NUCLEOLAR ORGANISER ACTIVITY IN WHEAT-BARLEY CHROMOSOME ADDITION LINES

J. L. SANTOS, J. R. LACADENA, M. C. CERMEÑO AND J. ORELLANA

Departamento de Genética, Facultad de Biología, Universidad Complutense, Madrid-3, Spain

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SUMMARY

The nucleolar organiser activity of cultivated barley, *Hordeum vulgare*, and the wheat-barley chromosome addition lines is analysed by a modified, highly reproducible, silver-staining procedure. Four Ag-NORs are observed in metaphase cells of barley corresponding to chromosomes 5H^v and 6H^v. Six Ag-NORs are detected in the chromosome addition lines 5H^v and 6H^v while only four Ag-NORs are observed in the rest of the addition lines analysed (2H^v, 3H^v, 4H^v and 7H^v). It is concluded that the nucleolar organiser chromosomes 1B, 6B, 5H^v and 6H^v are active. The chromosome 6H^v of barley seems to control the nucleolar activity of its homoeologous 6B of wheat judging from the diminution of the Ag-NOR size of chromosome 6B in the corresponding addition line.

1. INTRODUCTION

A modified, highly reproducible, Ag-staining procedure for plant material which enables us to analyse the nucleolar competition has been developed in our laboratory (Lacadena *et al.*, 1984; Cermeño *et al.*, 1983; Orellana *et al.*, 1984). It has been applied to study the activity of nucleolar organiser regions (NORs) since it is known that Ag-staining reaction of NORs (Ag-NORs) at mitotic metaphase can be considered as an indication for genetic activity during the preceding interphase (Miller *et al.*, 1976a, 1976b; Schwarzscher *et al.*, 1978; Schmiady *et al.*, 1979).

In this paper the NOR activity of barley and wheat-barley chromosome addition lines is analysed by using the silver procedure.

2. MATERIALS AND METHODS

Plants of common wheat *Triticum aestivum*, cv. Chinese Spring, barley, *Hordeum vulgare*, cv. Betzes and the Chinese Spring-Betzes addition lines for chromosomes 2H^v, 3H^v, 4H^v, 5H^v, 6H^v and 7H^v (kindly supplied by Dr Islam, Waite Agr. Res. Inst., The Univ. of Adelaide) were used (see Islam *et al.*, 1981). Homoeologous nomenclature of barley chromosomes (1H^v to 7H^v) has been used.

Seeds were germinated on wetted filter paper in Petri dishes at 20°C. Primary roots 1 cm long were excised and immersed in tap water at 0°C for 36–48 h to shorten the chromosomes. Subsequently the tips were fixed in acetic:alcohol 1:3.

Somatic metaphase cells were comparatively analysed by phase contrast, C-banding and Ag-staining techniques. The C-banding procedure was carried out according to Giraldez *et al.*, (1979) and the silver-staining method for nucleolar organiser regions as described by Lacadena *et al.*, (1984).

TABLE 1
Ag-NORs and nucleoli visualised, respectively, in somatic metaphase and interphase cells in barley and wheat-barley chromosome addition lines

Material	No of plants	Metaphases	Ag-NORs	Number of nucleoli at interphase*								Total cells
				1	2	3	4	5	6	7	8	
Barley cv. Betzes	2	10	4	65	85	43	7	-	-	-	-	200
Chinese Spring-Betzes addition lines (CS-B)												
CS-B2H ^v	5	82	4	84	267	215	100	27	7	-	-	700
CS-B3H ^v	5	22	4	76	235	224	130	30	5	-	-	700
CS-B4H ^v	5	33	4	77	290	298	105	30	3	-	-	803
CS-B5H ^v	5	11	6	186	355	271	124	41	12	-	1	990
CS-B6H ^v	4	14	6	65	271	258	142	51	12	1	-	800
CS-B7H ^v	2	4	4	50	142	130	62	12	4	-	-	400

* Contingency tests of the distributions observed in each type of plants were not significant and, consequently, the corresponding data were pooled.

PLATE. 1. C-banded metaphase cells of barley cv. Betzes, hexaploid wheat cv. Chinese Spring and wheat-barley 6H^v addition line. (a) C-banded metaphase cell of Betzes trisomic for chromosome 3H^v. (b) and (c) Chinese Spring hexaploid wheat, phase contrast and C-banding of the same cell. (d) and (e) wheat-barley 6H^v addition line, phase contrast and C-banding of the same cell. Arrows indicate the secondary constrictions.

PLATE. 2. Ag-stained somatic metaphase cells and interphase cells. (a) diploid barley cv. Betzes trisomic for chromosome 3H^v. (b) hexaploid wheat cv. Chinese Spring. (c) wheat-barley 6H^v addition line. (d) wheat-barley 5H^v addition line. (e and f) interphase cells with 7 and 8 nucleoli corresponding to 6H^v and 5H^v wheat-barley addition lines, respectively. Arrows indicate Ag-NORs.

3. RESULTS

The nucleolar organiser chromosomes were identified with both phase contrast and C-banding (plate 1). On the other hand, Ag-NORs were also clearly identified (fig. 2) being located in close correspondence with the secondary constriction as observed by phase contrast.

The results obtained are shown in table 1. Four Ag-NORs were observed in all the metaphase cells observed in both the Betzes barley (plate 2(a)) and Chinese Spring wheat (plate 2(b)). In the addition lines for chromosome 5H^v and 6H^v (the two nucleolar organiser chromosomes of barley), six Ag-NORs were observed in all the metaphase cells analysed (plates 2(c-d)) while in the remainder (2H^v, 3H^v, 4H^v and 7H^v) addition lines only four Ag-NORs were detected, corresponding to the wheat chromosomes 1B and 6B.

The frequency distributions of the number of nucleoli observed at interphase are also shown in table 1.

4. DISCUSSION

The C-banding patterns of the nucleolar organiser chromosomes enabled clear identification of the wheat chromosomes 1B and 6B (Lacadena *et al.*, 1984) and the barley chromosomes 5H^v and 6H^v (Islam, 1980; Linde-Laursen *et al.*, 1983) in both the parental species and the corresponding addition lines. In consequence, from the results obtained one can conclude that in the Betzes cultivar of barley the NORs of both the 5H^v and 6H^v chromosomes are active in interphase since four Ag-NORs are observed in metaphase cells. Both Ag-NORs seem to be of rather a similar size (plate 2(a)). However, Linde-Laursen (1983) has described a nucleolus organiser polymorphism in some barley cultivars. The maximum number of nucleoli observed agrees with the number of Ag-NORs present in the cells (table 1). Our results are in agreement with those of Nicoloff *et al.*, (1979).

From the results obtained in the wheat-barley addition lines it can be concluded that the NORs of both the 5H^v and 6H^v chromosomes of barley are active in addition to those of the wheat chromosomes 1B and 6B since six Ag-NORs (two from 1B, two from 6B and two others from, respectively, 5H^v or 6H^v) are detected in all the metaphase cells observed in the corresponding addition lines (see table 1) (plates 2(c-d)). The maximum number of nucleoli observed at interphase agree quite well with those expected taking into consideration that the Chinese Spring cultivar of wheat shows up to 5 and 6 nucleoli since the NOR of chromosome 5D is active although difficult to detect by silver procedure (Cermeno *et al.*, 1984). This explains the numbers of 7 or 8 nucleoli found in the 5H^v and 6H^v chromosome addition lines (plates 2(e-f)). It is worth mentioning that two nucleoli appear to be much smaller than the remainder. They are probably formed by the chromosome 5D of wheat.

On the other hand, it has been clearly observed in all (40) metaphase cells of the 6H^v chromosome addition line that the Ag-NOR size of chromosome 6B is greatly reduced in comparison with that of its homoeologous 6H^v (see plates 1(d-e) and 2(c)). Taking into account that the NOR of chromosome 6B possess approximately 60 per cent of the total rRNA gene complement of hexaploid wheat (Flavell and Smith, 1974; Flavell and O'Dell, 1976), one might deduce that its genetic activity has probably been diminished by the

presence of its homoeologous chromosome 6H^v of barley. This fact would correspond to a partial differential amphiplasty. It is worth mentioning that Martini *et al.*, (1982) reported a partial inactivation of wheat nucleolus organisers by the nucleolus organiser chromosomes from *Aegilops umbellulata*.

In previous work we have found that the NOR activity of chromosome 1R of rye is suppressed by the presence of wheat (Lacadena *et al.*, 1984; Cermeño *et al.*, 1984) and *Aegilops ventricosa* chromosomes (Orellana *et al.*, 1984). On the other hand, the behaviour of nucleolar organiser activity of *Ae. ventricosa* chromosomes are similar to those of barley chromosomes since in the amphiploids of several tetraploid wheats and *Ae. ventricosa* the *ventricosa* NORs are active (Orellana *et al.*, 1984). These observations are in agreement with those of Ramsay and Dyer (1983) who found that rye NORs are suppressed by the barley chromosomes in the corresponding interspecific hybrid. So, it seems that, for an unknown reason, the rye nucleolar organiser chromosome 1R is the weakest among the similar chromosomes of cereals and related species.

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