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Identification and chromosomal location of four subfamilies of the rubisco small subunit genes in common wheat

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Summary. Three different 3′ noncoding sequences of wheat rubisco small subunit (SSU) genes (*RbcS*) were used as probes to identify the gene members of different *RbcS* subfamilies in the common wheat cultivar Chinese Spring (CS). All genes of the wheat *RbcS* multigene family were previously assigned to the long arm of homoeologous group 5 and to the short arm of homoeologous group 2 chromosomes of cv CS. Extracted DNA from various aneuploids of these homoeologous groups was digested with four restriction enzymes and hybridized with three different 3′ noncoding sequences of wheat SSU clones. All *RbcS* genes located on the long arm of homoeologous group 5 chromosomes were found to comprise a single subfamily, while those located on the short arm of group 2 comprised three subfamilies. Each of the ancestral diploid genomes A, B, and D has at least one representative gene in each subfamily, suggesting that the divergence into subfamilies preceded the differentiation into species. This divergence of the *RbcS* genes, which is presumably accompanied by a similar divergence in the 5′ region, may lead to differential expression of various subfamilies in different tissues and in different developmental stages, in response to different environmental conditions. Moreover, members of one subfamily that belong to different genomes may have diverged also in the coding sequence and, consequently, code for distinguishable SSU. It is assumed that such utilization of the *RbcS* multigene family increases the adaptability and phenotypic plasticity of common wheat over its diploid progenitors.

Key words: Rubiso small subunit – Multigene family – Chromosomal location – Wheat – *Triticum*

Introduction

Ribulose 1,5-bisphosphate carboxylase/oxygenase (rubisco), the most abundant protein in leaves of light-grown plants, is a key chloroplast enzyme both in photosynthesis and in photorespiration (Miziorko and Lorimer 1983). This enzyme is a hexadecamer (McIntosh et al. 1980) consisting of eight chloroplast-encoded large subunits (Coen et al. 1977) and eight nuclear-encoded small subunits (SSU) (Kawashima and Wildman 1972). The SSU protein in wheat and in several other plant species was found to be encoded by a multigene family (Berry-Lowe et al. 1982; Broglie et al. 1983; Coruzzi et al. 1983; Sugita et al. 1987). Rubisco SSU genes (*RbcS*) were cloned both from dicotyledonous plants including soybean (Berry-Lowe et al. 1982), tomato (Sugita et al. 1987), tobacco (Mazur and Chui 1985), and petunia (Dunsmuir et al. 1983), as well as from the monocots *Lemna gibba* (Stiekema et al. 1983), maize (Lebrun et al. 1987), and wheat (Broglie et al. 1983; Smith et al. 1983). Based on sequence homology (Dean et al. 1985a), the *RbcS* multigene family of various plant species has been classified into several subfamilies. In most cases, the 3′ noncoding region, which functions in mRNA processing and poly-A addition, is highly conserved within subfamilies and diverged between subfamilies (Dean et al. 1989). Thus, DNA probes carrying different 3′ end sequences and lacking any sequence of the coding region may be used to distinguish between different *RbcS* subfamilies (Broglie et al. 1983; Coruzzi et al. 1984). To date, one genomic and four cDNA clones of wheat *RbcS* genes were isolated and characterized (Broglie et al. 1983; Smith et al. 1983). As deduced from their 3′ noncoding sequences, these clones represent three different subfamilies.

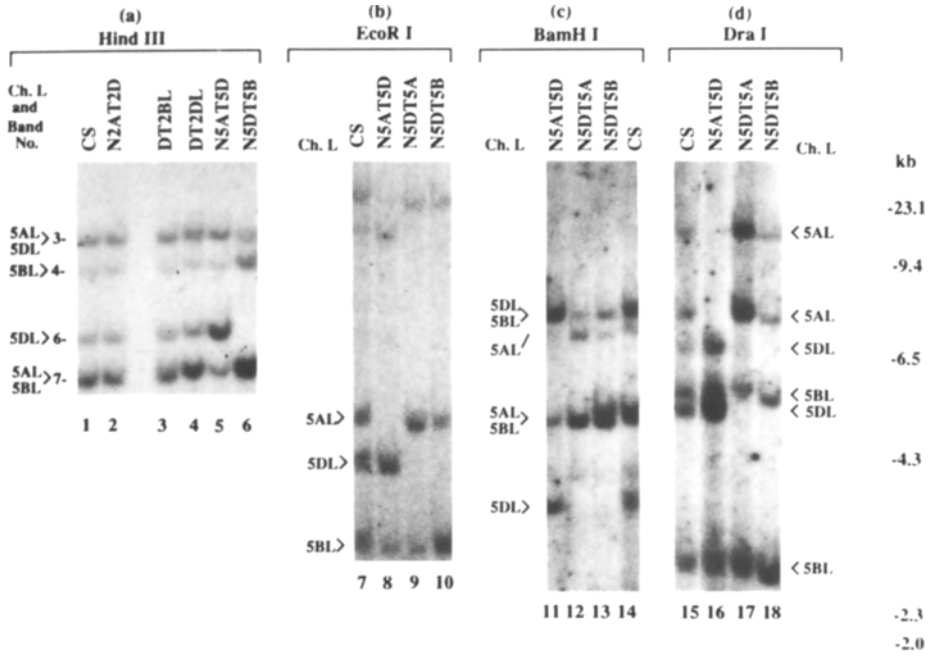

Fig. 1a–d. Autoradiogram from Southern hybridization of total DNA of the common wheat cultivar Chinese Spring (CS) and its aneuploids for groups 2 and 5 chromosomes digested with *Hin*dIII (**a**), *Eco*RI (**b**), *Bam*HI (**c**), and *Dra*I (**d**) and hybridized with probe I. The deduced chromosomal location (Ch. L) of each band is indicated on the *left*. For the *Hin*dIII digest (**a**) the indicated band numbers correspond to those of Fig. 4. Molecular weight marker (one DNA *Hin*dIII digest) is on the *right*. (As the patterns of different restriction enzymes were obtained in different gels, the approximate molecular weight is given)

Table 1. The 3′ end probes of wheat *RbcS* genes used in this study

Probe designation	Origin	Reference
Probe I	*Stu*I-*Xho*I fragment of the cDNA clone pTS512[a]	(Smith et al. 1983)
Probe II	*Stu*I-*Nsi*I fragment of the cDNA clone pW9[a]	(Broglie et al. 1983)
Probe III	*Stu*I-*Eco*RI fragment of the active genomic clone pWS4.3[a]	(Broglie et al. 1983)

[a] *Stu*I cuts two nucleotides upstream to the stop-codon

We have previously identified in the common wheat cv Chinese Spring (CS) 21 *Hin*dIII-digested DNA fragments that hybridized with a wheat rubisco SSU probe containing most of the coding sequence for the mature protein (Galili et al. 1991). As one of these bands was found in the present study to contain an additional fragment, CS contains altogether at least 22 *Hin*dIII-digested DNA fragments. Of these fragments, 16 were located on the short arm of group 2 – 4 on 2AS, 8 on 2BS, and 4 on 2DS – and the remaining 6 on the long arm of group 5 chromosomes – 2 on each of arms 5AL, 5BL, and 5DL. In the present work we have used three different 3′ end probes derived from different wheat *RbcS* genes. By applying these probes onto DNA isolated from aneuploids of homoeologous groups 2 and 5 of CS, we located to chromosomes the member genes of the different *RbcS* subfamilies. The obtained data provide a valuable tool for the study of the evolutionary and regulatory aspects of the different *RbcS* gene subfamilies in wheat.

Materials and methods

The following plant materials were used: the standard laboratory common wheat cultivar Chinese Spring (2n = 6x = 42; genome AABBDD); nullisomic-tetrasomic (NT) lines for homoeologous groups 2 and 5 of CS, each lacking a given pair of chromosomes and carrying an extra pair of one of their homoeologues; and ditelosomic (DT) lines for homoeologous group 2 of CS, each deficient for a given pair of chromosome arms. The methodology used in this study, including DNA extraction, electrophoresis in agarose gels, blotting onto GeneScreen plus membranes, and hybridization, were as previously described (Galili et al. 1991).

Three different 3′ end probes of wheat *RbcS* genes were used in this study. Data on these probes are given in Table 1.

Results

DNA from cv CS and from several aneuploids for homoeologous groups 2 and 5 was digested with the restriction enzymes *Hin*dIII, *Eco*RI, *Bam*HI, and *Dra*I and

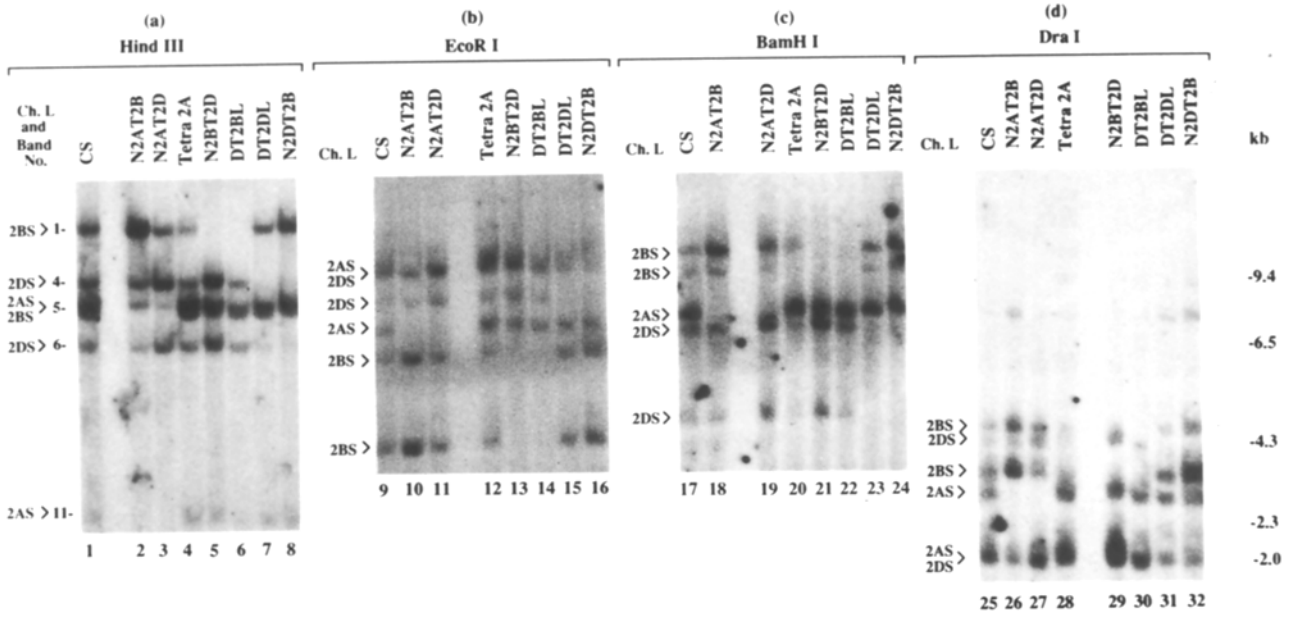

Fig. 2a–d. Same as Fig. 1, analyzing DNA of aneuploids for group 2 hybridized with probe II

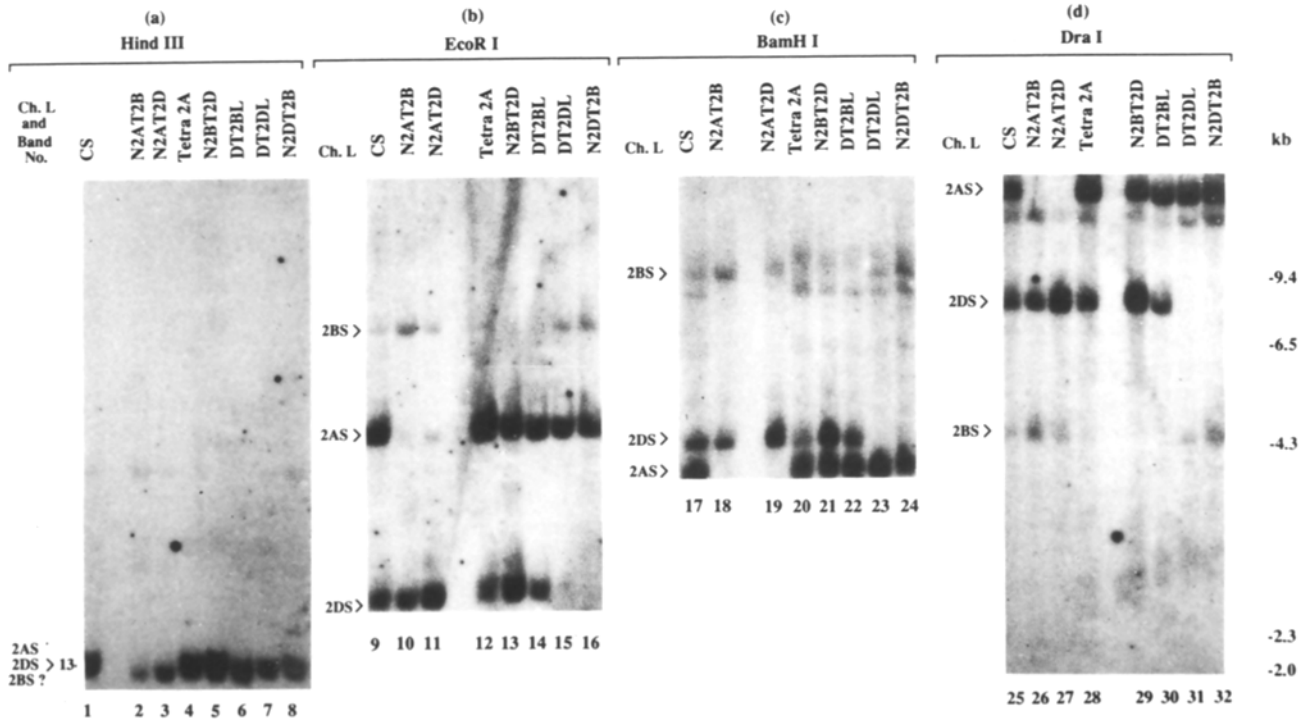

Fig. 3a–d. Same as Fig. 1, analyzing DNA of aneuploids for group 2 hybridized with probe III

hybridized with three 3′ end probes of wheat *RbcS* genes (Figs. 1–3). Figure 1 shows the results of such Southern analyses using probe I. In the *Hin*dIII and *Bam*HI digests (Fig. 1a and c, respectively), this probe hybridized to four bands. Judging by the relative intensity of bands in CS and by the relative intensity of bands, or their disappearance, in the relevant aneuploids, it was deduced that two bands in each digest contained two different DNA fragments. All six fragments were located on the long arm of group 5 chromosomes, two on each homoeologue (5A, 5B, and 5D). Moreover, all *RbcS* genes located on this homoeologous group were identified by this probe

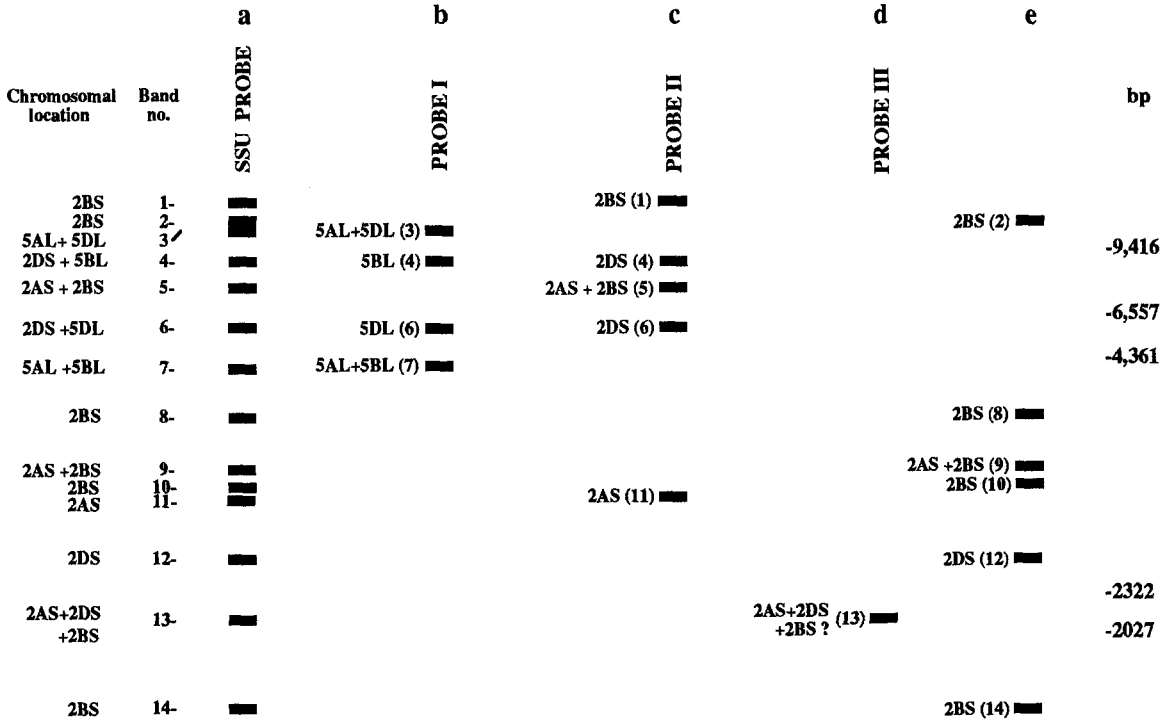

Fig. 4. Scheme of Southern hybridization of *Hin*dIII-digested DNA of common wheat cv CS probed with rubisco SSU probe (lane *a*), and the 3′ end specific probes, probe I (lane *b*), probe II (lane *c*), and probe III (lane *d*). Bands that did not hybridize with any of the 3′ specific probes are shown in lane *e*. Chromosomal location as well as band numbers (in parentheses) are indicated. The chromosomal location of bands shown in lane *a* are based on our previous report (Galili et al. 1991), with one exception: band no. 5 is controlled by chromosome arm 2AS as well as by 2BS

(Fig. 4). The presence of six fragments was confirmed by the other two restriction enzymes, *Eco*RI and *Dra*I (Fig. 1b and d, respectively). In the *Eco*RI digest the probe hybridized to three bands, each consisting of two fragments. (The two uppermost faint bands in *Eco*RI seem to have resulted from partial digestion). In the digest of *Dra*I the probe hybridized to six different bands. Thus, all four restriction enzymes revealed a similar number of six DNA fragments, all located to chromosomes of group 5, two on each of the homoeologues.

Using probe II, the Southern analyses of CS and several relevant aneuploids for group 2 are presented in Fig. 2. Following digestion with each of the four enzymes, this probe hybridized to five bands representing six DNA fragments, two on each of the chromosome arms 2AS, 2BS, and 2DS. Evidently, band no. 5 in the *Hin*dIII digest does not contain a single fragment on 2AS, as previously reported (Galili et al. 1991), but an additional one on 2BS. In the *Bam*HI digest (Fig. 2c), the band assigned to chromosome 2A had a higher intensity, suggesting that it represents two comigrating DNA fragments. Thus, this probe hybridized specifically to six *RbcS* genes located on the short arm of group 2 chromosomes, two on each homoeologue 2A, 2B, and 2D.

Figure 3 presents the Southern hybridization patterns of CS and several aneuploids for group 2 chromosomes probed with probe III. In the *Hin*dIII digest (Fig. 3a), this probe hybridized mainly to a single band representing three comigrating DNA fragments located on chromosome arms 2AS, 2BS, and 2DS. These three fragments were resolved by the other three restriction enzymes (Fig. 3b–d). In the *Eco*RI digest this probe hybridized to three different bands with the relative intensity of 2AS > 2DS > 2BS. In our previous study (Galili et al. 1991), hybridization of the *Hin*dIII digest with a probe carrying most of the *RbcS* coding sequence revealed no difference between the three fragments of this band (Fig. 3a) in respect to the number of gene copies. It is therefore assumed that the differential staining intensity between bands assigned to the three homoeologues reflected a greater homology between the gene located on 2AS and the probe. The additional bands that appeared in the *Bam*HI and *Dra*I digests may have resulted from partial digestion. All the fragments that hybridized to this probe were located on the short arm of chromosomes of homoeologous group 2.

Thus, altogether the 22 *RbcS* genes were classified into four subfamilies, one located on the long arm homoeologous group 5, and three on the short arm of homoeologous group 2. Three of these subfamilies were directly identified: subfamily (SF)-1, by probe I; SF-2 by probe II, and SF-3 by probe III. The remaining bands

Table 2. The designation of the *RbcS* genes in common wheat (cv Chinese Spring) and their classification into subfamilies. Subfamilies were defined by hybridization with 3′ noncoding sequences of three different SSU clone. Genes on the same chromosome arm are numbered in sequence (in parentheses). The indicated band number in the *Hin*dIII digest probed with rubisco SSU probe is after Galili et al. (1991) (see Fig. 4)

Subfamily (SF)	Gene designation	Chromosomal location	Band no. in *Hin*dIII
SF-1 (probe I)	*XRbcS-5A-HindIIIa* (1)	5AL	3
	XRbcS-5A-HindIIIa (2)	5AL	7
	XRbcS-5B-HindIIIa (1)	5BL	4
	XRbcS-5B-HindIIIa (2)	5BL	7
	XRbcS-5D-HindIIIa (1)	5DL	3
	XRbcS-5D-HindIIIa (2)	5DL	6
SF-2 (probe II)	*XRbcS-2A-HindIIIa* (1)	2AS	5
	XRbcS-2A-HindIIIa (2)	2AS	11
	XRbcS-2B-HindIIIa (1)	2BS	1
	XRbcS-2B-HindIIIa (2)	2BS	5
	XRbcS-2D-HindIIIa (1)	2DS	4
	XRbcS-2D-HindIIIa (2)	2DS	6
SF-3 (probe III)	*XRbcS-2A-HindIIIa* (3)	2AS	13
	XRbcS-2B-HindIIIa (3)	2BS	13
	XRbcS-2D-HindIIIa (3)	2DS	13
SF-4	*XRbcS-2A-HindIIIa* (4)	2AS	9
	XRbcS-2B-HindIIIa (4)	2BS	2
	XRbcS-2B-HindIIIa (5)	2BS	8
	XRbcS-2B-HindIIIa (6)	2BS	9
	XRbcS-2B-HindIIIa (7)	2BS	10
	XRbcS-2B-HindIIIa (8)	2BS	14
	XRbcS-2D-HindIIIa (4)	2DS	12

that did not hybridize with any of these probes comprise at least one subfamily (SF-4). The designation of the *RbcS* genes, their subfamily assignment, and chromosomal location are presented in Table 2.

Discussion

In the present study we have used as probes three different 3′ noncoding sequences of wheat *RbcS* genes, in order to identify the gene members of the different *RbcS* gene subfamilies in common wheat and map them to chromosomes. Such probes were used in the past to identify some *RbcS*-specific genes in wheat (Broglie et al. 1983), pea (Fluhr et al. 1986; Strittmatter and Chua 1987), petunia (Dean et al. 1985b), tomato (Sugita et al. 1987), *Lemna gibba* (Silverthorne et al. 1990), soybean (Shirley et al. 1990), and other plants. As each of the three probes used in the present study hybridized to different DNA fragments, three subfamilies of wheat *RbcS* were directly identified. The chromosomal location of the *RbcS* genes reported here confirmed our previous results (Galili et al. 1991) obtained by a wheat *RbcS* probe carrying most of the coding DNA sequence for the mature SSU protein (Fig. 4). In addition, two bands previously reported to contain fragments derived from two different homoeologous groups (i.e., bands 4 and 6, Fig. 4) were resolved in the present study by probes I and II into their specific chromosomes.

Out of the three subfamilies, the one located on group 5 (SF-1) contains all *RbcS* genes that belong to this homoeologous group, i.e., all group 5 *RbcS* genes comprise a single subfamily. The other two subfamilies were assigned to group 2 chromosomes (Table 2). Seven fragments located on group 2 chromosomes (one on 2A, one on 2D, and five on 2B) did not hybridize to any of these probes (Fig. 4 and Table 2) and therefore comprise an additional subfamily. Thus, homoeologous group 2 carries three different subfamilies.

In contrast to our finding of three bands in the *Bam*HI DNA digest probed with probe III (Fig. 3c), Broglie et al. (1983), using the same enzyme and a similar probe, reported a single band of 4.3 kb in size. This discrepancy could be explained in several ways. The most plausible one is that the 4.3-kb band consists of two DNA fragments, which in our study were resolved into two fragments of similar size, while the weaker band was below the level of detection in the previous study. Alternatively, the extra bands detected by our probe may be due to the difference in the probes used, as the *Stu*I site of our probe (Table 1) is located ca. 35 bp upstream of the *Mbo*I site used by Broglie et al. (1983).

Based upon hybridization to 3′ noncoding sequences of three different wheat *RbcS* clones, the 22 *RbcS* genes of common wheat, 6 of genome A, 10 of genome B, and 6 of genome D, were divided into four subfamilies (Table 2). The finding that each of the four *RbcS* subfamilies in common wheat contains genes from the three different genomes, A, B, and D, suggests that in the Triticinae the divergence into *RbcS* subfamilies preceded the differentiation into species. This divergence has been maintained within the species at the diploid level (S. Galili, in preparation) and within genomes at the polyploid level. In wheat, similar to petunia and tomato (Dean et al. 1989), the number of genes in the various subfamilies is not identical: SF-1 contains all six genes of homoeologous group 5, and SF-2, SF-3, and SF-4 contain, respectively, 6, 3, and 7 genes, out of the 16 genes of homoeologous group 2. Differences between subfamilies in the number of genes may have resulted from duplications. Since in SF-1 and SF-2 each genome contains two genes, it may be assumed that these duplications occurred before speciation. On the other hand, SF-4 contains one gene of genomes A and D and five genes of genome B, suggesting that the duplications in the B genome occurred after speciation. Indeed, studies of Southern hybridization patterns of the *RbcS* genes of various diploid *Triticum* and *Aegilops* species (Galili et al. 1991) showed that the *RbcS* probe hybridized to six bands both in *T. monococ-*

cum var. *urartu*, the donor of the A genome, and *Ae. squarrosa*, the donor of the D genome, but to 8–12 bands in *Ae. searsii* and *Ae. speltoides*, which are related to the B genome. Evidently, such duplications already exist in diploids of the S-genome group (related to the wheat B genome) and need not be the result of changes that occurred on the polyploid level.

The grouping of the *RbcS* genes into several subfamilies that are distinguishable from one another in the 3′ region of the genes, and in some cases also in the 5′ region and in the coding sequence for the mature SSU, characterizes all plant species in which these genes were studied (Dean et al. 1989). The universality of such organization suggests that it may have a functional significance. In general, the divergence in nucleotide sequence within the regions coding for the mature SSU and the transit peptide, as well as within the 3′ and 5′ regions, is much greater between subfamilies than within subfamilies. In wheat, members of different subfamilies were reported to code for distinguishable polypeptides (up to six different amino acids) (Smith et al. 1983) and thus produce heterogenous SSU. Actually, as suggested by Broglie et al. (1983), the rubisco enzymes of common wheat are not homogenous; rather, they are a group of heterogenous holoenzymes composed of dissimilar small subunit molecules. Moreover, since the 5′ and 3′ flanking regions are involved in the regulation of gene expression (Ingelbrecht et al. 1989), subfamilies diverging in these regions may be differentially expressed in different tissues and in different developmental stages in response to different environmental conditions (e.g., light intensity). In fact, variations in the level of gene expression of different members of different *RbcS* gene subfamilies, as well as in their tissue specificity, were reported in other plants (Dean et al. 1989). In allopolyploid organisms like common wheat, members of different genomes that belong to a given subfamily (homoeoalleles) possess slight differences, not only in their flanking regions but also in their coding sequence, and consequently code for variant species of SSU. Such divergence may increase the heterogeneity of the SSU in different tissues and/or developmental stages and thereby increase the adaptability and phenotypic plasticity of the polyploid. Actually, Smith et al. (1983) identified three cDNA clones that, based on the results of this work, are coded by the genes of SF-1. The predicted amino acid sequence of the mature SSU encoded by these genes would differ by at least two amino acids. Similarly, in allotetraploid soybean, Grandbastien et al. (1986) found that the predicted amino acid sequences of the mature proteins encoded by the two homoeologous genes, SRS1 and SRS4, differ by two residues. Hence, the ability to differentially regulate the expression of members of multigene families, and consequently produce greater heterogeneity of proteins encoded by such gene families, may be one of the molecular mechanisms that facilitates the wider adaptability of polyploids over their diploid progenitors in a wide range of ecogeographical conditions.

We have used in our Southern blot analyses two cDNA clones (probes I and II) and an active genomic clone (probe III) whose 3′ end region was shown to hybridize with total wheat RNA (Broglie et al. 1983; Smith et al. 1983). Thus, at least one gene of each of the two identified homoeologous group 2 subfamilies (SF-2 and SF-3, Table 2) and three genes of the group 5 subfamily (SF-1, Table 2) are active. Three different members of this subfamily were isolated as cDNA clones (Smith et al. 1983). The findings reported here may be useful in elucidating developmental and tissue-specific regulation of *RbcS* genes in wheat.

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