RIBOSOMAL RNA GENES AND SUPERNUMERARY B CHROMOSOMES OF RYE

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SUMMARY

The presence of ribosomal RNA genes on rye supernumerary B chromosomes from three different rye populations has been investigated by ribosomal RNA/DNA hybridisation. DNAs were purified from wheat plants containing zero, two, three or four rye B chromosomes and from individual plants from two rye populations containing different numbers of B chromosomes. The number of ribosomal RNA genes was highly correlated with rye B chromosome number in the wheat plants but not in the rye plants. It is suggested, therefore, that not all rye B chromosomes are the same and that those transferred into the wheat genetic background each carry approximately 650 ribosomal RNA genes.

1. INTRODUCTION

THE origin, function and behaviour of supernumerary or B chromosomes in plants have been investigated in a number of laboratories but B chromosomes still remain something of an enigma. As part of a series of experiments designed to provide information on the kinds of nucleotide sequences which are present in B chromosomes of rye and how these relate to rye A chromosome sequences (Rimpau and Flavell, 1974*a, b*) we have carried out a series of ribosomal RNA (rRNA)/DNA hybridisation experiments, to determine if rye B chromosomes carry rRNA genes.

2. MATERIALS AND METHODS

(i) *Plant genotypes*

Rye B chromosomes from three different sources were studied.

Source 1: seed from a population of rye containing B chromosomes was obtained from Dr R. N. Jones, Department of Agricultural Botany, University of Wales, Aberystwyth. These B chromosomes were first found in an accession of *Secale vavilovi* which was subsequently crossed to *Secale cereale*. The Aberystwyth population was derived from the F₁.

Source 2: these B chromosomes were first observed in a spring rye variety from Transbaikial, Siberia. These were transferred into a hexaploid wheat variety from Nepal by Lindström in Sweden (Müntzing, 1973). They have since been transferred into the genetic background of the hexaploid wheat variety, Chinese Spring, by Mr V. Chapman of this Department by a procedure involving backcrossing to Chinese Spring six times.

Source 3: these B chromosomes originated in a Japanese *Secale cereale* population. A population carrying B chromosomes derived from a Japanese accession has been maintained at Aberystwyth by open pollination by Dr R. N. Jones who kindly supplied us with seed.

The number of B chromosomes in each plant was determined from cytological analyses of contracted metaphase chromosomes in squashed Feulgen-stained root tips.

(ii) *DNA and ^3H rRNA purification and rRNA/DNA hybridisation*

DNA was purified from frozen aerial plant tissue as described in detail elsewhere (Smith and Flavell, 1974). Several wheat plants containing the same number of B chromosomes were pooled for DNA extraction and the frozen tissue finely ground in a coffee mill. The rye plants were extracted individually using liquid nitrogen to facilitate cell breakage instead of grinding in the coffee mill.

The DNA preparations from the wheat plants were further purified by banding in caesium chloride (Flamm, Bond and Burr, 1966) although extensive analyses have subsequently confirmed that with our DNA preparations further purification through caesium chloride gradients rarely makes any significant difference to rRNA/DNA hybridisation results. DNA from diluted caesium chloride gradient fractions was recovered by ethanol precipitation and centrifugation.

^3H labelled rRNA was purified from wheat embryos, variety Chinese Spring, after incubation in ^3H uridine exactly as described previously (Mohan and Flavell, 1974). The ^3H labelled rRNA preparations used had specific activities of 60,313 and 46,500 cpm/ μg .

The rRNA/DNA hybridisation procedure was exactly as described elsewhere (Mohan and Flavell, 1974; Flavell and Smith, 1974). Denatured DNA immobilised on nitrocellulose filters was incubated with ^3H RNA (6 $\mu\text{g}/\text{ml}$) for 3 hr in $6\times\text{SSC}$ at 70°C . The DNA content of each filter was determined after counting by hydrolysis at 100°C for 20 min in 1 N HCl and measuring the OD_{260} of the acid solution. $\text{OD } 27.8 \equiv 1 \text{ mg}/\text{ml}$ DNA (Brown and Weber, 1968). All results were corrected for the behaviour of filters lacking DNA. In every experiment five or six replicate filters of each genotype were included together with five or six replicate filters loaded with euploid Chinese Spring DNA to serve as internal controls. Standard deviations of the means of the replicate filters were calculated from an analysis of variance. The percentage of DNA hybridising to rRNA was calculated from the specific activity of the rRNA and the DNA bound to each filter. The number of genes in the genome was calculated from the percentage hybridisation and the DNA content per nucleus.

3. RESULTS

The effect of B chromosomes on rRNA gene multiplicity was estimated using DNAs isolated from one wheat and two rye lines carrying different numbers of rye B chromosomes from different sources. DNAs highly purified from batches of four to six wheat plants and from individual rye plants with different numbers of rye B chromosomes were immobilised on nitrocellulose filters and hybridised to ^3H -labelled rRNA in $6\times\text{SSC}$ at 70°C for 3 hrs. The percentage hybridisation values and numbers of genes were calculated from the percentage of the DNA on each filter binding to rRNA and from the DNA content per cell assuming that each B chromosome constituted 5.5 per cent of the rye diploid genome (Jones and Rees, 1968) and 2.9 per cent of the wheat hexaploid genome.

Figure 1 shows that the addition of rye B chromosomes to Chinese Spring wheat increased the numbers of rRNA genes in the genome. The linear regression relating rRNA gene number and B chromosome number is highly significant (correlation coefficient = 0.81). The slope indicates that each B chromosome increases the rRNA gene number per cell by approximately 650.

Because the production of the B chromosome carrying wheat plants involved backcrossing to Chinese Spring wheat six times we have assumed the wheat genome is the same in all these plants.

FIG. 1.—Effect of rye B chromosomes (source 2) on rRNA gene number in wheat, variety Chinese Spring. Each point is the mean of 10-12 hybridisations in two experiments. The standard error of each mean is approximately 250 to 300 genes.

The number of rRNA genes in the genomes of 21 individual rye plants carrying different numbers of B chromosomes from *Secale vavilovi* (source 1) is shown in fig. 2. The linear regression correlating rRNA gene number and B chromosome number is not significant (correlation coefficient = 0.17) although the slope is positive. In addition, within each group of plants with the same number of B chromosomes there was significant variation in rRNA gene multiplicity. This indicates heterogeneity within this rye population for the numbers of rRNA genes at the nucleolar organiser sites of the A chromosomes. This is not, perhaps, too surprising in view of the origin of this population (see Materials and Methods). Analyses of rRNA gene multiplicity in the rye plants from Japan carrying B chromosomes (source 3) gave similar results. There was no significant increase in the number of rRNA genes with increasing numbers of B chromosomes (correlation coefficient = 0.30) but there was significant variation between single plants having the same number of B chromosomes.

FIG. 2.—Effect of rye B chromosomes (source 1) on rRNA gene number in rye. Each point is the mean of 6, 12 or 18 hybridisations in three experiments. The standard error of each mean is approximately 200 genes. The arrows indicate the mean values for plants with the same number of B chromosomes.

4. DISCUSSION

The significant correlation between the number of rRNA genes in the Chinese Spring wheat plants and the number of rye B chromosomes (fig. 1) indicates that these B chromosomes carry rRNA genes. It seems unlikely that the increased number of rRNA genes in the presence of B chromosomes is due to modification of the number of rRNA genes in the wheat chromosomes by the rye B chromosomes. This would require modification of rRNA gene multiplicity after fertilisation since the number of B chromosomes is determined only at fertilisation; the B chromosome complement of male and female gametes may not be the same, even in inbreeding situations, owing to unequal segregation of B chromosomes at pollen grain mitosis.

The absence of a significant correlation between the number of rRNA genes and the number of B chromosomes in the two rye populations carrying B chromosomes of different origins implies that these B chromosomes carry no or only few rRNA genes. The heterogeneity of rRNA gene number on the A chromosomes could mask a correlation between rRNA gene number and B chromosome number. However, the number of rRNA genes in the plant with eight B chromosomes strongly supports the conclusion that these

B chromosomes carry no or only very few rRNA genes. Whether or not these latter types of rye B chromosomes carry rRNA genes, it seems certain that they carry fewer than the B chromosomes from the *Secale cereale* accession from Transbaikal (source 2). Thus not all B chromosomes in rye are the same.

The overall similarity of the DNAs of plant B chromosomes to the DNAs of the A chromosomes (for rye, Rimpau and Flavell, 1974a; for maize, Chilton and McCarthy, 1973; for *Aegilops*, Dover, 1974) suggests that plant B chromosomes originate by fragmentation of A chromosomes. This has been discussed for rye by Kranz (1971). The presence of rRNA genes on at least one of the types of rye B chromosomes suggests that this supernumerary chromosome may have originated from one of the nucleolar organiser regions of the rye A chromosome complement. The other B chromosomes may have come from different segments of the A chromosome complement.

The variation in the number of rRNA genes within and between each of the two rye populations, independently of B chromosome number, provides further examples of heterogeneity within a species for rRNA gene number. Such variation has already been described for wheat and rye (Flavell and Smith, 1974).

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