Theor Appl Genet (1992) 84:1–5

Dosage response of rye genes in a wheat background

2. Secalin genes on 1RS*

D. C. Bittel[1,**] **and J. P. Gustafson**[1,2]

[1] Department of Agronomy, 208 Curtis Hall, University of Missouri, Columbia, MO 65211, USA
[2] United States Department of Agriculture – Agricultural Research Service, Plant Genetics Research Unit, University of Missouri, Columbia, MO 65211, USA

Received May 10, 1991; Accepted October 9, 1991
Communicated by J. W. Snape

Summary. A series of hexaploid wheat lines containing zero, two or four doses of rye chromosome arm 1RS was used to investigate the response to changes in dosage by the rye genes when in a wheat background. The quantity of protein produced by the secalin protein genes contained on 1RS was directly related to the number of copies of 1RS present in the line. No response could be detected by representative wheat proteins suggesting that the increase in secalin protein observed was due to an increase in mRNA produced when four copies of the secalin gene was present. These results suggest that increasing the dosage of alien genes introgressed into wheat may be a useful tool for enhancing their expression.

Key words: *Triticum aestivum* – *Secale cereale* – Alien gene expression – Endosperm protein

Introduction

Techniques for alien gene transfer into wheat (*Triticum aestivum* L. em Thell.) are well established. However, it is difficult to predict how alien genes will function when put into a new genetic background. There have been many studies examining the regulation of seed storage protein (SSP) gene expression in wheat (Aragoncilli et al. 1978; Galili and Feldman 1984; Bartels and Thompson 1986; Galili et al. 1986; Halford et al. 1988; Rogers et al. 1990), but there have been few studies examining the regulation of foreign SSP genes when they are transferred into a wheat background. When the short arm of chromosome 1 (1RS) of rye (*Secale cereale* L.) is transferred into the wheat genome, the opportunity arises to examine the interactions between the wheat genome and the genes contained on 1RS.

Chromosome arm 1RS contains many genes which have proven useful in wheat breeding programs such as disease resistance, stress tolerance, improved nutritional quality and yield enhancement (Rajaram et al. 1983; Zeller and Hsam 1983; Gustafson 1988). In addition, 1RS contains genes that code for SSP denoted as secalins and the rRNA genes. This particular chromosomal segment has been used in wheat breeding programs around the world and is found in cultivars covering significant world acreage (Rajaram et al. 1983; 1990). However, 1RS does confer some negative properties on many of the cultivars that possess the segment, most notably sticky dough.

The mechanisms regulating wheat SSP production tend to maintain a threshold level of protein in the seed. When SSP genes are lost, the remaining genes increase protein production to compensate for the lost genes (Galili et al. 1986). Wheat SSP genes exhibit a nonlinear dosage response. As the gene dosage increases so does the amount of protein produced by that gene (Aragoncillo et al. 1978; Galili and Feldman 1984). Galili et al. (1986) postulated a complex series of interconnected mechanisms regulating SSP production at the levels of both transcription and translation.

Because of the importance of alien chromosome segments in general, and 1RS in particular, to wheat breeding programs a series of lines were constructed which contained zero, two or four doses of 1RS in a wheat

* Contribution from the Missouri Agricultural Experiment Station. Journal Series No. 11,413
Mention of a trade name or proprietary product does not constitute a guarantee, warranty or recommendation of the product by the U.S. Department of Agriculture or the University of Missouri and does not imply its approval to the exclusion of other products that may be suitable
** Correspondence to D. C. Bittel
Present address: Department of Agronomy and Plant Genetics, Borlang Hall, University of Minnesota, St. Paul, MN 55108, USA

background (Bittel et al. 1991). These lines enabled the evaluation of the effect of differences in dosage of 1RS on the expression of SSP genes carried by the rye arm.

Materials and methods

The wheat varieties used were: 'Anza', a hexaploid wheat; 'Anza/S149' (hereafter referred to as S149), a 1R/1D substitution line derived from 'Anza' (Gustafson 1988); 'Glennson', a hexaploid wheat from the Centro Internacional de Mejoramiento de Maíz Y Trigo (CIMMYT, Mexico), containing a 1BL/1RS translocation; and 'Amigo', containing a 1AL/1RS translocation (Sebesta and Wood 1978). Additional lines were constructed by crossing the three varieties 'S149', 'Amigo' and 'Glennson'. From F_2 or F_3 plants lines were selected which contain zero, two or four copies of 1RS as determined by the C-banding method of Lukaszewski and Gustafson (1983). Those lines containing the rye translocations or intact rye chromosome were missing the corresponding wheat chromosome; e.g. line 7841-34-4 contained two copies of 1BL/1RS and 1R but did not contain 1B or 1D. Thus, all lines contained 42 chromosomes. Table 1 lists the lines used in the present study and their chromosome constitution. All lines are maintained at the USDA-ARS, University of Missouri seed storage facility.

Acid-PAGE (A-PAGE) essentially followed the procedure of Ng et al. (1988) but modified to accommodate a Biorad mini-protein II electrophoresis unit. A Bradford assay (Bradford 1976) was performed on each sample, and the amount of protein loaded was adjusted so that total protein in each lane was equal. Samples were loaded onto 2 mm-thick 6% acrylamide gels. The proteins were fractionated at a constant voltage of 180 V for approximately 60 min until the tracking dye ran off the gel. The gels were stained in 12% trichloroacetic acid (TCA) with 0.04% coomassie brilliant blue R-250 and destained in 12% TCA.

The fractionated proteins were scanned on an LKB scanning laser densitometer. The intensity of each band was recorded, as well as the percentage of the total protein for the lane. The values for two replicates for each sample were averaged, and the percentage of total protein due to the secalins was compared between lines by *t*-tests. By averaging the values and comparing only the percentage of total protein in the lane due to a particular band we ensured that the comparison of bands between lines represented an increase in protein relative to the total protein present in the sample.

Table 1. Lines and chromosome constitution

Line number	Cross	Rye arm present	(Number of 1RS arms)
7841-46-1	S × G	No rye	0
7841-28-6	S × G	1R	2
7841-31-2	S × G	1BL/1RS	2
7841-34-4	S × G	1R, 1BL/1RS	4
88-265-5	S × A	No rye	0
88-30	S × A	1R	2
88-48	S × A	1AL/1RS	2
88-41-5	S × A	1R, 1AL/1RS	4
48-144-1	G × A	No rye	0
48-148	G × A	1AL/1RS	2
48-136-1	G × A	1BL/1RS	2
48-145-8	G × A	1AL/1RS, 1BL/1RS	4
48-145-5	G × A	1AL/1RS, 1BL/1RS	4

S = S149, A = Amigo, G = Glennson

Results and discussion

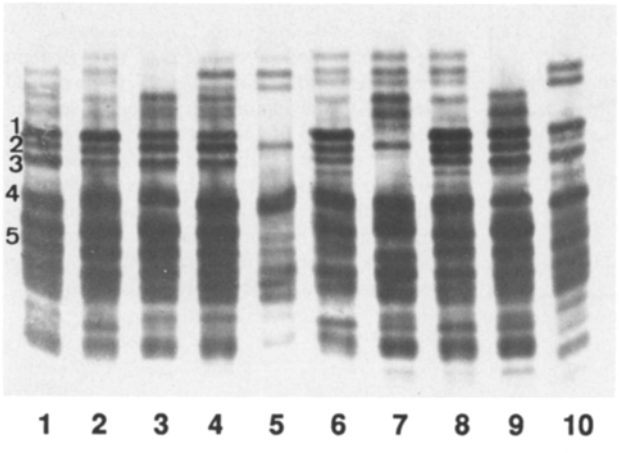
Figures 1 and 2 show the A-PAGE patterns of the lines selected for variable numbers of 1RS. The secalin proteins coded for by genes on 1RS are labeled 1 and 3, (Figs. 1 and 2). Bands 1 and 3 were determined to be ω secalins when run on an SDS-PAGE gel and subsequently compared to the pattern of 'Imperial' rye (unpublished data). Lanes 12, 17 and 18 (Fig. 2) contained samples from lines containing four doses of 1RS. These lines had six doses of 1RS in the endosperm: four maternal and two paternal. Bands 1 and 3 are noticeably darker in lanes 12, 17 and 18, which have six doses of 1RS in the endosperm, in contrast to the other lanes, which have zero or three copies of 1RS in the endosperm. The bands labeled 2, 4 and 5 (Figs. 1 and 2) are wheat gliadins measured as controls in order to gauge the response of the rye secalins. Figure 3 shows the percentage of total protein in the lane accounted for by each band.

The percentage of total protein due to the two ω secalin bands was greater in the samples from the lines with four copies of 1RS than in the lines which have only two doses. The percentage of total protein due to band 1 was 7.07% in the samples with six doses of 1RS in the endosperm in contrast to 3.83% in lines with three doses in the endosperm, which is a highly significant difference ($P < 0.001$). The percentage due to band 3 was 7.83% in samples with six doses of 1RS, which differed significantly from the 4.40% found in the samples with three doses ($P < 0.001$). This observation is in agreement with Galili et al. (1986) who saw an increase in protein in response to increases in wheat SSP gene copy number. Rogers et al. (1990) also observed a correlation between bread-making quality and an increase in the copy number of glutenin genes associated with quality characteristics. Apparently, as the number of doses of the genes which produced favorable proteins increased, so did the quality of the dough.

The bands labeled 2, 4 and 5 represent wheat gliadins. The percentage of total protein due to each of the five bands is shown in Fig. 3. The wheat gliadin bands varied within groups, but no pattern was distinguishable. However, there was a tendency to maintain a constant level of the total of the three wheat bands. Figure 4 shows the sums for the three wheat bands and for the two rye bands. Although each individual gliadin varied, both within groups and between groups, the percentage of total protein accounted for by the sum of the three gliadins remained relatively constant regardless of the number of rye arms present. The only significant change in band intensity was with respect to the ω secalin bands when they were compared between lines that contained two doses of 1RS and those with four doses.

Since all of the lines studied were hexaploid, each rye arm replaced a corresponding homoeologous wheat arm.

Figs. 1, 2. A-PAGE. Lines with zero, two or four doses of 1RS. *Note:* line 88-41-5 is not shown in photographs. It was analyzed in a separate gel



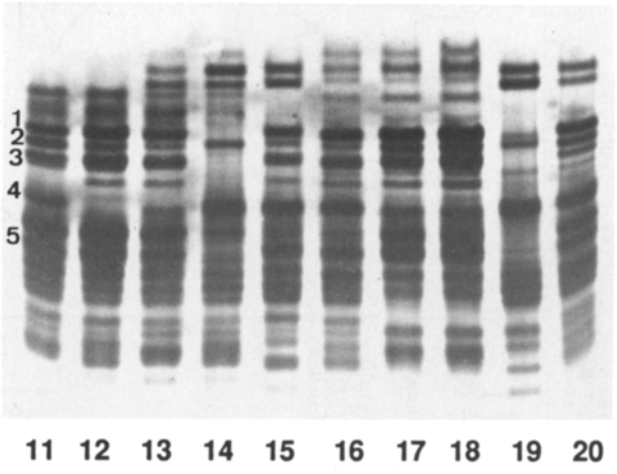


Fig. 1. A-PAGE. Lines with 0, 2 or 4 doses of 1RS

Lane	Sample	(Number of 1RS arms)
1	S149 × Glenn (F_1)	(2)
2	Amigo × Glenn (F_1)	(2)
3	S149	(2)
4	Anza × S149 (F_1)	(1)
5	Anza	(0)
6	Amigo	(2)
7	88-256-5	(0)
8	88-48	(2)
9	88-30	(2)
10	Glennson	(2)

Fig. 2. A-PAGE. Lines with 0, 2 or 4 doses of 1RS

Lane	Sample	(Number of 1RS arms)
11	7841-28-6	(2)
12	7841-34-4	(4)
13	7841-31-2	(2)
14	7841-46-1	(0)
15	Glennson	(2)
16	48-148	(2)
17	48-145-5	(4)
18	48-145-8	(4)
19	48-144-1	(0)
20	48-136-1	(2)

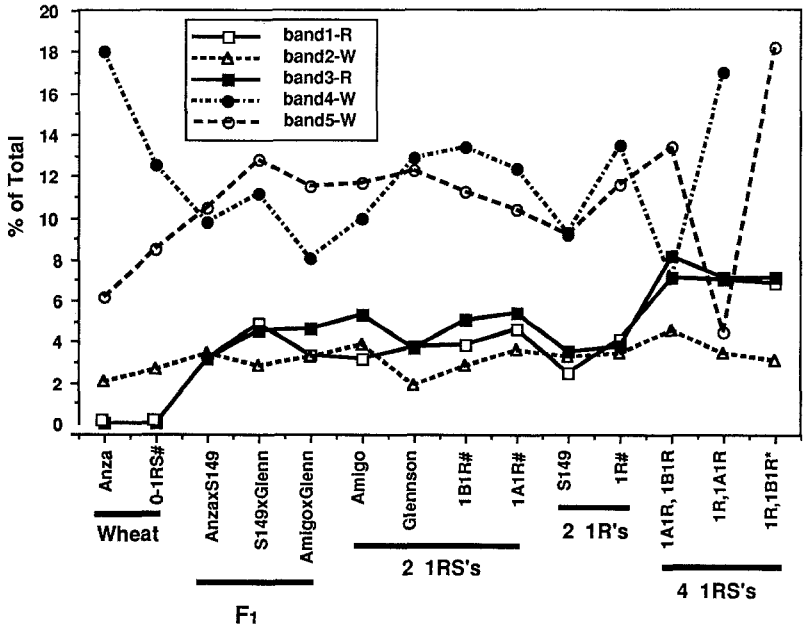


Fig. 3. Percentage of total protein due to secalins and gliadins. *Dashed lines* represent gliadins, *solid lines* represent secalins. # Entries representing lines with the same chromosome configuration, which were pooled as their protein percentages did not differ significantly; * band 4 was absent, no value recorded for gliadins

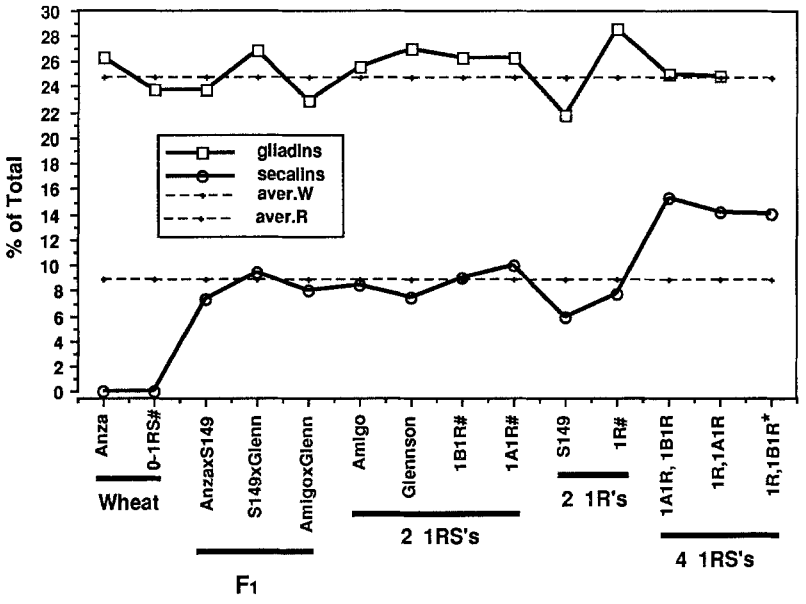


Fig. 4. Summed percentages of total protein for gliadins and secalins. *Aver. W* is the pooled average of the gliadins in the lines selected with two doses of 1RS; *aver. R* is the pooled average of the secalins in the lines selected with two doses of 1RS. # Entries representing lines with the same chromosome configuration, which were pooled as their protein percentages did not differ significantly; * band 4 was absent, no value recorded for gliadins

Galili et al. (1986) showed that when SSP genes were removed, more protein was produced by the remaining genes to compensate for the loss. It is possible that a mechanism exists which regulates the translation of mRNA to ensure an adequate level of protein is provided for the germinating embryo. However, if this were the case, then the translation of all SSP mRNAs would be expected to increase. It seems unlikely that specific mRNAs would be selectively singled out for changes in translation. In the present study there was no evidence of nonspecific increases in protein levels. Rather, the only SSPs which were present at an increased level were those coded for by the ω secalin genes on 1RS. It is unlikely that nonspecific translation of only the secalin mRNAs would occur. Therefore, it appears that when more copies of the secalin genes were present, more protein was produced as a result of an increase in the amount of mRNA from the secalin genes.

Lane 4 contained a sample from an F_1 hybrid between 'Anza', which has no rye chromosomes, and 'S149', which contains a 1R/1D substitution. It therefore, has one copy of 1RS in the endosperm originating from the paternal parent, 'S149'. In the F_1 the value for band 3 was 3.15% (Fig. 1, lane 4), which was less than that found in any other sample. It was less than half of the average value observed in samples from lines with six doses of 1RS (7.65%) in the endosperm and only two-thirds of the average value of samples with three doses of 1RS (4.77%) in the endosperm. For this polypeptide the change in quantity was directly related to the number of copies present. However, the relationship was not linear, which agrees with the observations of Galili et al. (1986).

Although the dosage of 1RS affected the quantity of secalin produced, environmental and other genetic regulating mechanisms also play an important role in regulating protein production. This was illustrated when the level of protein in band 1 extracted from the F_1 from the cross 'Anza' × 'S149', (Fig. 1, lane 4) was compared with the rest of the samples. The percentage of total protein recorded for band 1 in the F_1 (3.15%) was less than that found in all but 2 other samples: 'S149' (2.45%) and 'Glennson' (2.50%), (Fig. 1, lanes 3 and 10 respectively). However, 'S149' is the male parent and contained three doses of 1R in its endosperm, whereas the F_1 seed contained only one dose of 1R. Putting the rye chromosome into a new genetic background caused an increase in the amount of protein produced by the secalin genes. Obviously, factors other than dosage are important for regulating the amount of protein produced by the secalin genes.

Lanes 1, 2 and 4 contain F_1 hybrids between various lines. Comparisons between the F_1s and the parent lines reveal that the bands present in the parent are all present in the F_1. Although there are changes in intensity, all of the genes were expressed in the F_1s. Apparently, mechanisms exist which modify the amount of protein produced by each individual gene, but no mechanism was found that was capable of completely suppressing gene expression in the F_1s examined.

The results of the analysis of the dosage response of the rye secalin genes indicated that SSP genes from rye respond to regulating factors in the wheat genome much the same as the wheat SSP genes. Investigations are in progress to determine if other genes carried by 1RS also respond to changes in dosage in the same manner as the ω secalin genes. If alien genes are generally found to respond positively to increases in dosage, it may prove to be a useful mechanism for enhancing the expression of desirable alien sequences when they are introgressed into cultivated wheat.

References

Aragoncillo C, Rodriguez-Loperena MA, Salcedo G, Carbonero P, Garcia-Olmedo F (1978) Influence of homeologous chromosomes on gene dosage effects in allohexaploid wheat (*Triticum aestivum* L.). Proc Natl Acad Sci USA 75:1446–1450

Bartels D, Thompson RD (1986) Synthesis of mRNAs coding for abundant endosperm proteins during wheat grain development. Plant Sci 46:117–125

Bittel DC, Fominaya A, Jouve N, Gustafson JP (1991) Dosage response of rye genes in a wheat background 1. Selection of lines with multiple copies of 1RS. J Plant Breed

Bradford M (1976) A rapid and sensitive method for the determination of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 72:248–254

Galili G, Feldman M (1984) Intergenomic suppression of endosperm protein genes in common wheat. Can J Genet Cytol 26:651–656

Galili G, Levy AA, Feldman M (1986) Gene-dosage compensation of endosperm proteins in hexaploid wheat *Triticum aestivum*. Proc Natl Acad Sci USA 83:6524–6528

Gustafson JP (1988) Evaluation of a 1R(1D) substitution in wheat. In: Miller TE, Koebner RMD (eds) Proc 7th Int Wheat Genet Symp. Cambridge, UK, pp 193–196

Halford NG, Forde J, Anderson OD, Greene FC, Shewry PR (1988) The structure and expression of genes encoding the high molecular weight (HMW) subunits of wheat glutenin. In: Miller TE, Koebner RMD (eds) Proc 7th Int Wheat Genet Symp. Cambridge, UK, pp 745–750

Lukaszewski AJ, Gustafson JP (1983) Translocations and modifications in triticale × wheat hybrids. Theor Apl Genet 64:239–248

Ng PKW, Scanlon MG, Bushuk W (1988) A catalog of biochemical fingerprints of registered Canadian wheat cultivars by electrophoresis and high-performance liquid chromatography. University of Manitoba, Winnipeg, Canada

Rajaram S, Mann CE, Ortiz-Ferrara G, Mujeeb-Kazi A (1983) Adaptation, stability and high yield potential of certain 1B/1R CIMMYT wheats. In: Sakamoto S (ed) Proc 6th Int Wheat Genet Symp. Kyoto, Japan, pp 613–621

Rajaram S, Villareal R, Mujeeb-Kazi A (1990) The global impact of 1B/1R wheats. Agronomy Abstr 105. Annual Meeting ASA, CSSA, SSA

Rogers WJ, Rickatson JM, Sayers EJ, Law CN (1990) Dosage effects of chromosomes of homoeologous groups 1 and 6 upon bread making quality in hexaploid wheat. Theor Appl Genet 80:281–287

Sebesta EE, Wood Jr EA (1978) Transfer of greenbug resistance from rye to wheat with X-rays. Agronomy Abstr 61. Annual Meeting ASA,CSSA, SSA

Zeller FJ, Hsam SLK (1983) Broadening the genetic variability of cultivated wheat by utilizing rye chromatin. In: Sakamoto S (ed) Proc 6th Int Wheat Genet Symp. Kyoto, Japan, pp 161–173