Nucleolar dominance in triticales: control by unlinked genes

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Hybrid plants and animals often show suppression of activity of ribosomal genes (rDNA) originating from one of the parental or ancestral species. In the wheat × rye amphiploid triticale, containing 28 chromosomes of wheat origin and 14 from rye, rDNA of rye origin (on chromosome 1R) is not normally expressed, while the 1B- and 6B-origin rDNA from wheat shows strong expression. Expression of rDNA can be accurately assessed by the silver staining method, which stains both interphase nucleoli and metaphase rDNA sites that were actively expressed at the previous interphase. We show here that substitution of another rye chromosome, 2R, by a chromosome from hexaploid wheat, 2D (triticale-2D(2R)), prevents suppression of the rye-origin rDNA, and leads to activity of all six major rDNA loci. These results were found in two different triticales and supported by rDNA behaviour in wheat–rye chromosomal addition lines. Models for chromosomal interactions leading to control of rDNA expression are presented.

Key words: gene expression, nucleolar dominance, rDNA, substitution lines, triticale

Introduction

Rapid progress has been made in understanding the regulation and control of expression patterns of organ-specific genes (Goldberg et al. 1989, Dillon et al. 1993, Higgins 1993, Lu et al. 1993). Constitutively expressed genes are in general more difficult to study, although there are clear regulatory mechanisms. In the present paper, we examine the genetic control of nucleolar dominance, an extreme form of gene suppression: in many hybrid organisms, the 18S–5.8S–25S rRNA genes (rDNA) from one parent are often suppressed, while those from the other parent are preferentially expressed (Reeder 1985). Systems involving preferential inactivation of particular rRNA gene loci are found widely in plant and animal hybrids. In itself, the system is of interest because the gene product, rRNA, is important, but the interaction is also a valuable model for studying gene expression because the regulation of individual known loci can be examined directly by cytological methods.

In wheat (Triticum aestivum (L.) em. Thell) × rye (Secale cereale L.) hybrids and in the artificial amphiploid triticale (× Triticosecale Wittmack, 2n = 6x = 42), nucleolar dominance of wheat is observed, resulting in almost total inactivation of rDNA of rye origin (Thomas & Kaltsikes 1983, Cermen˜o et al. 1984, Lacadena et al. 1988). Rye carries a single pair of nucleolar organizing regions (NORs, the site of rRNA genes) on the 1R satellited chromosome, while tetraploid (A and B genomes) and hexaploid (A, B and D genomes) wheat have major rDNA loci on the short arms of satellited chromosomes 6B and 1B, and minor loci on non-satellited chromosomes, 5DS and 1AS, and additional, probably normally unexpressed and perhaps variable loci, on 7DL, 5AL, 1BL, 7DS and 3DS (see Mukai et al. 1991, 1993, Jiang & Gill, 1994). In the large majority of cells of the hybrids, the rye NOR is virtually undetectable by silver staining (Cermen˜o et al. 1984, Lacadena et al. 1984, Silva et al. 1995), a method that reveals interphase nucleoli and stains the metaphase NORs, which were transcribed during the previous interphase (Hubbell 1985, Jimenez et al. 1988).

In the present work, we aimed to examine the genetic control of rRNA gene suppression in lines derived from wheat × rye hybrids using molecular cytogenetic methods. These are able to provide insight into interactions between genomes, genes and loci controlling expression.

Materials and methods

Plant material

The hexaploid triticale cultivars, Drira, Juanilho, Lasko, Bacum, Rosner and Rhino, were used. Cultivars Juanilho, Drira and Bacum were supplied by the Estac ¸ao Nacional de Melhoramento de Plantas, Elvas, Portugal; cultivars Lasko (Poznan Plant Breeders, Poland) and Rosner (University of Manitoba, Canada) were obtained from the National Institute of Agricultural Botany, Cambridge, UK; cultivar Rhino, N. Neves, M. Silva (joint first authors) and W. Viegas are at the Departamento de Botanica e Eng. Biologica, Instituto Superior de Agronomia, 1399 Lisbon, Portugal. J. S. Heslop-Harrison (corresponding author) is at the Karyobiology Group, John Innes Centre, Colney, Norwich NR4 7UH, UK. Tel: (+44) 01603 452571; Fax: (+44) 01603 456844; email: Pat.Heslop-Harrison@BBSRC.AC.UK.

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Rhino-2D(2R) and associated unpublished data were kindly supplied by Dr W. Pfeiffer, CIMMYT, Mexico. Cultivars Drira, Juanilho and Lasko are AABBRR triticales (2n = 6x = 42, with full sets of seven pairs of chromosomes from each of the genomes A, B and R). Cultivar Rosner has a chromosome substitution (2n = 6x = 42; AABBRR(2D(2R))(2D(2R)), with chromosome pair 2R from rye substituted by the homoeologous chromosomes 2D from hexaploid wheat; Apbels et al. 1982). Cultivar Bacum, analysed in in situ hybridization during this work, also has a 2D(2R) chromosome substitution. The varieties are not known to be related. Cultivar Rhino is a AABBRR hexaploid triticate; the 2D(2R) substitution line directly derived from the AABBRR line was used in this study and will be referred to throughout the text as Rhino-2D(2R). The hexaploid wheat line (cv. Chinese Spring) with the disomic addition of chromosome pair 1 from rye (cv. Imperial) was also used in these studies (CS + 1R, Driscoll & Sears 1971, supplied by T. E. Miller and S. Reader).

Silver staining

Silver staining was performed on all lines studied following the technique described in Neves et al. (1995). Briefly, seeds of each cultivar were germinated in tap water, cold treated for 48 h to synchronize the cells, recovered at 25°C for 26–30 h to maximize metaphase number, and root tips were ice treated for accumulation of condensed metaphases before fixation in FAA [1:1:18 (v/v) formaldehyde (37%)-ethanol (50%)-glacial acetic acid] for 2–3 days at 4°C. The intact root tips were then washed in distilled water to remove the fixative, immersed in a 15% AgNO3 solution (pH 5.5 adjusted with formic acid) overnight at 60°C, washed in distilled water, developed in 1% hydroquinone-10% formaldehyde (1:1) for 5–10 min at room temperature and fixed in freshly prepared photographic fixative. The preparation was made by squashing in 45% acetic acid. The number of nucleoli per interphase cell and the number of silver-stained subterminal metaphase NOR (Ag-ST-NOR) chromosomes were scored for each cultivar. In this study, only the activity of the satellite NORs (present on chromosomes 1B, 6B and 1R) is analysed at metaphase; other loci show little (5D, 1A) or no (7D) expression detectable by silver staining at metaphase (Silva et al. 1995), while the terminal heterochromatin of many rye chromosomes also stains with silver. The expression of 5D and 1A NORs can be seen at interphase when they form nucleoli.

In situ hybridization

To analyse the chromosome constitution of the hexaploid triticate cultivars, Lasko and Bacum, non-radioactive fluorescence in situ hybridization following Schwarzacher et al. (1989) was used with genomic rye DNA cv. Petkus Spring as a probe, in the probe, in the presence of an excess of unlabelled wheat DNA (Annanthawat-Jonsson et al. 1990), to distinguish the parental origin of the chromosomes. Two cloned repetitive sequences, pTa71 (a 9-kb fragment containing the 18S–5.8S–25S rRNA genes and intergenic spacers isolated from wheat, Gerlach & Bedbrook 1979) and pSc119.2 (a 611-bp fragment consisting of a repetitive sequence isolated from rye, Bedbrook et al. 1988, subcloned and kindly provided by McIntyre et al. 1990), were used to help individual chromosome identification. Both sequences are homologous to wheat and rye DNA.

Results

Table 1 summarizes the frequency of interphase nuclei with different numbers of nucleoli (scored after silver staining), and of metaphase cells with different numbers of silver-stained satellite nucleolar organizing regions. Analysis of the nucleolar number by ANOVA (Snedecor & Cochran 1987) showed a very highly significant difference (P < 0.001) between cultivars Rosner and Bacum and the three other cultivars in the analysis (Drira, Juanilho and Lasko). The cultivars Rosner and Bacum also showed additional classes of cells with seven and, for Bacum, eight nucleoli, never observed in the other three cultivars in which the maximum number of nucleoli observed was 6. Because of nucleolar fusion during the cell cycle, the maximum number of nucleoli regularly observed is of greater importance than averages. The analysis of metaphase cells with silver-stained subterminal NORs (Ag-ST-NORs) showed that most metaphase cells in cvs Juanilho, Drira and Lasko had four Ag-ST-NORs (86%; Table 1, Figure 1a). In triticale cultivars Bacum and Rosner, six Ag-ST-NORs were observed in 92% and 78% of metaphase cells (Table 1 & Figure 1b).

To test the hypothesis that the chromosome constitution of cv. Bacum was directly correlated with the unusual expression of rye rRNA genes in a triticale, nucleolar activity was studied in a hexaploid wheat line with the disomic addition of 1R chromosomes (CS + 1R; 2n = 6x + 2 = 44). To evaluate any genotypic influence of the wheat progenitor genome on rye origin NOR expression, nucleolar activity was studied through silver staining in cv. Rhino and Rhino-2D(2R) (Table 1). AABBRR Rhino showed only wheat origin NOR expression in the great majority of cells (75%), as occurred in cultivars Drira, Juanilho and Lasko. Rhino-2D(2R) showed 1R NOR activity in more than 85% of cells, and the ANOVA test indicates that this distribution is similar to the ones observed in cultivars Bacum and Rosner. After silver staining, the CS + 1R line showed a high frequency of metaphase cells with five or six Ag-ST-NORs (68%, Table 1); this distribution was significantly different from the ones observed in both AABBRR and 2D(2R) substitution triticales.

Double target fluorescence in situ hybridization was used to allocate rDNA loci observed by silver staining to the different genomes in metaphase cells of cultivars Lasko and Bacum using rye genomic DNA and the wheat rDNA unit as probes. When probed with rye genomic DNA, triticale Lasko showed a balanced amphiploid chromosome constitution with 14 chromosomes of rye origin and 28 chromosomes of wheat origin (Figure 2). Six major rDNA sites were identified on satellite chromosomes 1B and 6B of wheat origin and on chromosomes 1R (Figure 2). In situ hybridization on triticale Bacum revealed 12 chromosomes of rye origin and 30 chromosomes of wheat origin (Figure 3a). rDNA hybridization confirmed the identification of two pairs of major NORs on wheat chromosomes.