possessed 1RS and secalin proteins. The competitive ELISA experiment demonstrated specific determination for secalin that was originated from rye chromosomal parts. The result of 1D SDS-PAGE for identifying rye secalin subunits showed all three rye specific secalin protein subunits (75 KDa, 45 KDa, and 40 KDa) for rye and triticale, and 1RS specific secalins (45 KDa and 40 KDa) for 1AL/1RS and 1BL/1RS translocated wheats. All Korean wheats were lacking 1RS of rye chromosome and secalin.

Key words : secalin, enzyme-linked immunosorbent assay, wheat, rye, translocation.

Gluten (glutenin and gliadin), the major wheat endosperm protein, constitutes over 80% of the total grain protein and is responsible for the unique properties of wheat end-use quality. It provides one of the best systems for analysing gene expression in crop because their expressions are genetically fixed manner (Graybosch et al., 1990).

Secalins are the major seed storage prolamins of rye and account for about 50% of the total nitrogen of the matured grains (Shewry et al., 1983). Although the prolamins of rye are related to those of wheat, rye secalin has the higher proportion of the disulfied-bonded polymers that are solubilized in alcohol-water mixtures under the non-reducing conditions (Field et al., 1983b). Previous studies have indicated the possible use of prolamins in studying biochemical genetics and quality aspects (Graybosch et al., 1993; Dhaliwal & MacRitchie, 1990; MacRitchie et al., 1988; Payne, 1987; Field et al., 1983a,b).

The selection for disease resistance among progenies from crosses between rye and wheat has produced several wheat-rye chromosomal translocations in which some portions of rye chromosomes have replaced the homoeologous chromosome arms in wheat (Koebner & Shepherd, 1988). In the 1AL/1RS or 1BL/1RS translocated lines, the short arm of 1A or 1B has been replaced by the short arm of 1R, respectively. Chromosome 1RS carries valuable genes for disease (Zeller & Hsam, 1984) and insect resistance (Hollenhorst & Koppa, 1983; Sebesta & Wood, 1978). Therefore, the advantages of high grain yield and disease resistance make wheat breeders use translocation germplasm throughout the world (Rajaram et al., 1983; Merker, 1982). Despite of numerous agronomic advantages, 1RS in the form of wheat-rye translocation was also known for decreasing low-molecular glutenin proteins that resulted in sticky dough and defect on end-use qualities for breadmaking (Dhaliwal and MacRitchie, 1990; MacRitchie et al., 1988; Martin and Stewart, 1986).

Enzyme-linked immunosorbent assay (ELISA) have the capability to meet in assessing early generation screening within crop breeding programs. Immunological similarity of target proteins was used for producing antibodies among endosperm proteins of various cereal species (Skerritt & Lew 1990). ELISA has advantages of taking low running costs and handling large numbers of samples in a short time period. This includes prediction of wheat end-use quality parameters and identification of 1BL/1RS chromosomal translocation (Seo et al., 1995).

The objectives of this study were; Ⅰ) to evaluate antisecalin antibody which is specific to proteins unique to cultivated rye using competitive ELISA, Ⅱ) to apply one-dimensional SDS-PAGE and ELISA for identifying 1RS translocation in Korean wheat cultivars and experimental lines, Ⅲ) to examine various cereal crop species and genotypes for the presence or absence of secalin encoding genes.

MATERIALS AND METHODS

Seed samples

* Department of Agronomy, Korea University, Anam-dong, Sungbuk-gu, Seoul 136-701, Korea.
† Corresponding author
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Table 1. Competition enzyme-linked immunosorbent assay analysis of cultivars and experimental lines from different cereal crop species and check wheat varieties.

Crops	Entries	Mean absorbances
Wheat	Urimil(.249)†, Tapdongmil(.232), Eunpamil(.202), Geurumil(.224), Jokwang(.242), Namhaemil(.232), Cheonggeamil(.250), Dahongmil(.236), Olmil(.196), Alchanmil (.217), Olgrumil(.280), Jaeraesomac(.211), Jangkwang 1(.227), Jinpeong(.57), Yookseong#3(.229), Sinkwang(.218), Naemil(.210), Youngkwang(.208), Kyeongkwang (.226), Gobunmil(.212) Jaeraejong I (.224), Somacjaerae(.204), Jaeraeneumil (.210), Jaeraemil(.243), Chungnamjaerae(.240), Tongmil(.225), Jaeraejong(.253), Geumgangmil(.240), Suwon 85(.252), Suwon 86(.191), Suwon 185(.238), Suwon 205(.258), Suwon 207(.243), Suwon 209(.216), Suwon 210(.216), Suwon 211(.252), Suwon 213 (.271), Suwon 218(.249), Suwon 225(.274), Suwon 229(.289), Suwon 230(.137), Suwon 234(.195), Suwon 236(.246), Suwon 239(.267), Suwon 241(.257), Suwon 243 (.245), Suwon 244(.197), Suwon 245(.201), Suwon 246(.254), Suwon 249(.250)	.233b‡
Rye	Geuruhomil(.084), Jangkwang(.083), Chochun(.077), Chungchu(.072), Chilbogisik(.082), Paldang(.077), Homil 8(.072), Homil 10(.079), Homil 12(.084), Homil 21(.089), Homil 23(.098)	.082f
Triticale	Suwon 15(.103), Suwon 18(.117), Suwon 23(.121), Suwon 24(.111), Josik 4(.115)	.113e
1AL/1RS translocation	TAM 107(.123)	.123c
1BL/1RS translocation	Siouxland(.117)	.117d
Non-translocation	Scout 66(.254)	.254a

† ELISA absorbence of individual plant
‡ means with same letter are not significantly different

The competition ELISA was made using grain meals from fifty Korean wheat cultivars and experimental lines. The antibody specific to secalin was characterized by mixtures of three rye meals, "Geuruhomil", "Jangkwang" and "Chochun". Some of Korean rye and triticale cultivars were also evaluated for the presence of secalin. All Korean wheat, rye, and triticale seeds tested in this study were kindly provided by National Crop Experimental Station, RDA (Suwon, Korea). As check wheat sources, 1AL/1RS and 1BL/1RS wheat-rye translocated lines and a non-translocated line were tested. These check materials were provided by Dr. R.A. Graybosch (USDA/ARS) and were increased at the Korea University. All seed sources used in this study are listed in Table 1.

Sample preparations for 1D SDS-PAGE

Each grain meal sample of 25 mg was vortexed with 1 ml 70% EtOH and shaked for 1 hour at 18℃. After centrifugation for 2 minutes, 100 μl of each supernatant was transferred to a new microtube. Samples were dried in a SpeedVac Concentrator. Same volume of 1x lane buffer (2% SDS, 10% glycerol, 0.06 M Tris pH 8.8, 0.002 M EDTA pH 8.0) was added and boiled 10 minutes for each sample.

SDS-PAGE for total gliadin

The procedures for preparation and running gel were followed as described by Graybosch & Morris (1990) with some modifications. Unreduced protein samples were obtained by omission of the dithiorythritol. Treated samples of 8 μl were loaded in 12% SDS-polyacrylamide gel and run for 3 hours at 40 watts.

Coated protein preparation

Proteins were extracted from grain meal mixture of three rye cultivars ("Geuruhomil", "Jangkwang", and "Chochun") with 1 ml of 70% ethyl alcohol by vortexing a few seconds and shaking for 30 minutes. After centrifugation for 3~4 minutes at 14×10^3 rpm, 800 μl of supernatant was transferred to the tube filled with 7.2 ml of 1×PBS. Sample was mixed gently and 50 μl of suspension was placed into each well of micro well plate (Dynatek Immulon 2). The plate was incubated to dry for overnight. After the incubation, unbounded protein areas were blocked with 200 μl of 1% polyvinyl alcohol diluted

in 1x PBS. After blocking solution was removed by flicking plate, the plate was kept in refrigerator until used.

Competitive Enzyme-linked Immunosorbent Assay (ELISA)

Proteins were extracted from 25 mg seed meal of wheat, rye, and triticale with 1 ml of 0.04 M NaCl by vortexing and shaking for 30 minutes at 210 rpm on orbital shaker at 20℃. After centrifugation for 3 minutes at 14 × 10^3 rpm, 20 μl of supernatant was transferred to each microtitre plate well. Same amount (20 μl) of 1× PBS was filled in each "control" well which does not contain coated protein. In order to avoid possible "outside-row" effects, the edged wells were not included in the design. Each well was added with 20 μl anti-secalin antibody (Seo et al., 1995) diluted to 1 : 500 (antibody : 1x PBS). Plate was incubated for 90 minutes at 20℃. After excess antibody was removed by flicking the plate, each plate was washed with solution (PBS, 0.5% Triton x-100) 4 times, and washed twice with deionized water by filling the wells and flicking the plate. Each well was incubated with 40 μl of goat anti-mouse AP (alkaline phosphotase) diluted to 1 : 1000 in diluent buffer (1 mM $MgCl_2$, 50 mM Tris-HCl, 0.04% NaN_3, 2% BSA) for 60 minutes at 20℃. After unbound antibody was removed by flicking the plate and washed as described above, 150 μl of 0.05% (w/v) p-nitro-phenyl-phosphate with 30 ml substrate buffer (2M diethanolamine, 2mM $MgCl_2$, pH 9. 7) was added to each well. Plate was incubated for 30 minutes at 20℃. Absorbances of alkaline phosphotase reaction product were measured by microplate reader at 405 nm.

Data analysis

Quantitative analysis was conducted on the basis of immunological cross reactivities (absorbances) measured by ELISA. All tested plant materials were arranged with three replications for competitive ELISA experiment. The averaged ELISA absorbances were evaluated for identifying the presence or absence of secalin proteins and their relative quantification.

RESULTS

The reactions with antibody for secalin obtained from 0.04 M NaCl extraction of grain meal mixture of three rye cultivars (Geuruhomil, Jangkwang, and Chochun), and triticale ("Suwon 15") were compared with those for proteins solubilized by 0.04 M NaCl from non-translocated check over a range of protein dilutions by competitive ELISA technique. To determine the optimum concentration for the protein-antigen reactions from the titration curve, serially diluted antigens were used (Fig. 1).

Results demonstrate specific antibody reactions with proteins solubilized from rye meal mixture and little

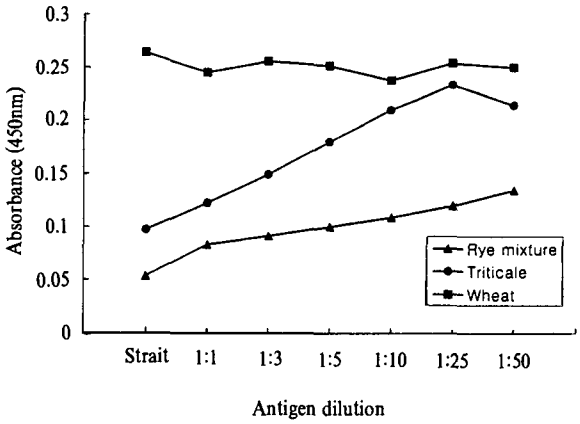

Fig. 1. Cross-reaction of antibody to proteins extracted from wheat (Scout 66), triticale (Suwon 15), and mechanical mixture of rye grain meal (Geuruhomil, Jangkwang, Chochun) by 0.04 M NaCl solution. Antigen dilution was prepared by volume: volume (strait protein solution : 1×PBS).

cross-reactions with normal wheat proteins. The regression coefficients for absorbances of antigen dilutions ranged from 1 : 1 (strait protein solution : 1×PBS) to 1 : 25 gave high linearities for both rye (R^2=0.86) and triticale (R^2=0.89). However, non-translocated wheat without epitopes for antibody bounding sites showed stable and high absorbances throughout various protein dilutions.

From the result of consistent specificity of antibody to rye secalin, the competitive ELISA using antisecalin antibody could be employed for identifying different cereal species and wheat-rye translocated wheat from non-translocated wheat. Since the antigen dilution of 1 : 1 (strait protein solution : 1×PBS) for antibody dilution of 1 : 500 well distinguished rye and triticale from non-translocated wheat, we used this antigen-antibody dilution throughout the competitive ELISA experiment.

The competitive ELISA was conducted for various cereal species and was applied for identifying 1RS wheat-rye translocation in Korean wheat varieties and experimental lines (Table 1). The three control varieties (1AL/1RS, 1BL/1RS, non-translocated wheat) evaluated by 1D SDS-PAGE assigned to each plate were used for adjusting absorbances associated with laboratory procedures. Duncan's multiple range test was conducted to test significance of relationships among the various cereal species (Table 1). The antigenic relationships among rye, triticale and wheat-rye translocated wheat could be evaluated for rye-specific secalin proteins.

The absorbances of rye cultivars (mean=0.082) which produced all three rye-specific secalin proteins offering epitopes to anti-secalin antibody were always lower than those of triticale (0.113) and wheat-rye translocated wheat (0.120) and non-translocated wheat (0.254).

Although rye and triticale had one set of R genome group, the genetic composition of cultivated hexaploid triticale (AABBRR) had contributed to lower concentration of cross-reacting antigen(secalin) than that of rye (RR). This result might be ascribed by allohexaploid nature of triticale in that secalin genes located on rye genome group were interfered by genes on homoeologous chromosomal parts.

The absorbances of 1RS translocations were significantly higher than those of rye and triticale. The relatively less amount of 45 KDa and 40 KDa secalins in 1RS translocated wheat than that of polypeptides of 75 KDa, 45 KDa and 40 KDa (Graybosh et al., 1993) might also be resulted in higher absorbances.

Significant difference of the mean absorbance between wheat-rye translocations and non-translocation was detected. The higher ELISA absorbances of 1AL/1RS and 1BL/1RS translocations than those of rye and triticale were the result of antisecalin antibody binding of only 45 KDa and 40 KDa secalins which were encoded by the gene on 1RS. Although a limited number was used in the test, the lower mean absorbances of 1BL/1RS than that of 1AL/1RS indicated that secalin expressions by the genes resided on 1RS could be different based on the translocated forms.

Our results indicated that the ELISA was suitable for identifying rye chromosomal segments and secalin proteins in different cereal genetic backgrounds.

Competitive ELISA experiment was also conducted to evaluate Korean wheat varieties and experimental lines for their presence of 1RS. All 50 wheat lines exhibited higher absorbances than those of check cultivars which possessed 1RS. Although "Olmil" (0.196), "Suwon 86" (0. 191), "Suwon 230" (0.137), "Suwon 243" (0.195), and "Suwon 244" (0.197) gave low absorbances, the values were still higher than those of 1RS checks. We analysed those lines by using electrophoretic separation and found none had rye secalin proteins [Fig. 2(A)].

Although ELISA absorbance of "Scout 66", non-translocated check variety, was the highest value, its absorbance was still fit to ranges of the absorbance values of Korean wheats (Table 1). Therefore, Korean wheat lines were shown to be absent of 1RS.

Since alcohol-soluble prolamins formed a polymeric series of polypeptides and could be used for studying biochemical genetics (Miflin & Shewry, 1979), we compared polypeptide profiles from rye, triticale, and wheat-rye translocated wheat and non-translocated wheat (Fig. 2). Results of the one-dimensional electrophoretic separation of unreduced 70% EtOH extractable proteins by 12% SDS-PAGE was shown in Fig. 2(A) and (B). It was possible to distinguish three rye specific protein subunits in the silver-stained gel. All 5 rye and 3 triticale lines produced 75 KDa encoded by the genes resided on 2RS and 45 KDa and 40 KDa subunits encoded by the genes on 1RS. The check varieties for 1AL/1RS and 1BL/1RS translocation lines showed only 45 KDa and 40 KDa subunits.

As shown in Fig. 2(A) and (B), Korean wheat lines were analysed for either the presence of 1RS or secalin proteins. All Korean lines tested showed that they were lacking 1RS and secalin subunits. This result was consistent with results of competitive ELISA in which high absorbance values were obtained for Korean wheat lines (Table 1).

When the polypeptide profiles of the rye and triticale were compared, the rye-specific polypeptide groups (75 KDa, 45 KDa, 40 KDa) were also observed in triticale (Fig. 2). However, ELISA absorbances between two species revealed that rye had more secalin proteins than triticale (Table 1). The similarity of those three-rye specific subunit groups between rye and triticale, but their weak specificity in triticale indicated that ELISA could be used for identifying secalin proteins and quantifying their relative amounts.

DISCUSSION

Wheat-rye (1RS) translocations are still useful in wheat breeding programs for introducing disease / insect resistance genes, agronomic advantage of tolerance in harsh growing conditions, and other end-use quality properties. In addition, a perceived grain yield advantage is associated with the presence of 1RS (Rajaram et al, 1983). In spite of some reports of detrimental effects on bread making associated with 1RS of wheat-rye translocation, the effects could be different based on various wheat genetic backgrounds.

Rye has been used as the most useful alien genetic material provider throughout the world because the allohexaploid nature of wheat can tolerate introduction of whole chromosome or chromosomal fragments. Furthermore, the end-use quality variation is derived from genetic and environmental factors, and their interactions (Peterson et al., 1992). End-use quality is not only influenced by the compositions of protein subunits, but also by other factors including grain hardness, lipid composition, and starch granule distribution. For these reasons, we are able to take advantages of both pest resistance and good quality properties when wheats with superior quality properties were used as recurrent parents. Thus, after chromosomal segments of 1RS are introduced, diagnostic tools based on genetically fixed secalin subunit composition will be necessary. The use of ELISA technology combined with 1D SDS-PAGE test has its significant advantages of low running costs and handle large number of samples in short periods of time with small amount of samples can meet this purpose.

Once 1RS translocation is applied in the wheat breeding program, use of wheat lines with high gliadin property should be considered as recurrent parents in the backcross breeding. In this way, we may reduce the risk of possible quality decrease caused by a reduced amount of gliadin and presence of secalin. Since secalin proteins could be used as an indication of the presence of 1RS, we could also apply either immunological assay or 1D

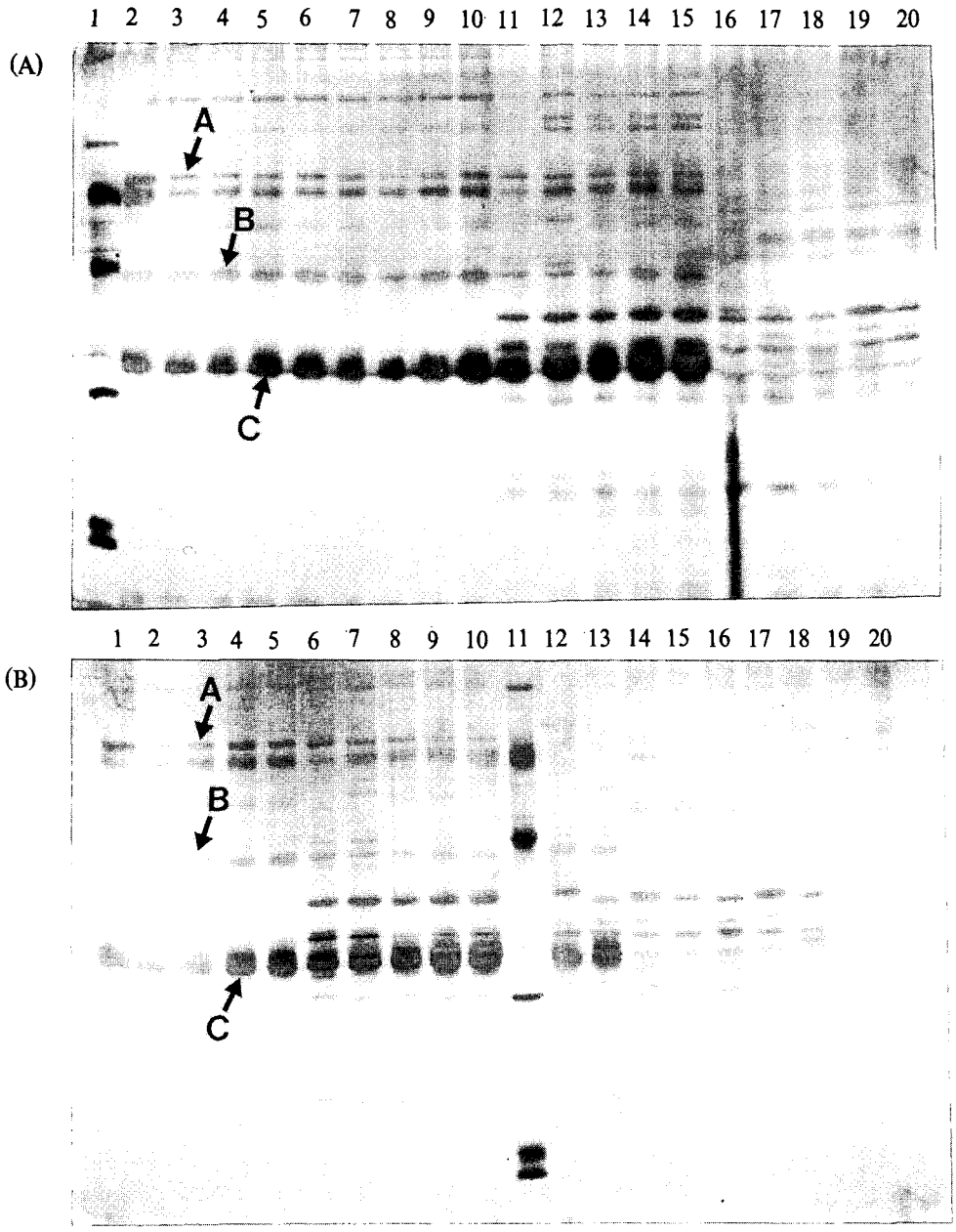

Fig. 2. (A). One dimensional SDS-PAGE separation of unreduced proteins extracted by 70% EtOH from various cereal crop species. Lane 1: size marker, lane 2-10: rye (2=Geuruhomil, 3=Jangkwang, 4=Chochun, 5=Chungchu, 6=Chilbogisik, 7=Paldang, 8=Homil 8, 9=Homil 10, 10=Homil 12), lane 11-15: triticale (11=Suwon 15, 12=Suwon 18, 13=Suwon 23, 14=Suwon 24, 15=Josik 4), lane 16-20: wheat (16=Olmil, 17=Suwon 86, 18=Suwon 230, 19=Suwon 243, 20=Suwon 233). Arrows indicate rye-specific secalin protein subunits (A=75 KDa, B=45 KDa, and C=40 KDa).

(B). One dimensional SDS-PAGE separation of unreduced proteins extracted by 70% EtOH from various cereal crop species. Lane 1-5: rye (1=Homil 21, 2=Homil 23, 3=Geuruhomil, 4=Jangkwang, 5=Chungchu), lane 6-10: triticale (6=Suwon 15, 7=Suwon 18, 8=Suwon 23, 9=Suwon 24, 10=Josik 4), lane 11: size marker, lane 12: TAM107 (1AL/1RS), lane 13: Siouxland (1BL/1RS), lane 14: Scout 66 (non-translocated wheat), lane 15-20: wheat (15=Urimil, 16=Tapdongmil, 17=Eunpamil, 18=Geurumil, 19=Jokwang, 20=Namhaemil). Arrows indicate rye-specific secalin protein subunits (A=75 KDa, B=45 KDa, and C=40 KDa). Both 1AL/1RS and 1BL/1RS lack 75 KDa secalin encoded by the genes on 2RS.

SDS-PAGE to evaluate wheats with 1RS for pest resistance. Since early generation screening for breeding programs requires methodologies that can accomodate the large number of samples in short period of time, use of ELISA can provide rapid screening breeding materials. Use of 1D SDS-PAGE will also provide accurate analysis of plant materials for detection of rye chromosomal segments in wheat-rye translocation.

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REFERENCES

Dhaliwal, A. S., and F. MacRitchie. 1990. Contributions of protein fractions to dough handling properties of wheat-rye translocation cultivars. J. Cereal Sci. 12:113-122.

Field, J. M., P. R. Shewry, S. R. Burgess, J. Forde, S. Parmar, and B. J. Miflin. 1983a. The presence of high molecular weight aggregates in the protein bodies of developing endosperms of wheat and other cereals. J. Cereal Sci. 1:33-41.

_____, _____, and B.J. Miflin. 1983b. Solubilization and characterization of wheat gluten proteins: correlations between the amount of aggregated proteins and baking quality. J. Sci. Food Agric. 34:370-377.

Graybosch, R. A., and R. Morris. 1990. An improved SDS-PAGE method for the analysis of wheat endosperm storage proteins. J. Cereal Sci. 11:201-212

_____, C. J. Peterson, L. E. Hansen, and P. J. Mattern. 1990. Relationships between protein solubility characteristics, 1BL/1RS, high molecular weight glutenin composition, and end-use quality in winter wheat germplasm. Cereal Chem. 67:342-349.

_____, Y. W. Seo, and C. J. Peterson. 1993. Detection of wheat-rye chromosomal translocation using an anti-secalin monoclonal antibody. Cereal Chemistry 70:458-463

Koebner, R. M. D., and K. W. Shepherd. 1988. Isolation and agronomic assessment of allosynthetic recombinants derived from wheat/rye translocation 1D.1RS, carrying reduced amounts of rye chromatin. In Proc. 7th Int. Wheat Genet. Symp. Miller T. E., and R. M. D. Koebner, eds. Institute of Plant Science Research, Cambridge, United Kingdom. pp. 343-348.

_____, _____, D. Worrall, D. R. Shelton, and A. Lukaszewski. 1993. Comparative flour quality and protein characteristics of 1BL/1RS and 1AL/1RS wheat -rye translocation lines. J. Cereal Sci. 17:95-106.

Hollenhorst, M. M., and L. R. Koppa. 1983. Chromosomal location of genes for resistance to greenbug in 'Largo' and 'Amigo' wheats. Crop Sci. 23:94-96.

MacRitchie, F., D. L. du Cros, and C. W. Wrigley. 1988. Flour polypeptides related to wheat quality. in: Advances in Cereal Science and Technology, Vol. X. Y. Pomeranz, (ed). Am. Assoc. Cereal Chem. St. Paul, MN, U.S.A. pp. 78-145.

Martin, D. J., and B. G. Stewart. 1986. Dough mixing properties of a wheat-rye derived cultivar. Euphytica 35:225-232.

Merker, A. 1982. "Veery" - A CIMMYT spring wheat with the 1B/1R chromosome translocation. In Cereal Research Communications. Vol. 10, No. 1-2. pp. 105-106.

Miflin, B. J., and P.R. Shewry. 1979. The biology and biochemistry of cereal seed prolamins. In Seed protein improvement for cereals and grain legumes. Int. Atomic Energy Authority, Vienna. pp 137-158.

Payne, P. I. 1987. Genetics of wheat storage proteins and the effect of allelic variation on bread-making quality. Ann. Rev. Plant Physiol. 38:141-153.

Peterson, C. J., R. A. Graybosch, P. S. Baenziger, and A. W. Grombacher. 1992. Genotype and environment effects on quality characteristics of hard red winter wheat. Crop Sci. 32:98-103.

Rajaram, S., C. E. Mann, G. Ortiz-Ferrara, and A. Mujeeb-Kazi. 1983. Adaptation, stability and high yield potential of certain 1B/1R CIMMYT wheats. Proc. 6th Int. Wheat Genet. Symp. Plant Germ-Plasm Inst., Fac. Agric., Kyoto Univ. pp. 613-621.

Sebesta, E. E., E. A. Wood. 1978. Transfer of greenbug resistance from rye to wheat with X-rays. Agronomy Abst. 70:61-62.

Seo, Y. W., R. A. Graybosch, C. J. Peterson, and D. R. Shelton. 1995. Assessment of enzyme-linked immunoassay of rye secalins as a tool in the prediction of 1RS wheat quality. Cereal Chem. 72:252-254.

Shewry, P. R., S. Parmer, and B. J. Miflin. 1983. Extraction, separation, and polymorphism of the prolamin storage proteins (Secalins) of rye. Cereal Chem. 60:1-6.

_____, A. S. Tatham, J. Forde, M. Kreis, and B. J. Miflin. 1986. The classification and nomenclature of wheat gluten proteins: A reassessment. J. Cereal Sci. 4:97-106.

Skerritt, J. H. and P. Y. Lew. 1990. Homologies between grain proteins of different cereal species. 1. Monoclonal antibody reaction with total protein extracts. J. Cereal Sci. 11:103-121.

Zeller, F. J., and S. L. K. Hsam. 1984. Broadening the genetic variability of cultivated wheat by utilizing rye chromatin. In Proc. 7th Int. Wheat Genet. Symp. Sakamoto S. ed. Plant Germ Plasm Institute. Kyoto University, Kyoto, Japan. pp. 161-173.