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Cytogenetically Engineered Rye Chromosomes 1R to Improve Bread-making Quality of Hexaploid Triticale

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ABSTRACT

Hexaploid triticale (X *Triticosecale* Wittmack) is rarely used for human consumption because of its poor bread-making quality. To create the genetic potential for bread-making quality similar to that of bread wheat (*Triticum aestivum* L), rye (*Secale cereale* L.) chromosome 1R in triticale cv. Presto was cytogenetically engineered to remove secalin loci *Sec-1* and *Sec-3*, and to introduce wheat storage protein loci *Gli-1* and *Glu-1*. The manipulations were by homoeologous recombination between rye chromosome 1R and 1B or 1D of wheat, followed by homologous recombination of primary recombinants with translocation breakpoints in desired locations. This approach generated three classes of multi-breakpoint translocation chromosomes named Valdy, FC and RM. Chromosome Valdy is a three breakpoint translocation with loci *Gli-D1*, *Sec-1* and *Glu-D1*; chromosomes FC1 and FC2 are five breakpoint translocations with loci *Gli-D1* and *Glu-D1*, and chromosomes RM are six breakpoint translocations with loci *Gli-B1* and *Glu-D1*. Preliminary tests of the effects of these chromosomes show a 230 to 250% increase of the SDS-sedimentation value over the recipient triticale Presto. While the impact of these chromosomes on the agronomic value of triticale is not clear, they restore to triticale the genetic load of gluten-encoding loci similar to that of bread wheat, thereby creating the genetic potential for breeding bread-making triticales.

TRITICALE, A MAN-MADE HYBRID of wheat (*Triticum s*pp.) and rye was created to combine the high yielding capacity of wheat with the stress tolerance of rye. Early efforts concentrated on octoploids generated from bread wheat and rye, but their inherent meiotic instability prevented breeding progress. Only hexaploid triticales, mostly the so-called secondary triticales selected from hybrids of octoploids with newly-synthesized hexaploids, have truly demonstrated triticale's promise.

Despite its high yielding capacity and presumed lower nutrient and soil requirements than wheat, triticale's acreage worldwide has been increasing only slowly (Arseniuk and Oleksiak, 2002). This may be a consequence of its rather limited end uses (Briggs, 1991; Pena, 1996). Triticale is bred and produced mainly for animal feed and forage. Despite some early attempts in several countries, it has never gained status as a human food. This is largely because, by wheat standards, its bread-making quality is poor. Good quality baked products can be obtained from triticale but by using different baking technologies (Rakowska and Haber, 1991).

The bread-making quality of bread wheat is determined by several factors of which the quantity and quality of gluten-forming storage proteins is critical (Bietz, 1987). Gluten is formed on dough mixing by polymerization of shorter subunits into long chains and interactions with lipids, giving dough its strength and elasticity (Simmonds, 1981). In bread wheat, gluten-forming proteins are encoded by loci located on chromosomes of homoeologous groups 1 and 6 (Payne et al., 1984; Payne, 1987). In group 1 the long arms carry loci *Glu-1* for high molecular weight glutenin subunits; the short arms have loci *Gli-1* for gliadins and *Glu-3* for low molecular glutenin subunits. In group 6 the short arms carry loci *Gli-2* that encode gliadins. The number of active genes in each locus may range from zero to many; the exact number of active gliadin genes has not yet been determined (Anderson et al., 1997). Each class of storage proteins contributes to a specific dough characteristic, such as strength, elasticity, extensibility and so on, that, in combination, determines loaf characteristics.

Storage proteins in rye are known as secalins, and loci controlling them are located in positions corresponding to those of wheat (Shewry et al., 1984). Chromosome 1R has a *Sec-1* locus on its short arm that encodes alcohol-extractable proteins similar to gliadins of wheat; the long arm has locus *Sec-3* in a position corresponding to the *Glu-1* loci of wheat. The *Gli-2*-related rye locus, *Sec-2*, is located on the short arm of chromosome 2R. This is because of an ancient chromosome translocation, 2R–6R that differentiates wheat from rye (Devos et al., 1993).

Rye cannot be baked using wheat bread-making technology. Because introgressions of rye chromatin in commercial wheats are frequent, the effect of secalins on the bread-making quality of wheat should be well known. It is generally believed to be detrimental, but this belief may be valid only for the *Sec-1* locus (Kumlay et al., 2003). As all introgressions of *Sec-1* into wheat have been accompanied by concomitant removal of one set of *Gli-1*/*Glu-3* loci, it is not clear whether it is the presence of *Sec-1* or the absence of wheat loci that negatively impact the bread-making quality. Not all secalins are detrimental to bread-making quality: replacement of some *Glu-1* wheat loci by the *Sec-3* locus produced a small positive effect on dough parameters in bread wheat (Kumlay et al., 2003), and in all rankings of the relative contribution of individual group-1 chromosomes to bread-making quality of triticale, rye chromosome 1R has always placed higher than wheat chromosome 1A (Kazman and Lelley, 1996; Lukaszewski, 1996, 1998; Wos et al., 2002; Kumlay et al., 2003). No effect of *Sec-2* on any parameters of wheat dough has so far been detected (Gupta et al., 1989).

The genomic constitution of hexaploid triticale is AABBRR whereas in bread wheat it is AABBDD. In effect, in triticale the rye (R) genome replaces the D genome of bread wheat. This difference in genomic consti-

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tution eliminates one third of wheat storage protein loci with their known positive effects on bread-making quality, and replaces them with a set of secalin loci that at best do not harm. Correspondingly, the total amount of gluten in triticale is low, and bread-making quality is poor (Pena, 1996). If triticale is to become a bread cereal, especially if it is to be used for leavened bread, its genetic constitution of storage protein loci has to be restored to the wheat level. Such an operation can be performed using several different technologies and approaches: transformation with known storage protein loci from the D-genome of wheat (Shewry et al., 1995) and concomitant silencing of the detrimental secalin loci; chromosome substitution, or cytogenetic engineering of specific segments of chromosomes that carry important loci. This article summarizes all aspects of cytogenetic engineering of rye chromosome 1R in hexaploid triticale to remove its original secalin loci and to introduce into it known pertinent wheat storage protein loci.

MATERIALS AND METHODS

Plant Material and Genetic Stocks

The basic line used in this study was hexaploid triticale cv. Presto, a winter cultivar originally obtained from the late Dr. E. Tymieniecka, then at Poznan Plant Breeding, Laski, Poland. All cytogenetic stocks of Presto used in this study have been developed by the author, including monosomics and single D-genome substitution lines (Lukaszewski, 1991), as well as the ditelosomic 1RL line (Presto Dt 1RL) extensively used in testing of various recombinant chromosomes here (Lukaszewski, unpublished data, 1998). Presto Dt1RL is lacking the short arm of 1R. Whenever convenient or necessary, chromosome manipulations were performed in hexaploid wheat cv. Pavon 76. Pavon 76, henceforth referred to as Pavon, is a white spring bread wheat from the International Center for the Improvement of Maize and Wheat (CIMMYT), El Batan, Mexico with very good bread-making quality (Kumlay et al., 2003). Engineering of the 1RS.1BL translocation for bread wheat was done in Pavon (Lukaszewski, 2000). A sample of Pavon was obtained from Dr. S. Rajaram, CIMMYT, Mexico. All chromosome constructs developed in wheat or obtained in wheat were transferred to Presto by backcrosses and selection in each generation. Given that in transfers from wheat to triticale only two of the three genomes are subject to recombination, six backcrosses were deemed sufficient in most cases to produce an acceptably uniform triticale material.

Handling of Plant Material

All plants during the chromosome development phase were grown in pots in a greenhouse on the University of California, Riverside, campus under the long day (usually 16h day/8h night) regime and at temperatures suitable for growing cereals of temperate climates. Vernalization was under 12 h day/12 h night regime in temperatures of approximately 4 to 7°C for 7 to 8 wk. All crosses were made by hand and all grain used in any part of the experiments originated from bagged heads. This did not include larger quantities of grain from homozygotes, produced for preliminary tests of bread-making quality.

The lengths of heads in the translocation lines were determined by counting the numbers of spikelets in the primary heads of plants grown in the greenhouse and in random samples of normally developed heads from plots grown at the East Farm of Oregon State University, Corvallis, Oregon. A standard sample was ten heads per line. All comparisons were made to lines of standard Presto grown under the same conditions and in the immediate vicinity of tested plants on lines.

Nomenclature

The nomenclature for engineered chromosomes, in effect multi-breakpoint translocations, is the same as proposed and used previously, when introgressing the *Glu-D1* locus into rye chromosome 1R (Lukaszewski and Curtis, 1992), and in the engineering project of the 1RS.1BL translocation in wheat (Lukaszewski, 2000). Single breakpoint translocations obtained after one round of induced homoeologous recombination are called primary recombinants; chromosomes obtained by homologous recombination of selected primary recombinants are called secondary recombinants (these are at least two-breakpoint translocations). Homologous recombination of secondary recombinant chromosomes produces tertiary recombinants, and so on. The nomenclature for the storage protein loci used in this article is that of Payne et al. (1984) for wheat and Shewry et al. (1984) for rye, notwithstanding numerous later changes.

Chromosomes

Some manipulations were performed using chromosomes obtained from others, or chromosomes created earlier in wheat or in triticale. These chromosomes were: 1RS-1DS recombinants WR2 and WR4 created and described by Koebner and Shepherd (1986) and Rogovsky et al. (1991) and kindly provided for this project by Dr. K.W. Shepherd, University of Adelaide, Australia. Chromosome 1R.1D$_{5+10}$-2 was created in hexaploid triticale cv. Rhino by Lukaszewski and Curtis (1992) and transferred to cv. Presto by backcrosses with ten completed. Chromosome MA1 is an engineered translocation 1RS.1BL of Kavkaz origin in the genetic background of bread wheat cv. Pavon (Lukaszewski, 2000). All remaining chromosomes were generated in the course of this study and their origin and structure, as well as some specific details of their development, are explained and illustrated in the Results section of this paper.

Chromosomes WR2 and WR4 have their short arms composed of short terminal segments of 1DS, long proximal segments of 1RS; their long arms are 1DL. The translocation breakpoints in the short arms of both chromosomes are distal to the *Sec-1* locus but apparently proximal to the block of disease resistant loci, including *Sr 31*. Therefore, both WR2 and WR4 carry *Gli-D1*/*Glu-D3* of wheat and *Sec-1* loci of rye on their short arms. The terminal 1DS segment is from the cv. Gabo, the rye segment is from the cv. Imperial.

Chromosome 1R.1D$_{5+10}$-2 is a chromosome 1R with an intercalary insert on the long arm from wheat chromosome 1D. This wheat insert replaces a segment of 1R carrying locus *Sec-3* encoding a secalin, and introduces a segment of 1DL with locus *Glu-D1*, allele *d*, encoding high molecular weight glutenin subunits 5+10. The wheat insert originated from the cv. Wheaton.

Chromosome MA1 is a 1RS.1BL-centric translocation originating from the Aurora/Kavkaz source (Rajaram et al., 1984), with its short arm engineered by homoeologous recombination (Lukaszewski, 2000). Starting from the telomere, this engineered short arm consists of a short terminal rye segment with a prominent telomeric C-band, a small intercalary segment of 1BS with loci *Gli-B1* and *Glu-B3*, a rye segment with a block of disease resistance loci, another intercalary segment of 1BS that removes the *Sec-1* locus, and a long proximal rye segment all the way to the centromere. The long arm of the chromosome is 1BL. The chromosome is a five-breakpoint translocation.

Cytology

The standard approach used throughout this study was that of E.R. Sears (Sears, 1981) for the development of intercalary alien inserts into wheat chromosomes. All cytological selection was by high through-put C-banding as described earlier (Lukaszewski and Xu, 1995). Some end products of the manipulations were verified using in situ probing with total genomic DNA following the protocol of Massoudi-Nejad et al. (2002). Depending on the specific application, total genomic DNA of either wheat or rye was labeled with digoxigenin using standard nick translation kits following the manufacturer's instructions (Roche Applied Science, Indianapolis, IN), hybridized to chromosome preparations in the presence of the other species DNA serving as a block, and detected by Anti-DIG-FITC (Roche Applied Science). The usual proportion of the probe to block was about 1:100, but higher block concentration were also used.

All observations were made using a Zeiss Axioscope 20 research microscope (Thornwood, NY). Photographs were taken with a digital Spot RT camera from Diagnostic Instruments Inc. (Sterling Heights, MI), and processed using Adobe Photoshop 6.0 software (San Jose, CA).

Identification of Storage Protein Loci

Identification of the storage protein loci present or absent from individual chromosome constructs was by polyacrylamide gel electrophoresis (PAGE) of individual kernels. Screening for high molecular weight glutenins and their corresponding *Sec-3* secalins was by SDS-PAGE as described by Brzezinski and Lukaszewski (1998). In the early stages of the project, gliadins and *Sec-1* secalins were resolved by Acid-PAGE following the protocol adapted for mini gels by Dr. A. Rybalka and kindly demonstrated to the author; in the later stages a modification of the protocol for gliadins of Lee et al. (1999) was used, whereby the ethanol-soluble fraction was extracted from crushed fragments of individual kernels in 70% ethanol for approximately 2 h at room temperature, air dried overnight, suspended in a SDS extraction buffer and resolved on the same type of gels as described in Brzezinski and Lukaszewski (1998). All separations were done using the Hoeffer Mighty Small II gel system. Detailed screening in the advanced stages of chromosome development was performed by the electrophoretic laboratory WIBEX, in Borowiec, Poland.

For all tests, individual kernels were cut with a razor blade. The germ parts were saved; the brush portions were crushed and used for protein extraction. Whenever it was deemed necessary to screen the same kernels for two different classes of protein, alcohol extraction of gliadins/*Sec-1* secalins was done first, followed by air-drying and the 2-mercaptoethanol- SDS-buffer extraction of HWM glutenins. Kernels with the desired constitution of the proteins were identified, individually germinated, and karyotyped by C-banding to confirm the desired/expected chromosome structure.

Preliminary Tests of Bread-Making Quality

Preliminary testing of bread-making quality was done using the micro SDS-sedimentation test for durum wheat (Dick and Quick, 1983). The tests were performed using standard laboratory glassware and can only be used to assess sedimentation levels of tested lines relative to the controls run in all experiments. The results for each translocation line were expressed as fractions of the value for the control present in each test and averaged over replications. Grain samples of controls originated from plants grown at the same time and in the immediate vicinity of the tested material. In more advanced stages, grain samples from replicated field trials were used.

All other tests and protocols used in the course of this study, such as screening for new primary recombinants, genetic mapping of translocation breakpoints in some primary recombinants, including screening for the presence/absence of disease resistance loci, were the same as in Lukaszewski (2000).

RESULTS

Chromosomes Valdy and Valdy II

To provide a quick solution to the bread-making quality problems of triticale, two chromosomes were created from chromosome 1R.1D$_{5+10}$-2 and two recombinant chromosomes WR2 and WR4. The combination involving WR4 was made first (Fig. 1).

Differences in banding patterns of chromosomes 1R.1D$_{5+10}$-2 on the one hand and WR2 and WR4 on the other, permitted monitoring recombination in two segments along the chromosome length, namely the long proximal rye segments on the short arms of WR2 and WR4 with their corresponding segments in 1RS of 1R.1D$_{5+10}$-2, and the intercalary segment from 1DL in the long arm of 1R.1D$_{5+10}$-2 with its corresponding segment in the 1DL arms of WR2 and WR4. Only two of the four possible configurations were of further interest and only their frequencies were noted.

During the transfer of chromosomes WR4 to Presto 1R.1D$_{5+10}$-2 only intact chromosomes WR4 were present among the total of 35 BC_1 and BC_2 plants screened. Among 70 BC_3 plants screened there were seven recombined chromosomes, two of these seven were rye chromosomes 1R.1D$_{5+10}$-2 with the WR4 translocation on the short arms and a terminal segment of 1DL on the long arms, and five were chromosomes WR4 with terminal rye segments on the long arms. The first two chromosomes must have originated from a crossover event in the proximal rye segment on the short arm. Because these two represent only one configuration of the two possible, the actual recombination rate of the proximal 1RS segment common to both chromosomes must have been about 4% (four events among 105 BC_1–BC_3 progeny screened). It should be noted that the segment in question comprises at least the proximal 75% of the 1RS length. The other five recombinant chromosomes must have originated from crossover events in the intercalary 1DL segment present on the long arm in 1R.1D$_{5+10}$-2. Again, with only one of two possible configurations scored, the actual crossover rate for the segment must have been approximately 9.5%.

The two chromosomes that originated from crossover events in the proximal segments of 1RS were named Valdy (Fig. 1 and 2). They were backcrossed an additional four times to Presto 1R.1D$_{5+10}$-2, and following selfing homozygotes were selected. Lines of Presto Valdy were tested for some parameters of bread-making quality and found to have reached the levels of commercial bread-making wheats (data not shown).

Upon distribution to winter triticale breeders it quickly became apparent that the presence of the Valdy translocation was associated in some environments with a

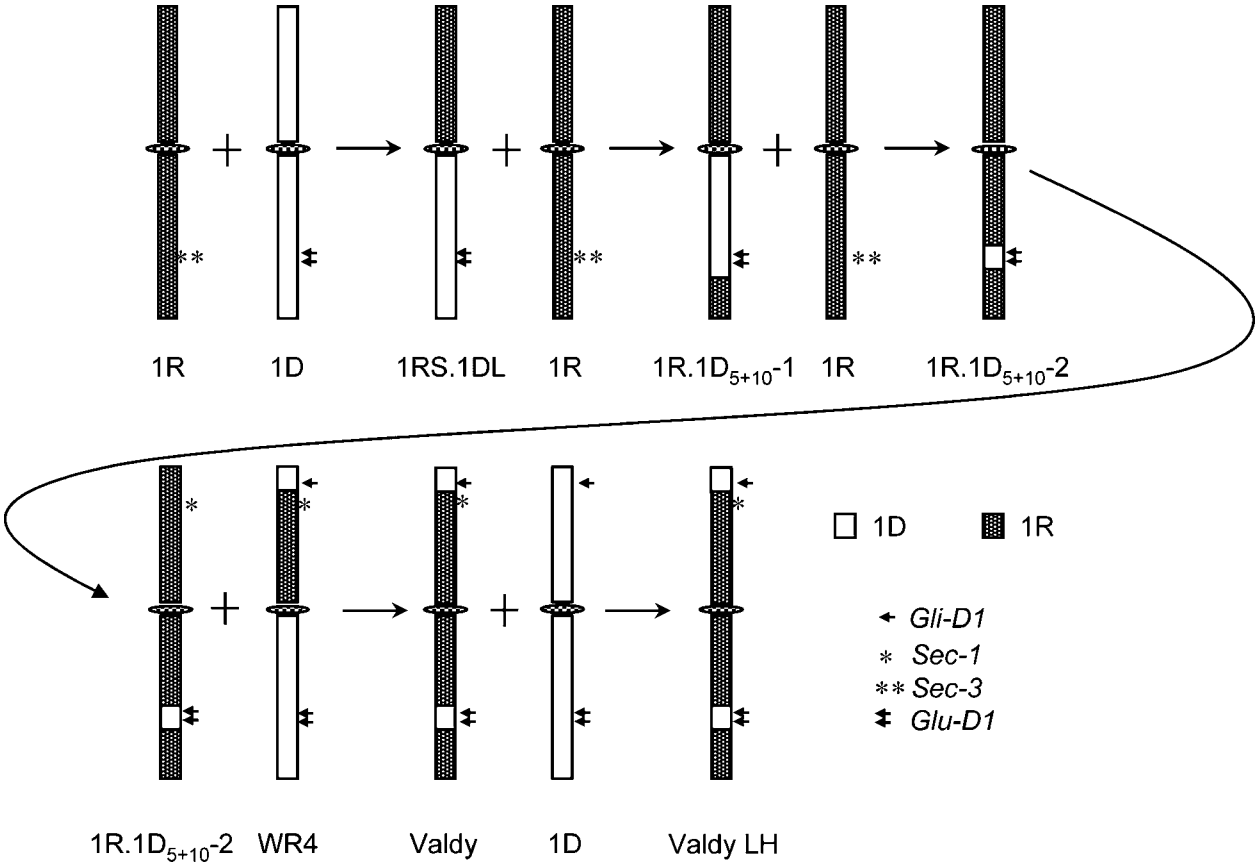

Fig. 1. The development of chromosomes 1R.1D$_{5+10}$-2 (after Lukaszewski and Curtis, 1992), and Valdy and Valdy LH from chromosomes 1D of wheat and 1R of rye, and their recombinants.

serious reduction in head length, by almost 50% as measured by the number of spikelets. This might have been responsible for about 15% yield reduction in some tests (R.J. Metzger, H. Wos, personal communication, 2002). Head length reduction went unnoticed in the greenhouse. Later counts of spikelets on samples of heads from plants grown in the greenhouse revealed that on average Valdy translocations reduced head length in the greenhouse-grown Presto lines by about two to three spikelets per head (Table 1).

The presence of chromosome 1R.1D$_{5+10}$-2 has never been associated with any morphological effects in triti-

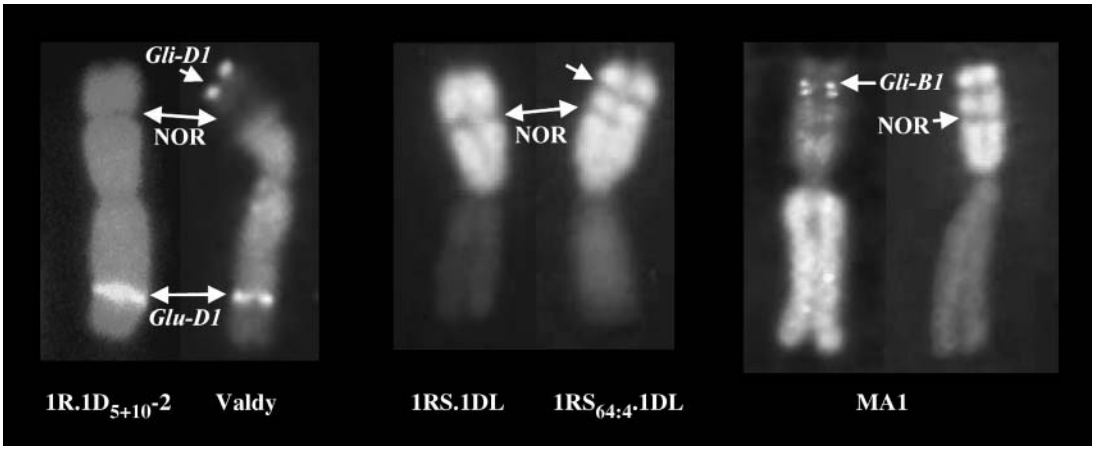

Fig. 2. In situ probing with total genomic DNA in engineered chromosomes 1R. Probe signal is white; block signal is gray. Pairs from left to right: chromosomes 1R.1D$_{5+10}$-2 and Valdy, probed with total genomic DNA of wheat with rye genomic DNA used as the block. Chromosomes 1RS.1DL and 1RS$_{64:4}$.1DL probed with rye genomic DNA and wheat genomic DNA as the block. Two chromosomes MA1: the one on the left probed with wheat genomic DNA and rye genomic DNA as the block; the one on the right with rye genomic DNA and wheat DNA as the block. Probing with rye probe in 1RS$_{64:4}$.1DL and MA1 shows a band of weaker signal in the middle of the satellite (single arrows). Probing with wheat probe in MA1 shows two distinct hybridization sites: the upper one delimits the 1BS insert with *Gli-B1*; the lower one the 1BS insert that eliminates the *Sec-1* locus. Positions of signals relative to the telomere vary because of the presence/absence or size of the telomeric C-band from 1RS on some chromosomes.

Table 1. Average numbers of spikelets in the primary heads of various translocation and substitution lines of Presto. Numbers of heads scored in parentheses. Heads in Corvallis, OR were from dense field planting.

Line	Greenhouse, UCR	Field, Corvallis, OR
Presto	25.9 ± 1.1 (18)	34.0 ± 0.8 (48)
Presto Valdy	23.3 ± 0.6 (12)	24.6 ± 1.3 (32)
Presto Valdy LH	25.1 ± 0.5 (18)	27.4 ± 1.2 (52)
Presto 1D(1R)	24.1 ± 0.6 (6)	32.3 ± 1.0 (12)

cale, and especially not with a reduction of spikelet number. It was assumed, therefore, that the short arm of the WR4 translocation was the culprit. This arm consists of a segment of 1DS and a segment of 1RS. The 1RS arm is quite frequent in commercial wheats the world over (Braun et al., 1998) and is generally associated with a yield increase (Rajaram et al., 1984; Singh et al., 1998), while the cv. Gabo, the source of the 1DS segment in WR4, is known for low number of spikelets under some conditions (R. Metzger, personal communication, 2001). Consequently, on the assumption that the wheat segment was the problem, Presto Valdy was crossed to two Presto 1D(1R) substitution lines. The first of these two lines involved chromosome 1D from the cv. Luna, a winter wheat from the breeding program of the late Dr. T. Wolski, then at Poznanska Hodowla Roslin, Laski, Poland; the other line involved chromosome 1D from the cv. Glenlea. Both substitution lines have heads with the number of spikelets similar to Presto. The F_1 hybrids were backcrossed as male to Presto Valdy and the resulting progeny were screened cytologically to identify plants with two Valdy-type chromosomes. In the backcross cross of F_1 involving $1D_{luna}$ 60 such progeny plants were identified; in the backcross involving $1D_{glenlea}$ 50 lines were identified. All 110 lines were grown and self pollinated in isolation. The samples were split into two, with one half grown in Corvallis, OR, and the other in Borowo, Poland.

The following summer the two sets of 50 and 60 lines in both locations were screened for head length, courtesy of Drs. R.J. Metzger and H. Wos. The set originating from the cross to $1D_{luna}$ was found to contain, in both locations, the same six lines segregating for long and short heads. The remaining 54 lines in each place had uniformly short heads. The lines with long heads were #16, 18, 22, 31, 67, and 70. These lines were named Presto Valdy LH 16, 18, 22, 31, 67, and 70, respectively. There were no lines segregating long heads in the set of 50 lines originating from the cross to $1D_{glenlea}$. The following year the six long-head (LH) lines of Presto Valdy were tested again for head length in Corvallis, OR under different sowing densities, from 10-cm spacing to commercial sowing density. Under spaced planting no head length reduction was apparent. However, increased sowing densities revealed some reduction, but to a lesser extent than that in the original Presto Valdy. Clearly, recombination with 1D of the cv. Luna mitigated but did not remedy the problem.

While the issue of head length in the original Valdy chromosome was being investigated, another Valdy type chromosome was generated from a wheat-rye recombinant WR2 and chromosome $1R.1D_{5+10}$-2. In this instance, the cross and the first two backcrosses of WR2 were to Presto $1D_{luna}(1R)$. BC_3 was to Presto $1R.1D_{5+10}$-2, and in the following backcross, among eleven progeny screened, a single recombined chromosome was identified. The short arm of this chromosome had the structure of WR2; the long arm was of $1R.1D_{5+10}$-2. The crossover event that produced this chromosome must have occurred in the proximal rye segment of the short arm. The plant heterozygous for the recombined chromosome was self pollinated and among its progeny a homozygote was identified, grown, backcrossed to Presto Dt 1RL and self pollinated. Electrophoretic verification among the selfed progeny confirmed that this chromosome carried loci *Gli-D1*, *Sec-1* and *Glu-D1* (*d*), and did not have the *Sec-3* locus. This chromosome was named Valdy II.

The status of head length in Presto Valdy II is not clear. As the primary recombinant WR2 was originally crossed and backcrossed to a Presto 1D(1R) substitution line, where heads are of the same length as in standard Presto, it is possible that its terminal 1DS segment is already recombined, similar to the Valdy LH chromosomes. Chromosome Valdy II has not yet been tested under field conditions.

Chromosome FC1

To prepare stocks for the eventuality that the Imperial 1RS present in both Valdy chromosomes negatively impacts head length in triticale it was decided to exploit the recombinants of the Aurora/Kavkaz 1RS generated in the 1RS.1BL chromosome engineering project (Lukaszewski, 2000). That project was centered on the 1RS.1BL translocation, but along the way 3228 progeny were screened to select recombinants of 1RS with 1DS (Lukaszewski, 2000) and 13 primary recombinants were isolated. Among these, nine were in the configuration with terminal rye segment and proximal wheat segment and the remaining four were in the reciprocal configuration. Following the convention adopted in that study (Lukaszewski, 2000) the former were labeled 1D+ and the latter labeled T−; both followed by the number indicating the order in which they were found. Genetic mapping of the translocation breakpoints indicated that most were proximal to the *Sec-1* locus and did not permit generation of the desired secondary recombinants. Therefore, in this project, 3618 progeny were screened of Pavon plants with chromosome constitution 19" + 1RS.1DL + 1D + 5B*ph1b*" where 1RS originated from the Aurora/Kavkaz 1RS.1BL translocation and 37 recombinants of 1RS were identified. Of these, 31 involved 1DS, one involved 1BS and five involved 1AS. Among recombinants 1RS-1DS, five were in the configuration with a terminal rye segment (1D+) and 26 were with a proximal rye segment (T−). Preliminary mapping of the translocation breakpoints using the same approaches as in Lukaszewski (2000) revealed that again, most translocation breakpoints were proximal to the *Sec-1* locus, and none in the configuration with a proximal rye segment were located between *Lr26* and *Gli-D1*. Therefore, the existing re-

combinants could only be used to eliminate the *Sec-1* locus from 1RS, but not to introduce the *Gli-D1* locus, while preserving the block of resistance genes between the two.

Secondary recombinant chromosome arms 1RS with intercalary inserts from wheat 1DS that eliminate the *Sec-1* locus were produced in Presto. For this purpose, primary recombinants T−62 and T−64, which have proximal rye segments and terminal 1DS segments, with breakpoints proximal to *Sec-1,* and primary recombinants 1D+4, 1D+9, and 1D+11 with terminal rye segments and breakpoints proximal to the disease resistance loci but distal to *Sec-1*, were crossed and backcrossed twice to Presto 1R.1D$_{5+10}$-2. The two sets of breakpoints (in 1D+ vs. T− primary recombinants) flank the *Sec-1* locus and homologous recombination in the overlapping wheat segment would produce 1RS with an intercalary insert of 1DS that eliminates *Sec-1* (Fig. 2 and 3).

In backcrosses of the primary recombinants to Presto 1R.1D$_{5+10}$-2, selection was for recombination events in the 1DL segments common to the 1DL arms in the primary recombinants and chromosome 1R.1D$_{5+10}$-2, or in primary recombinants in the T− configuration (that is, with proximal rye segments on their short arms), in their proximal 1RS segments and corresponding segments of 1R.1D$_{5+10}$-2. In BC$_1$ chromosomes T−62 and T−64 were identified with terminal rye segments on the long arms. The structure of their long arms is identical to that of chromosome 1R.1D$_{5+10}$-1 (Lukaszewski and Curtis, 1992, Fig. 1). The BC$_2$, plants with chromosomes 1D+4, 1D+9, and 1D+11 on the one hand, and chromosomes T−62 and T−64 recombined in their long arms on the other, were intercrossed and heterozygotes selected. Their backcross and selfed progenies were screened for secondary recombinant chromosomes 1RS.

Of the six combinations tested only three produced secondary recombinants in the samples screened: T−62 vs. 1D+4, T−62 vs. 1D+11 (data not shown), and T−64 vs. 1D+4. In the combination T−62 vs. 1D+4, among 260 progeny chromosomes screened, there were three that must have resulted from crossovers in the targeted overlapping segments of 1DS, for a frequency of 1.15%. In the combination T−64 vs. 1D+4 there were two recombinants among 360 progeny chromosomes from a backcross as male, and three chromosomes among 180 progeny from self pollination, for the total of five recombinants among 718 progeny chromosomes tested, for a frequency of 0.7%. These secondary recombinant chromosomes, 1RS$_{64:2}$.1RL.1DL$_{5+10}$-1 and 1RS$_{64:4}$.1RL.1DL$_{5+10}$-1 were backcrossed again to Presto 1R.1D$_{5+10}$-2 and Presto Valdy, and used in attempts to generate tertiary recombinant chromosomes.

Plants with translocation Valdy and secondary recombinant chromosomes 1RS$_{62:4}$.1RL.1DL$_{5+10}$-1 and 1RS$_{64:4}$.1RL.1DL$_{5+10}$-1 were backcrossed as female to Presto Dt1RL. Among 224 progeny kernels screened from the cross involving 1RS$_{62:4}$.1RL.1DL$_{5+10}$-1 and Valdy no tertiary recombinants from crossovers in the intercalary rye segment on the short arm have been identified. Among 95 progeny from the cross involving 1RS$_{64:4}$.1RL.1DL$_{5+10}$-1 screened by PAGE, one kernel was identified that was null *Sec-1*, had *Gli-D1*, and had both *Sec-3* and *Glu-D1*(*d*) loci. This half-kernel was germinated and its root tips analyzed cytologically by C-banding. The chromosome in question lacked the terminal rye C-band on the short arm, indicating that the terminal segment was from 1DS of Valdy, and had a terminal segment of 1RL on the long arm (see Fig. 3 and 4). Electrophoretic screening of the progeny of this plant confirmed that the chromosome in question indeed had the desired storage protein loci present: *Gli-D1* and *Glu-D1*(*d*), absent *Sec-1* and *Sec-3* (Fig. 5). It must have resulted from a crossover event in the overlapping segment of 1RS distal to the 1D+4 breakpoint in 1RS$_{64:4}$.1RL.1DL$_{5+10}$-1 and proximal to the breakpoint WR4 in Valdy. Using the system proposed for engineered chromosomes 1RS.1BL in Pavon, the complete notation for this chromosome is 1RS$_{64:4\cdot WR4}$.1RL.1DL$_{5+10}$-1. To simplify the matter, it will now be referred to as Chromosome FC1.

The crossover event that produced FC1 was a lucky one. In an attempt to increase the number of FC1-like chromosomes, an additional 388 kernels were screened by SDS-PAGE that originated from a cross of a plant with 20" + 1RS$_{64:4}$.1RL.1DL$_{5+10}$-1 + Valdy to Presto Dt1RL. Two crossover events in the targeted rye segment on the short arm were detected but both produced chromosomes with *Sec-1* present and *Gli-D1* absent (reciprocals to the FC1 product). Overall, the crossover rate in the targeted segment of 1RS was 3/483, or 0.62%, suggesting that the WR4 breakpoint may be more proximal than the T−9 breakpoint used in the construction of MA and Te chromosomes in Pavon (Lukaszewski, 2000).

Plants with chromosome FC1 were crossed and backcrossed to several different stocks of Presto containing

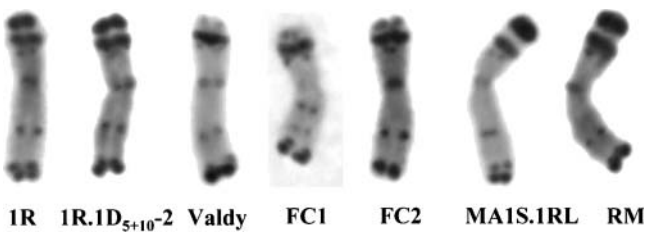

Fig. 3. C-banding patterns of the original chromosome 1R of the cv. Presto, and of various engineered chromosomes 1R produced in this study.

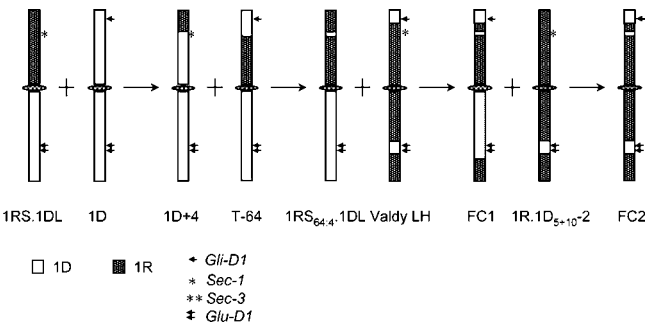

Fig. 4. The development of chromosomes FC1 and FC2 from various primary and secondary recombinants 1R-1D.

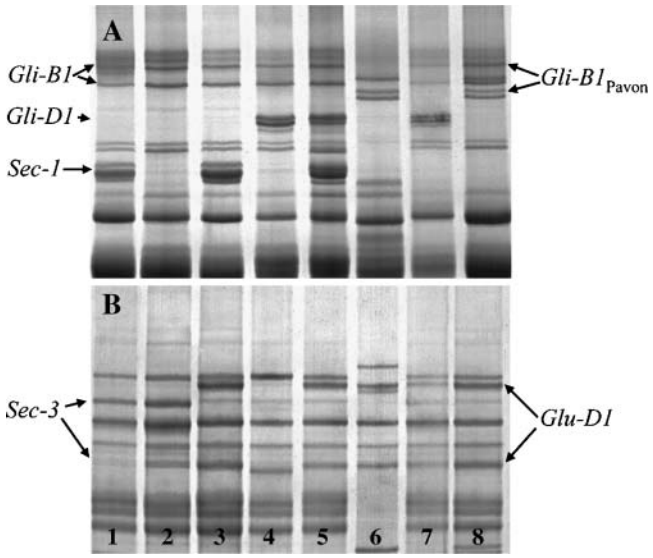

Fig. 5. Sequential SDS-PAGE separation of gliadins (A) and high molecular weight glutenins (B) from the same kernels of various triticale lines. Lanes 1: Presto, 2: Presto Dt1RL, 3: Presto 1R.1D$_{5+10}$-2, 4: Presto 1D(1R), 5: Presto Valdy, 6: 1RS$_{64:4}$.1DL in an early backcross to Presto; *Glu-A1* and *Gli-B1* alleles of Pavon are present, 7: Presto FC1, 8: Presto RM1. Chromosome 1D in substitution for 1R (lane 4) carries *Glu-D1)(a)* (subunits 2+12). There is a clear difference in the patterns of bands in *Gli-B1* of Presto (lanes 1, 2, 3, 4, 5, and 7) and that of Pavon (lanes 6 and 8–lane 8 has both alleles present, that of Presto present on chromosome 1B and that of Pavon present on chromosome RM). Photo courtesy of Dr. W. Brzezinski.

chromosome 1R.1D$_{5+10}$-2. Opportunities were created for crossovers in the proximal rye segment of the short arm of FC1 that would produce an FC1 chromosome with a normal long arm of 1R containing a small intercalary insert of the Glu-D1 locus. Three such chromosomes have been found so far (Fig. 3) but in samples that do not permit generalizations on crossover rates in the targeted region of FC1. The structure of these chromosomes needs to be confirmed in subsequent generations. These chromosomes will be referred to as chromosomes FC2.

Attempts to generate tertiary recombinants based on the secondary recombinant 1RS$_{62:4}$.1RL.1DL$_{5+10}$-1 and the primary recombinant WR2 have failed. Among 278 kernels from the backcross of plants with the two chromosomes to Presto Dt1RL, no crossovers in the targeted segment of 1RS were recovered.

Chromosomes Based on MA1

Lukaszewski (2000) engineered chromosome arm 1RS in bread wheat to remove the rye secalin locus *Sec-1* and to introduce wheat storage protein loci *Gli-B1* and *Glu-B3*. That effort resulted in chromosomes MA1 (Fig. 2) and MA2, and Te1 and Te2. Apart from the fact that wheat inserts in these chromosome arms are from wheat chromosome 1B and not from 1D, all four chromosome arms meet the requirements of the triticale improvement project. Therefore, chromosome MA1 was introduced into Presto 1R.1D$_{5+10}$-2 with the expectation that a crossover in the part of 1RS proximal to the two wheat inserts would produce chromosome 1R in triticale with all the wheat storage protein loci (*Gli-B1*, *Glu-B3*, *Glu-B1*), and no secalin loci (*Sec-1* and *Sec-3*). In both attempts this chromosome (MA1, 1RS$_{40:9;44:38}$.1BL) placed itself in substitution for chromosome 1B; that is, it was always present in addition to the pair of chromosomes 1R.1D$_{5+10}$-2. Perhaps because of this, the short arm of MA1 has never been observed in recombination with the short arm of 1R.1D$_{5+10}$-2. To make such recombination possible, Presto MA1 was crossed and backcrossed to the Presto 1D(1B) substitution line, and plants were selected with chromosome constitution 19" + MA1 + 1D" + 1R. Among 171 of their progeny from self pollination screened cytologically, 20 short-arm crossover events were identified. Of these, seven produced chromosomes 1R with heavy telomeric C-bands, originally in the telomere of the short arm of MA1, and 13 were MA1 chromosomes that had lost the telomeric C-band. The latter group was discarded.

Plants with the seven recombined 1R chromosomes were crossed to Presto Dt1RL and the resulting progeny were tested by SDS-PAGE for the presence/absence of the *Gli-B1* loci from Presto and Pavon, and the status of *Sec-1, Sec-3,* and *Glu-D1(d)* loci. Fortuitously, there is a clear difference in the pattern of protein bands generated by the Presto and Pavon alleles of *Gli-B1* (Fig. 5). Of the seven chromosomes, three must have resulted from crossovers in the rye segment between the most proximal translocation breakpoint on the short arm (that involved in the elimination of *Sec-1*) and the centromere, transferring the engineered segment of 1RS to chromosome 1R.1D$_{5+10}$-2. Another chromosome resulted from a crossover in the intercalary rye segment on the short arm. This produced a chromosome with both *Gli-B1* and *Sec-1* present. The status of *Gli-B1* and *Sec-1* loci of the remaining three chromosomes was unclear in the first test and they were never re-tested. The first three chromosomes, labeled A, B and D, respectively, were in essence chromosomes 1R.1D$_{5+10}$-2, where *Sec-3* had been replaced by *Glu-D(d)*, and the MA1-type short arms, with *Gli-B1* present and *Sec-1* absent (Fig. 5 and 6). As *Gli-B1* has always been completely linked to *Glu-B3*, it is assumed to be present.

In a parallel approach, a chromosome MA1S.1RL was constructed in Pavon 76 wheat by centric fission-fusion in double monosomics 20" + MA1' + 1BS.1RL'. Among 180 progeny screened by C-banding, 34 misdivision or misdivision-fusion products were present. Among the latter, there was one chromosome that by C-banding appeared to be a normal 1R (Fig. 3). This chromosome has its short arm from the engineered chromosome MA1 (1RS$_{40:9;44:38}$.1BL) and the long arm from the centric translocation 1BS.1RL. The long arm in 1BS.1RL originated from chromosome 1R found in CIMMYT wheat line E12165 (Lukaszewski, 1997b). This new chromosome is abbreviated as MA1S.1RL$_e$.

The plant with chromosome MA1S.1RL$_e$ was crossed and backcrossed three times to Presto 1R.1D$_{5+10}$-2. Following the second backcross, a cross to Presto Valdy LH16 was also made. BC$_3$ heterozygotes 20" + MA1S.1RL + 1R.1D$_{5+10}$-2 and 20" + MA1S.1RL + V-16 were back-

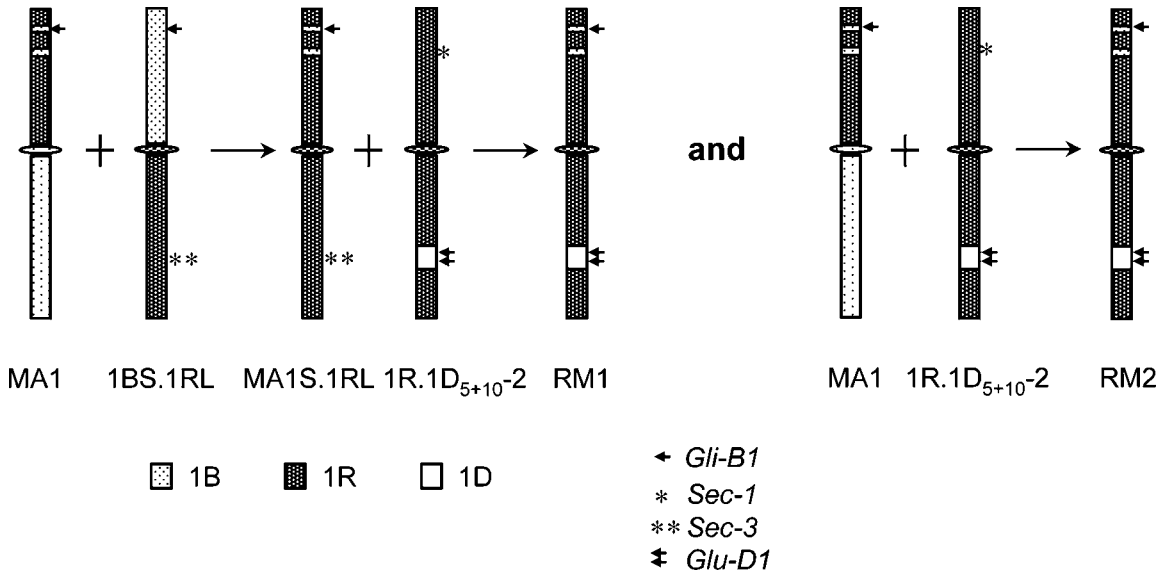

Fig. 6. Two approaches in the development of chromosomes RM from the engineered translocation 1RS.1BL in wheat (MA1) and chromosome 1R.1D$_{5+10}$-2 in triticale Presto. On the left, engineered short arm of MA1 is translocated onto a normal long arm of 1R by centric fission-fusion in wheat, and on transfer to triticale it is recombined with chromosome 1R.1D$_{5+10}$-2; on the right, MA1 is transferred into triticale and recombined with 1R.1D$_{5+10}$-2 in the presence of chromosome 1D.

crossed as female to Presto Dt 1RL and the resulting kernels were screened by SDS-PAGE. In both progenies kernels were sought that had the MA1 storage protein pattern on the short arm (*Gli-B1* of Pavon and no *Sec-1*), and *Glu-D1* (*d*) replacing *Sec-3* on the long arm (Fig. 5). In the second cross kernels were also sought, but not found, that had *Gli-D1* and no *Sec-1* on the short arm, and *Glu-D1(d)* and no *Sec-3* on the long arm. The first group of chromosomes, theoretically possible in both combinations, would be generated by crossovers in the rye segment stretching from the proximal wheat insert on the short arm that eliminates the *Sec-1* locus and the wheat insert on the long arm that replaces *Sec-3* with *Glu-D1*. In the combination MA1S.1RL$_e$ + 1R.1D$_{5+10}$-2 four such chromosomes were found in a sample of 83 progeny screened; four such chromosomes were found among 222 screened kernels from the combination involving MA1S.1RL and V-16. The new chromosomes are six breakpoint translocations that cytologically appear as normal chromosomes 1R but carry three intercalary wheat inserts from the short arm telomere to the long arm telomere: a distal insert on the short arm that introduces *Gli-B1* (and, presumably, *Glu-B3*) from Pavon; a proximal insert on the short arm that eliminates *Sec-1,* and an insert on the long arm that introduces *Glu-D1(d)* and removes *Sec-3* (Fig. 5). The complete notation for these chromosomes is 1RS$_{40:9;44:38}$.1RL.1DL$_{5+10}$-2. These chromosomes will be referred to as RM, followed by a number indicating the order in which they were constructed.

Preliminary Tests of Bread-Making Quality

Older chromosomes, such as the 1R.1D$_{5+10}$-2 and Valdy have already been tested for their effects on a range of bread-making parameters (Lukaszewski, 1998; Wos et al., 2002). In summary, the effect of 1R.1D$_{5+10}$-2 on the SDS-sedimentation value depends on the genetic background, with up to 230% increase in poor bread-making backgrounds and a multiyear average of 173.9% increase in Presto, a good bread-making triticale. Addition of the WR4 translocation to 1R.1D$_{5+10}$-2 in Valdy chromosomes further increased the SDS-sedimentation value in Presto by about 25%, to a level of about 209% of Presto (Table 2). Single SDS-sedimentation tests of Presto FC1 and Presto RM1 conducted on selfed seed from the first greenhouse-grown homozygotes of the two chromosomes produced values of 236.5 and 253.9% of the value for Presto, respectively. The latter is 92% of the multiyear SDS-sedimentation value for Pavon, a wheat with high parameters of bread-making quality (Kumlay et al., 2003), and 103% of the value for the cv. Wheaton, the donor of the 1DL segment with the *Glu-D1* locus in all chromosomes discussed in this article. Test bakes of Presto Valdy have shown loaf volumes similar to those of commercial wheat flour used as control (Wos et al., 2002; P.S. Baenziger, W. Brzezinski, R. Metzger, personal communication, 2003, 2004).

DISCUSSION

The simplest solution of the bread-making quality problem in triticale would be substitution of chromo-

Table 2. Average values of the micro SDS-sedimentation tests on Presto lines with chromosomes 1R.1D$_{5+10}$-2, Valdy, FC1, RM and the 1D(1R) substitution. Chromosome 1D in the substitution line also carries *Glu-D1*(*d*) locus.

Line tested	Number of tests	SDS-sedimentation value (% of Presto)
Presto	16	100
Presto 1D(1R)	5	250.4 ± 9.7
Presto 1R.1D$_{5+10}$-2	14	173.9 ± 4.9
Presto Valdy	10	209.0 ± 10.1
Presto FC1	1	236.5
Presto RM	1	253.9

some 1D for chromosome 1R. This substitution is available in several triticales (Lukaszewski, 1991, 1996; Kazman and Lelley, 1996) and involves chromosomes 1D from different sources and of different allelic composition at the storage protein loci. A whole chromosome substitution introduces all storage protein loci from 1D and eliminates both *Sec-1* and *Sec-3* loci, and would perhaps be simpler to maintain and track in a breeding program than multipoint translocation chromosomes produced in this study. Substitution 1D(1R) dramatically improves such parameters of bread-making quality of triticale as SDS-sedimentation, mixing time, mixing tolerance, and loaf volume (Kazman and Lelley, 1996; Lukaszewski, 1996, 1998; Wos et al., 2002; Kumlay et al., 2003; Budak et al., 2004).

Surprisingly, in all comparisons of the relative contribution of individual group-1 chromosomes to bread-making quality in triticale and wheat, chromosome 1R always ranked higher than wheat chromosome 1A with its *Glu-A1* and *Gli-A1/Glu-A3* gluten loci (Kazman and Lelley, 1996; Lafferty and Lelley, 2001; Lukaszewski, 1998; Wos et al., 2002; Kumlay et al., 2003; Budak et al., 2004). Therefore, substitution of 1D(1A) would appear more favorable. However, it leaves in triticale a set of suspect secalins loci on chromosome 1R which may be considered risky for long term bread-making improvement. In addition, substitution of 1D(1R) has several agronomically unacceptable characteristics. In triticale Presto, it reduced yield by about 25% relative to its sister lines with normal chromosome constitution and to Presto itself (Wos et al., 2002; Budak et al., 2004). Of the two arms of 1R involved, the short arm appears critical in triticale: translocation homozygotes 1RS.1DL or the combination 19" + 1RS.1AL" + 1D" are morphologically normal and no fertility effects have been observed (Lukaszewski, unpublished data, 1993). The short arm of rye chromosome 1 was shown to increase root biomass in bread wheat (Ehdaie et al., 2003). It is likely that this effect also stretches to triticale and accounts for some of its advantage over other cereals, especially in marginal environments. The loci that control root characteristics appear to be located in the distal part of 1RS (J.G. Waines, personal communication, 2005) in the general region of manipulations described in this study. Therefore, at the very least, the 1RS arm would have to be retained in hexaploid triticale, and manipulated carefully enough to remove or inactivate *Sec-1* and to introduce *Gli-1* and *Glu-3* loci while retaining as much of 1RS chromatin as possible, and especially the region responsible for the root characteristics, lest triticale loses its competitive advantage over other cereals in more demanding conditions. Apart from the specific loci located on 1RS itself, it appears that the level of intergenomic heterosis in the combination AABBRR is greater than in AABBDD (normal bread wheat). Therefore, any unnecessary reduction in the total amount of rye chromatin would likely have some adverse effect on triticale's performance.

This work has been performed using standard tools of cytology and the traditional approach of generating secondary alien introgressions pioneered by E.R. Sears (Sears, 1981). The primary screening was by C-banding, using presence/absence or size polymorphism of C-bands as a tracking tool for individual operations, combined with electrophoresis of storage proteins as needed. This approach worked reliably, as long as the operations were scheduled one at a time. Any desired intermediate product identified and selected was then matched with another chromosome construct, usually with a contrasting C-band marker, and the subsequent step was made. This approach guaranteed relatively cheap and efficient screening, at times of samples of considerable size, but it made for a fairly long procedure to construct the final chromosomes. All attempts made here to proceed by two steps at a time, that is, to search for products of multiple crossovers in any single step, ended in failure and necessitated a return to the last confirmed intermediate to start over again (data not shown). There is little doubt that techniques with a better resolution over longer stretches of chromosomes would permit a more thorough analyses and, presumably, selection of multiple crossover events in any one step. DNA markers with sufficient coverage in the areas of interest would likely meet such criteria. However, they would also make the process incomparably more expensive, even disregarding the cost of labor of DNA trained personnel. It may also be worth mentioning that although all crosses were made by hand, on hand-emasculated, bagged heads and under greenhouse conditions, and all heads were threshed by hand, contamination was frequent enough to produce a steady stream of false positives in electrophoretic screening of single kernels. This contamination was presumably from free pollen landing on the stigmas during hand pollination. Given that the frequencies of some desired crossovers were below one percent, even very low contamination levels produced false positives at a rate higher than the actual confirmed desired recombination events. In all instances, cytological screening of seedlings from the kernels with the desired constitution of storage protein loci weeded out such false positives. With other methods of screening regular progeny tests would have to be conducted, further slowing down progress.

Given the frequencies of secondary and tertiary recombinants recovered in the course of this study it does not appear likely that a much higher precision of chromosome engineering could have been achieved in reasonably sized samples, and especially without dense marker coverage of the critical chromosome areas in the vicinity of the targeted storage protein loci. Even the crude approach used here indicates that breakpoint T−62 is more distal than T−64, therefore it must be closer to the *Sec-1* locus; therefore it would be more suitable for the construction of chromosomes FC1 and FC2. This breakpoint was used to generate two secondary recombinants (with 1D+4 and 1D+11, data not shown), but these secondary recombinants in turn failed to produce any tertiary recombinant chromosomes. This is somewhat surprising, or may indicate bad luck. As the structure of the secondary chromosomes is understood, it should be the position of 1D+4 or 1D+11 breakpoints that would determine the success in recombination with

WR4 or even the proximal breakpoint of the distal insert in MA1. It is these breakpoints that determine the length of the intercalary rye segment. However, the relationship between chromosome structure and its ability to form chiasmata is not a simple one and appears to defy any attempts of straight-line predictions (Lukaszewski, 1994). As a rule in generation of secondary and tertiary recombinants, the donor chromosomes are of contrasting structure: one has a telomeric rye and proximal wheat segments and the other has telomeric wheat and proximal rye segments. As wheat chromosomes rely on the telomeric initiation of synapsis with no backup for it (Lukaszewski, 1997a) apart perhaps from purely random encounters in the nucleus, misalignment at the telomeres produced by structural heterozygosity has a major effect on pairing frequency hence the low crossover rate. In the process of generating secondary and tertiary recombinants 1RS-1BS, Lukaszewski (2000, 2001, and unpublished data, 2000) observed that crossover rates of such contrasting chromosomes were on average three times lower than predicted on the basis of the genetic lengths of homology shared by them.

The electrophoretic tests used in this study were for secalins, gliadins and high molecular weight glutenins. Low molecular weight glutenins were not routinely monitored. However, on chromosome 1B the low molecular weight glutenin locus is tightly linked to the *Gli-B1* locus (Pogna et al., 1990). It has never clearly separated from *Gli-B1* in a high density mapping in the distal end of 1BS, even when intralocus recombinants in *Gli-B1* were identified (Lukaszewski and Brzezinski, 2003). They did not separate in homoeologous recombination 1BS-1RS (Lukaszewski, 2000). The *Gli-1* and *Glu-3* loci, both multigenic, may overlap (Lee et al., 1999; Lukaszewski and Brzezinski, 2003). It appears quite certain that both are present in all engineered 1R chromosomes described here that carry the *Gli-B1* or *Gli-D1* loci. Preliminary screening for their presence (data not shown) did not give indications of their absence.

No comprehensive multi-year quality tests have been performed to date on the more advanced newer stocks described here. However, all tests so far indicate that even Valdy chromosomes, with the *Sec-1* locus present, produce loaves with parameters directly comparable to those obtained with commercial samples of wheat flour (Wos et al., 2002; R. Metzger, P.S. Baenziger, personal communication, 2003, 2004). By triticale standards, Presto is a good bread maker with a relatively high SDS-sedimentation value and loaf volume (Lukaszewski, 1998; Wos et al., 2002). The effects of the chromosomes described here on the properties of poor bread-making triticales are unknown but are likely to be substantial. Still, they cannot be expected to transform poor bread-making triticales into good bread-making stocks. If wheat is a good predictor here, addition of even the best and the most important gluten subunits to a poor genetic background will not produce a satisfactory end product. Therefore, if breeding of triticale for bread-making is to be initiated, nurseries specifically devoted to bread-making need to be developed so that the best germplasm is concentrated and slowly improved, while providing sufficient genetic variation for steady progress by breeding. To the best knowledge of the author, no bread-making nurseries of triticale exist at this point, and considerable variation exists in the glutenin subunit composition in breeding programs (Brzezinski and Lukaszewski, 1998; Pena et al., 1998).

The effects of the chromosomes described in this article on characteristics other than bread-making are far more difficult to predict. The experience with short head associated with translocation Valdy illustrates the perils involved in manipulation of chromosome segments. At times, a highly positive effect may be inadvertently introduced with a chromosome segment, such as yield increases associated with the 1RS.1BL translocation and with Agropyron introgressions in wheat (Singh et al., 1998). More often, the unanticipated associations are detrimental. Here the head length reduction by Valdy chromosomes was surprising and completely unexpected. It is still unclear what genetic factor causes it, where it is located, and what environmental factor triggers its development. It is almost undetectable in the greenhouse on UCR campus under artificial long day. It does not manifest itself in the field in Oregon from late fall or February planting. It is the most pronounced in moderately early fall planting (Oct. 1 through Oct. 15) in high density stands in Oregon and in Poland, where it has been tested extensively. Recombination of the presumed offending wheat segment in WR4 with a normal 1D seems to have mitigated the problem in Oregon, where the character still shows up in high density stands from fall planting, albeit to a lesser degree (R. Metzger, personal communication, 2004, 2005). Still, it needs to be pointed out that the chromosomes developed in this project are mosaic not only for pieces of wheat and rye chromatin, but also for rye segments originating from several different sources. Chromosomes FC1 and FC2 have segments of 1R from triticales Presto and Rhino, and from ryes 'Petkus', the donor of the Kavkaz 1RS (Zeller and Hsam, 1984), and from Imperial (Koebner and Shepherd, 1986; Rogovsky et al., 1991). RM chromosomes have segments from Presto, Rhino, and Petkus. The wheat segments originate from the cv. Wheaton (the insert on 1RL with *Glu-D1(d)*, and from the cultivars Pavon, Luna, and perhaps others. Pedigree and genetic mapping can assign the crossover points to some genetic intervals, some very narrow, but others sometimes as long as 2/3 of the entire chromosome. Given their mosaic nature, it will not be surprising if these chromosomes have an impact on agronomic characteristics of the recipient Presto. It has already been observed (R.J. Metzger, personal communication, 2002) that chromosome 1R.1D$_{5+10}$-2 has a small negative effect on yield in Presto. Its effect on yield in other lines has not been extensively tested, perhaps because no other isogenic sets of lines exist. Because the chromosomes described herein are far more complex and mosaic than 1R.1D$_{5+10}$-2, their effects may be more severe. Therefore, their true potential can only be assessed in wider sets of materials, following several breeding cycles that may find genetic backgrounds suitable to demonstrate their potential. At this point they only create the genetic

potential for good bread-making quality and in preliminary tests they seem to perform up to expectation.

As multiple breakpoint translocations, the chromosomes described herein will disassemble by crossing over in hybrids with normal chromosomes. The frequencies are not clear yet, but they are expected to be low. This is based on the very low crossover rates observed in the process of generating these chromosomes, and on preliminary small-sample tests of their breeding behavior. The disassembly rates appear lower than those of similar chromosomes MA and Te in wheat (Lukaszewski, 2001), but then even small differences in the positions of the translocation breakpoints may dramatically alter the pattern of pairing and crossing over. There may well be less recombination in triticale than in wheat. Nevertheless, given the positions of the wheat inserts in engineered 1R and lengths of the intervening 1R segments that separate them, any disassembly of the engineered chromosomes is most likely to separate *Glu-D1* on the long arm from the two inserts in the short arm. It is less likely, but still possible, that the two inserts on the short arm may separate. As each of such separations should have a discernible effect on quality, they ought to be easily eliminated by selection.

Elements of chromosome MA1 present in chromosomes RM create a situation where two segments of 1BS are present in four rather than in two copies. In theory it generates a potential for meiotic instability. In practice, this potential appears very low. Again, chromosomes depend on terminal initiation of synapsis and in the terminal regions there are only pairs. So it is not very likely that multivalents will be formed with noticeable frequency. So far, no recombination of RM with 1B was been observed in any triticales used, but the samples tested were relatively small. More interesting in this context is the effect of four doses of *Gli-B1/Glu-B3* loci on certain parameters of bread-making quality. In a study of 'Chinese Spring', a poor quality wheat, Rogers et al. (1990) found that while an increased dosage of chromosome 1B had a positive effect on bread-making quality, most of this effect was associated with the long arm; the effect of the short arm was small.

The chromosomes described here remove two of three secalin loci present in triticale with standard chromosome constitution. Extensive tests of *Sec-2* in wheat failed to detect any effect on bread-making quality (Gupta et al., 1989). No quality effects of *Sec-2* were observed when single chromosome substitution lines were tested in triticale (Wos et al., 2002). Therefore, it is not expected that *Sec-2* will be detrimental to quality and no attempts have been made to remove it. However, primary recombinants 2BS-2RS and 2DS-2RS do exist to address the issue (Lukaszewski et al., 2004), if needed. It is unclear what the effect may be of the absence of the *Gli-2* locus from chromosome 6DS. In the study of Rogers et al. (1990), there was an identifiable positive effect of the 6DS arm in Chinese Spring wheat. In a test of single D-genome substitutions in triticale Presto, no effect of *Gli-D2* on the SDS-sedimentation value was detected in the 6D(6R) substitution line; the only significant reduction (by 5% of the value of Presto) in group-6 was in substitution 6D(6B) (Lukaszewski, 1998). The studies of Rogers et al. (1990) and Lukaszewski (1998) imply that the presence of specific alleles of *Gli-2* loci may be an important factor. *Gli-2* loci are multigenic with up to 150 gene copies identified (Anderson et al., 1997) and so it may well be possible that if *Gli-D2* contributes in a substantial manner to some parameter of bread-making in triticale, its absence can be compensated for by selection of alleles with higher numbers of active genes present on the other two homoeologues, 6A and 6B.

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