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Ribosomal DNA as a convenient probe to follow segregation and possible divergency from expected homozygosity after haploidization of an androgenetic process

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Summary. Restriction fragment length polymorphism of the wheat nuclear ribosomal DNA has been studied in several steps of a breeding scheme, including parental genotypes, F1 hybrid, F9 generation, and anther-derived doubled haploid lines obtained from F9. Ribosomal DNA represents a suitable molecular marker in following segregation and possible divergency from expected homozygosity after haploidization of an androgenetic process. It has been shown to undergo variations among the first cycle-doubled haploid lines in the relative amount of two different sizes of ribosomal DNA repeat units. The specificity and peculiar properties of the plant system used allowed us to assign an intrachromosomal location (short arm of the chromosomes 1B, 1R or 6B) to several ribosomal DNA repeat units that differ by the length of their nontranscribed spacer region.

Key words: Ribosomal DNA – Restriction fragment length polymorphism – Anther-derived doubled haploids – Gametoclonal variation – Wheat

Introduction

Ribosomal RNA (rRNA) genes consist of tandemly repeated units, each of them containing transcribed and nontranscribed DNA; the latter is usually referred to as nontranscribed spacer region, or NTS. In animals and plants, rRNA genes have been shown to undergo variation, within the same species and within related species, in their copy number and the length of the repeat unit.

In plants, copy number variation has been reported by Flavell and Smith (1974) in wheat, by Maggini et al. (1978) in *Alium cepa,* by Cullis (1981) in flax, and by Rivin et al. (1983) in maize. The occurrence of length heterogeneity in rRNA genes – shown to be confined to the NTS region – is also well documented (Gerlach and Bedbrook 1979; Oono and Sugiura 1980; Fodor and Beridze 1980; Kato et al. 1982; Appels and Dvorak 1982; Yakura et al. 1984). However, it is not a general rule since Varsanyi-Breiner et al. (1979) have not been able to detect any length heterogeneity in rRNA genes of soybean, and Delseny et al. (1983) have found in radish a heterogeneity confined to coding sequences.

It is now generally accepted that plants regenerated from somatic cell cultures may display a genetic variability, termed "somaclonal variation" (Larkin and Scowcroft 1981). As far as rRNA is concerned, Landsmann and Uhrig (1985) have shown a 70% decrease in the copy number of 25S gene of *Solanum tuberosum* plants regenerated from protoplasts. Brettell et al. (1986) detected a significant loss of rRNA genes in triticale regenerated from immature embryos, but did not find any evidence for changes in the length of the NTS region of rDNA. The use of rDNA as a specific probe may help assess the quality of in vitro culture experiments leading to the production of regenerated plants. In this way, Uchimiya et al. (1983), using callus cultures derived from *Nicotiana langsdorffii* and *Nicotiana glauca* and their somatic hybrids produced by protoplast fusion, have shown that regenerated plants contained rDNA possessing the particular features of both parents, allowing them to discriminate between somatic hybrids and parental lines.

Regenerant plants may also be obtained from in vitro cultures of gametophytic cells. The variability generated by this cell culture technique is referred to as

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"gametoclonal variation" (Evans et al. 1984). In this way, de Paepe et al. (1982) have been able to show that nuclear DNA of doubled haploids of *Nicotiana sylvestris* resulting from consecutive androgenetic cycles contained, on the average, increasing amounts of total DNA and increasing proportions of highly repeated sequences. More recently, Rode et al. (1987) detected a gametoclonal variation in the NTS region of anther-derived dihaploids of wheat.

The work presented here is an attempt to use nuclear rDNA as a specific molecular probe throughout several steps of a soft wheat-breeding scheme, including the genitors, the F1 hybrid, the ninth generation of selfing, and the regenerant plants obtained from two consecutive androgenetic cycles. The progeny from which androgenetic lines have been obtained was initiated from the cultivars (cv), Talent and Aurora (Henry and de Buyser 1985). The cv Aurora possesses a 1B/1R translocation (Mettin et al. 1976) in which the short arm of chromosome 1B is replaced by the short arm of chromosome 1R from rye. In wheat, rRNA genes are essentially located on the short arm of the chromosomes 1B (Nor1 locus) and 6B (Nor2 locus). In rye, the Nor1 locus is found on the short arm of the chromosome 1R. This work has taken advantage of the fact that only chromosomes 1B and 6B are satellited in wheat, and that the translocated chromosome 1BL-1RS is a nonsatellited chromosome. This allows for the number of satellited chromosomes to be ascertained and the presence of the translocated chromosome to be determined. Despite the clear homeology between the chromosomes of rye and wheat, there have been few reports of pairing between them. Where pairing has occurred, it has been very limited, even when the pairing control gene Ph was absent (Miller 1984).

We have been able to determine the chromosomal location (Nor1 or Nor2) of the major rDNA units that differ in length and to show that the NTS region of nuclear DNA is a convenient probe to search for the true genetical origin of the androgenetic plants obtained. Moreover, a significant quantitative variation of two different sizes of rDNA repeat units has been detected among two out of the first-cycle androgenetic regenerants.

Materials and methods

Plant material

The wheat (*Triticum aestivum* L.) genotypes used throughout this work were developed from an initial cross of cv Talent and cv Aurora. From generation 1 to 9, individual plants were selected, after selfing, for agronomical performances. Six first-cycle doubled haploid lines were randomly chosen out of the 24 obtained from anther culture of F9 plants (Henry and de Buyser 1985). Eighteen second-cycle-doubled haploid lines were randomly chosen out of the 52 obtained from anther culture of 1 out of the 6 first-cycle doubled haploid lines. This breeding program is summarized in Fig. 1.

Chromosomal observations

Root tips were treated by the Feulgen technique and squashed in acetocarmine. The cv Talent is a four satellite chromosome genotype (1B and 6B pairs), and the cv Aurora possesses only two chromosomes 6B with satellites.

Plant DNA preparation

Seedlings were grown under complete darkness at 20°–22°C, and the leaves were harvested when they were about 8–10 cm long. The material was surface-sterilized with sodium hypochlorite [5% (v/v), 5 min], rinsed with distilled water, and frozen in liquid nitrogen. Total DNA was prepared according to Dellaporta et al. (1983) with several modifications (Rode et al. 1987). Each DNA sample was extracted from young leaves from four to six seedlings of the same spike.

DNA restriction and agarose gel electrophoresis

DNA (3–5 μg) was completely digested with either EcoRI or EcoRI + BamHI in the presence of 3–4 m*M* spermidine. The restriction fragments were separated by electrophoresis on 0.4% or 0.8% agarose vertical slab gels in TEA buffer. After electrophoresis, gels were stained with ethidium bromide (1 μg/ml) and photographed under UV light.

Ribosomal DNA probes

A wheat (*Triticum aestivum*, cv Chinese Spring) rDNA probe [gift of Dr. R. B. Flavell, plasmid pTA 71 isolated by Gerlach and Bedbrook (1979)] containing the repeat unit defined by the EcoRI site inside the 26 S rRNA gene was used to prepare three subclones by restricting the rDNA insert with EcoRI + BamHI and cloning in pUC 19. The subclones correspond to those already obtained by Gerlach and Bedbrook (1979): pTA 250-1 (4.4 kb EcoRI–BamHI fragment) containing the entire nontranscribed spacer region and a small fraction of the 26 S and 18 S rRNA genes, pTA 250-2 (3.6 kb BamHI–

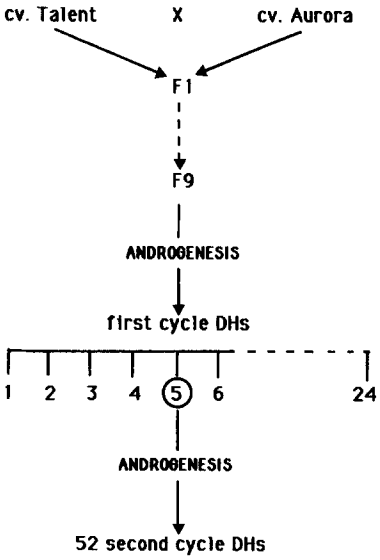

Fig. 1. Origin of the *Triticum aestivum* genotypes

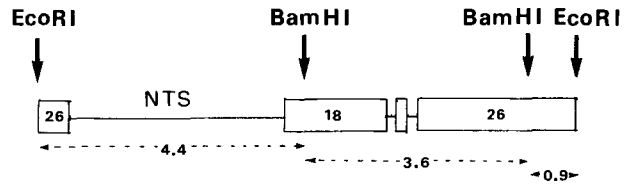

Fig. 2. Representation of the *T. aestivum* nuclear rDNA unit showing the location of the different probes used in this work. 26, 18: location of the genes coding for 26S and 18S rRNAs. NTS: nontranscribed spacer region (Appels and Dvorak 1982)

BamHI fragment) containing the major part of the 18 S and 26 S rRNA genes, the transcribed spacer, and the 5.8 S rRNA gene and pTA 250-3 (0.9 kb BamHI–EcoRI fragment) containing part of the 26 S rRNA gene. The location of these fragments within the rDNA repeat unit is shown in Fig. 2.

DNA–DNA hybridizations

Hybridizations between EcoRI and EcoRI + BamHI restricted wheat DNA and the three rDNA subclones were carried out as described by Rode et al. (1987). Autoradiograms were scanned using a double-beam microdensitometer in order to obtain an estimation of the relative amounts of the hybridization bands.

Results

Restriction fragment length polymorphism (RFLP) detected after EcoRI digestion

Total DNA of both parental lines (cv Talent and cv Aurora) was digested by EcoRI, which defines the rDNA repeat unit size, and probed with the entire rDNA unit (Fig. 3). Both cultivars exhibited one common hybridization band at 8.4 kb; this fragment is largely predominant in cv Aurora. In addition, cv Talent had two major hybridization bands at 8.6 kb and 8.9 kb, not detected in cv Aurora, while cv Aurora did exhibit an hybridization band at about 10 kb not found in cv Talent. These results are in agreement with those obtained by Gerlach and Bedbrook (1979), which deal with the length heterogeneity of the wheat rDNA repeat unit and indicate that the length heterogeneity depends on the cultivar checked.

As mentioned above, length heterogeneity within the rDNA repeat unit is due to a variation in the length of its nontranscribed spacer region occurring through the variation in the number of subrepeats (about 130 bp in cereals) within the NTS region (Appels and Dvorak 1982). In other words, the 4.4kb EcoRI–BamHI rDNA subclone may be used as a valuable probe in experiments based upon wheat rDNA RFLP. Indeed, hybridizations with EcoRI plus BamHI double digests of total DNA from all the wheat lines used in this work with either the 3.6 kb or the 0.9 kb rDNA subclones as a labelled probe did not show any RFLP (not shown here).

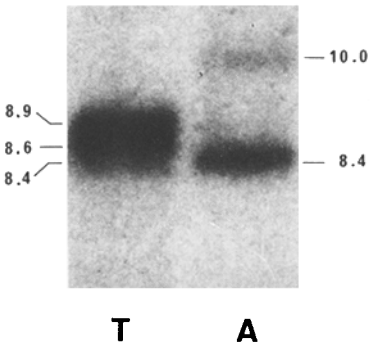

Fig. 3. EcoRI patterns of total DNA from *T. aestivum* cv Talent (*T*) and cv Aurora (*A*) hybridized with the entire rDNA unit clone. Electrophoresis on 0.4% agarose was run at 50 V for 17 h in TAE buffer (0.04 *M* Tris-acetate, pH 8, 0.002 *M* EDTA). The lengths of the hybridization bands are in kb. "1 kb ladder" (BRL) was used as a molecular weight marker

Table 1. Estimation of the relative amounts of the four major NTS regions found in the different wheat lines with respect to the total radioactivity detected on the autoradiograms. Autoradiograms were scanned using a microdensitometer and the area of each peak was estimated with the help of a microcomputer

	3.9 Kb.	4.15 Kb.	4.4 Kb.	5.4 Kb.
cv Talent	10	47	43	–
cv Aurora	25	–	4	67
F1 hybrid	15	26	25	29
F9 generation	15	36	4	44
first cycle DHs 2	19	–	< 2	75
4	31	–	< 2	62
6	40	–	< 2	57
1	< 2	53	< 2	44
3	< 2	55	< 2	41
5	5	62	< 2	29
Second cycle DHs (average)	4	56	2	36

RFLP of the NTS region during the course of a breeding scheme

In this series of experiments, total DNA from each of the wheat lines used throughout this work has been restricted with EcoRI plus BamHI, then hybridized with the 4.4 kb rDNA probe (NTS region). The results obtained with the 3.6 kb and 0.9 kb probes are shown only for the two parental genotypes.

Parental genotypes. Three major hybridization bands at 3.9 kb, 4.15 kb, and 4.4 kb are detected in cv Talent after hybridization with the 4.4 kb rDNA probe (Fig. 4). Table 1 gives an estimation – obtained from microdensitometer tracings of the autoradiograms – of the relative amounts of each of the four major bands detected within the different wheat samples used throughout this work. The detection of these three

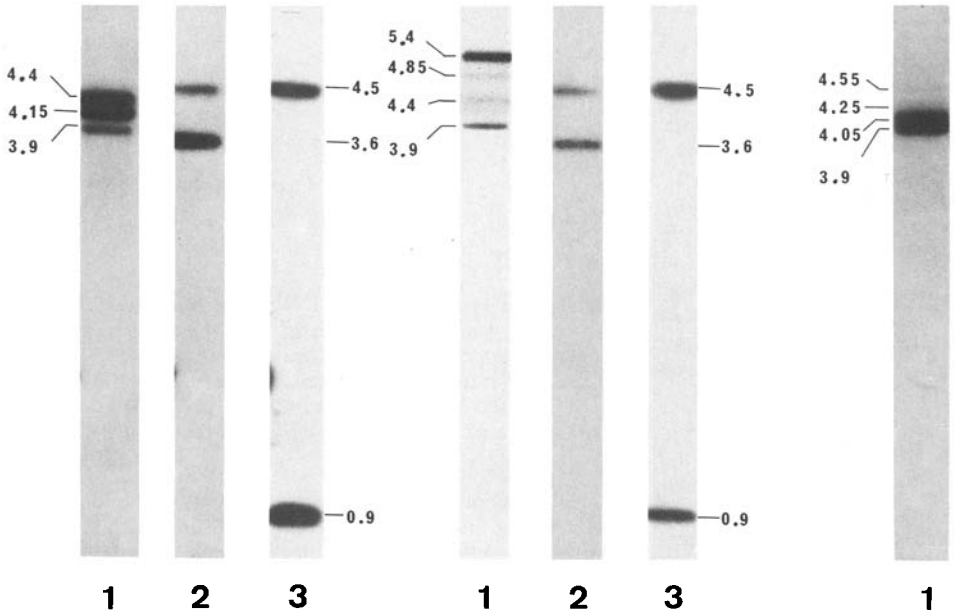

Fig. 4. EcoRI + BamHI double digest patterns of total DNA from *T. aestivum* cv *Talent* and cv *Aurora* and from *S. cereale* (*rye*) hybridized with: (*1*) 4.4 kb rDNA subclone, (*2*) 3.6 kb rDNA subclone, (*3*) 0.9 kb rDNA subclone. Electrophoresis on 0.8% agarose was run at 50 V for 10 h in TAE buffer. The lengths of the hybridization bands are in kb

bands is in agreement with the results obtained from EcoRI digestion, followed by hybridization with the entire rDNA repeat unit (Fig. 3). Indeed, the lengths of the major fragments obtained after EcoRI plus BamHI digestion correspond to the difference between the lengths of the entire repeat unit and that of the sum of both rDNA subclones (3.6 kb + 0.9 kb = 4.5 kb), corresponding to the coding regions of the repeat unit: 8.4 – 4.5 = 3.9 kb; 8.6 – 4.5 = 4.15 kb; 8.9 – 4.5 = 4.4 kb. In addition, a 7.2-kb-long hybridization band became visible after a prolonged exposure of the autoradiogram (not shown here).

Hybridization with the 3.6 kb probe (Fig. 4) lit up a band at 4.5 kb in addition to the fragment corresponding to the probe itself. However, as hybridization with the 0.9 kb probe (Fig. 4) lit up the same 4.5 kb band, it was likely that this additional 4.5 kb band arose from uncutting of the BamHI recognition site between the 3.6 kb and the 0.9 kb fragments due to a mutation arising in this site, or because of a methylation of the internal C of the recognition site (Fig. 2). A similar phenomenon has already been detected in *T. aestivum* cv César (Rode et al. 1987) and does not seem to proceed from a partial digestion of DNA since it has been observed whatever the conditions of hydrolysis. Therefore, the 3.9 kb, 4.15 kb, and 4.4 kb bands detected with the 4.4 kb rDNA probe are true NTS regions displaying RFLP.

In cv Aurora, the hybridization pattern resulting from the 4.4 kb probe is quite different from that of cv Talent (Fig. 4). Two major bands (3.9 kb and 5.4 kb) and two minor bands (4.4 kb and 4.85 kb) are detected, which represent 25%, 67%, 4%, and 4%, respectively, of the total radioactivity. When the time of exposure is prolonged, two weak hybridization bands at 6.3 kb and 8.0 kb may be detected (not shown here). The 5.4 kb and 4.85 kb hybridization bands – detected in cv Aurora and missing in cv Talent – might correspond, à priori, to 4.4 kb + 0.9 kb and 3.9 kb + 0.9 kb fragments, respectively, generated by either a mutation of the EcoRI recognition site (Fig. 2) or a methylation of the internal C of this site. To check this possibility, the same EcoRI + BamHI double digest was probed with the 3.6 kb and 0.9 kb rDNA subclones. As can be seen in Fig. 4, only the DNA fragment corresponding to the probe itself and a 4.5 kb fragment were detected. The same results as those for cv Talent are obtained, thus confirming the fact that the hybridization bands detected after probing with the 4.4 kb rDNA subclone correspond effectively to a variation in the length of the NTS region of the rDNA repeat unit.

In addition, probing a BamHI plus EcoRI double digest of total DNA of *S. cereale* (rye) with the 4.4 kb subclone lights up two major bands at 3.9 kb and 4.05 kb and two minor bands at 4.25 kb and 4.55 kb (Fig. 4). It must be noted that only the 3.9 kb band is

common to the wheat cultivar Aurora and to rye, thereby suggesting that this 3.9 kb fragment is located within the short arm of the chromosome 1R.

It is clear from these results that probing with the 4.4 kb rDNA subclone does allow one cultivar to be distinguished from the other without ambiguity. Nor1 and Nor2 loci are located in wheat within the satellited chromosomes and, as the 1B/1R translocation used generates a nonsatellited short arm, the study of rDNA RFLP associated with a cytological examination of chromosomes could represent a valuable genetic marker in the study of the progeny. Although the short arm of the chromosome 1R of rye displays fairly good homeology to the short arm of the chromosome 1B of wheat, they are sufficiently different to prevent pairing from occurring freely between them (Miller 1984).

FI hybrid. As expected, the hybridization pattern of total DNA restricted by EcoRI plus BamHI and probed with the labelled 4.4 kb rDNA subclone corresponds to the sum of the bands detected in cv Talent and in cv Aurora (Fig. 5): four major bands at 3.9 kb, 4.15 kb, 4.4 kb, and 5.4 kb representing 15%, 26%, 25%, and 29%, respectively of the total radioactivity (Table 1) and three minor bands at 4.85 kb, 7.2 kb, and 8 kb. Cytological examinations showed three satellited chromosomes, indicating the presence of an heterozygous 1B/1BL-1RS chromosome pair.

F9 generation. We show here the hybridization pattern between EcoRI + BamHI restricted total DNA and the labelled 4.4 kb probe exhibited by the sample, from which the first anther culture has been carried out. As can be seen in Fig. 5, this hybridization pattern is qualitatively identical to that of the F1 hybrid (bands at 3.9 kb, 4.15 kb, 4.4 kb, 4.85 kb, 5.4 kb, 6.3 kb, 7.2 kb, and 8 kb). However, some quantitative changes are observed in the relative intensities of several bands. Particularly, the 4.4 kb major band detected in the F1 hybrid becomes a minor band at the level of the F9 sample (Table 1). In the same way, the relative intensity of the 5.4 kb fragment becomes more important (Table 1). As for the F1 hybrid, only three satellited chromosomes were detected in this sample, in agreement with the hybridization data. It ensued that this F9 sample was suitable for following the development of an androgenetic process, as the chromosome segregation might theoretically be evidenced by probing with the 4.4 kb rDNA subclone within the anther-derived regenerant lines.

First cycle androgenetic lines. Twenty-four anther-derived regenerant lines have been initiated from the F9 sample described above, and 6 samples have been used in this work. As seen in Fig. 6, two different hybridization patterns were obtained. Three of the 6 samples (nos. 1, 3, and 5) exhibited two major bands at 4.15 kb and 5.4 kb, and 5 minor bands at 3.9 kb, 4.4 kb, 4.85 kb, 7.2 kb, and 8 kb. The 3 other samples (nos. 2, 4 and 6) exhibited 2 major bands at 3.9 kb and 5.4 kb, and 4 minor bands at 4.4 kb, 4.85 kb, 7.2 kb, and 8. kb, a pattern qualitatively identical to that of cv Aurora. The relative amounts of each of the four major bands are shown in Table 1. Samples 1, 3, and 5 had 4 satellited chromosomes, and samples 2, 4, and 6 had 2 satellited chromosomes, a result in agreement with the hybridization data and the cytological examinations dealing with the F9 sample.

If no qualitative change was found inside each distinct series of androgenetic lines, in contrast, we observed a quantitative change in both series: androgenetic line 2 shows an increase in the relative amount

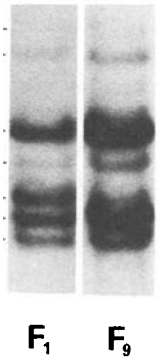

Fig. 5. EcoRI + BamHI double digest patterns of total DNA of F1 and F9 samples obtained from the initial cross Talent × Aurora hybridized with the 4.4 kb rDNA subclone. Electrophoresis on 0.8% agarose was run at 50 V for 12 h in TAE buffer. The lengths of the hybridization bands are in kb

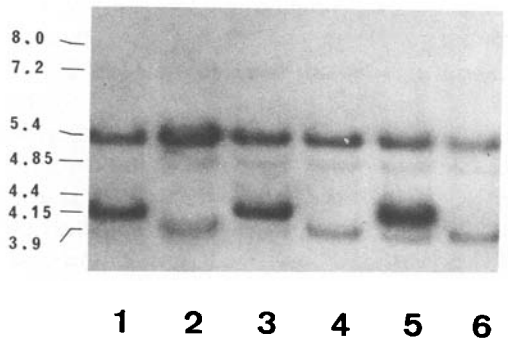

Fig. 6. EcoRI + BamHI double digest patterns of total DNA from the 6 first-cycle androgenetic lines (numbered 1–6) hybridized with the 4.4 kb rDNA subclone. Electrophoresis on 0.8% agarose was run at 50 V for 12 h in TAE buffer. The lengths of the hybridization bands are in kb

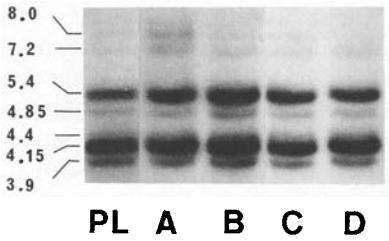

Fig. 7. EcoRI + BamHI double digest patterns of total DNA from 4 second-cycle androgenetic lines (lettered *A, B, C, D*) and from the corresponding parental line (*PL* = first-cycle doubled haploid line 5) hybridized with the 4.4 kb rDNA subclone. Electrophoresis on 0.8% agarose was run at 50 V for 10 h in TAE buffer. The lengths of the hybridization bands are in kb

of its 5.4 kb band, which is associated with a decrease in its 3.9 kb band (Table 1); androgenetic line 5 displays an increase in the relative amount of its 4.15 kb band, which is associated with a decrease in its 5.4 kb band (Table 1).

Second cycle androgenetic lines. Fifty-two androgenetic doubled haploid lines were obtained from anther cultures of first-cycle doubled haploid line 5, and 18 were used in this work. The hybridization patterns obtained with the 18 samples were all identical between themselves and with respect to the parental line. Figure 7 shows the hybridization patterns that correspond to only four samples. As expected, these second-cycle doubled haploid lines had 4 satellited chromosomes (1B and 6B pairs).

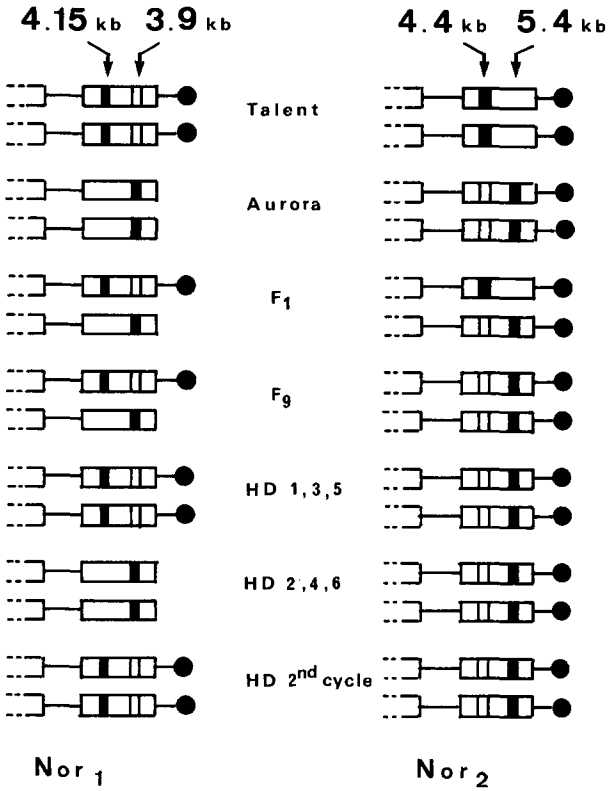

Fig. 8. Possible location of several variously sized NTS regions within Nor1 and Nor2 loci. ▬ NTS region detected as a major band; ▭ NTS region detected as a minor band. Only the short arm of the chromosomes 1B, 1R and 6B is schematized. ● Satellited chromosome 1B or 6B. The locations of these NTS regions within *Nor1* and *Nor2* loci have been arbitrarily chosen

Discussion

The ease with which chromosome 1B can be detected and characterized and the ease with which rRNA genes can be discriminated from the two parental genotypes are useful in assigning a chromosomal location (Nor1 or Nor2) for the major rDNA repeat units. The following discussion about this feature is diagrammed in Fig. 8. Only the 3.9 kb band is common to rye and to the wheat cultivar Aurora. Consequently, this NTS region must be located within the short arm of the chromosome 1R (Nor1 locus). As this 3.9 kb fragment is also present, although in a lesser amount, in the wheat cv Talent, it is expected that it must be located in this case within the short arm of the homeologous chromosome 1B. As no recombination can occur between these homeologous chromosomes, the relative amount of this fragment in the F9 sample must be intermediate between those of the two parental genotypes (Table 1). This fragment is a minor band in the first cycle dihaploid lines 1, 3, and 5 and a major band in lines 2, 4, and 6. This is in agreement with the fact that these dihaploid lines arise from only one haploid genome, which had not been able to recombine before the androgenesis experiment.

A similar approach may be carried out for the 4.15 kb fragment. The quantitative data concerning this fragment – particularly those dealing with the first- and seecond-cycle dihaploid lines (Table 1) – indicate that it has been transmitted from the parental genotypes to the dihaploid lines without the occurrence of noticeable recombination phenomena, thereby suggesting that it is located only within the short arm of the chromosome 1B (the wheat cv Aurora is devoid of this fragment).

The situation seems to be quite different as far as the 5.4 kb fragment is concerned. If we exclude first-cycle dihaploid lines 2 and 5, which are suspected to display a gametoclonal variation, then relative amounts of this fragment in lines 1 and 3 and in lines 4 and 6 (Table 1) suggest that the part of the chromosome pair encompassing this fragment has been able to recombine. These facts indicate a Nor2 location for the 5.4 kb fragment.

The 4.4 kb fragment is detected as a major band in the wheat cv Talent and as a minor band in the wheat cv Aurora. This fragment is present in all first-cycle dihaploid lines in very low amounts (2% of total radioactivity). As the Nor1 locus is located at the level of generation 9 in a heterologous chromosome pair (1BL-1BS/1BL-1RS structure), it is likely that the 4.4 kb fragment is located within the Nor2 locus.

From another point of view, this study has shown that a variation occurred among the first-cycle doubled haploid lines. This variation was only quantitative and restricted to the 4.15 kb and 5.4 kb fragments (i.e., to the 8.6 kb and 10 kb rDNA repeat units). It should be noted that the former is present only in cv Talent and is located within the short arm of the chromosome 1B, whereas the latter is present only in cv Aurora and is probably located within the short arm of the chromosome 6B. In other words, the translocated fragment (short arm of the chromosome 1R) would not be able to undergo a variation in the copy number of its rDNA repeat units. As this fragment cannot recombine (Miller 1984), one can ask whether a recombination phenomenon could be involved in the process leading to the quantitative variation observed. In an earlier work dealing with wheat androgenetic lines derived from another cultivar and demonstrating a qualitative variation of the NTS region, we emphasized the fact that the occurrence of variations in DNA organization in response to in vitro androgenesis could not be considered a general rule (Rode et al. 1987), but could depend markedly upon the nature of the genotype used. The present work, showing in this case a quantitative variation of the NTS region, is in agreement with this assumption and seems to confirm the fact that the major part of the variations observed after in vitro androgenesis could depend on events that are not related to the anther culture process per se.

One of the peculiarities introduced by the plant system used in this work is that some genes controlling certain desirable agronomic characters are known to be located on the short arm of the chromosome 1R of rye. The interest displayed by chromosome 1BL-1RS is the result of the presence in the rye 1RS chromosome arm of several genes controlling resistance to diseases (Pm8, Yr9, Sr31 and Lr26) and the positive effect on yield, which is associated with breeding lines carrying this 1B/1R translocation (Miller 1984). Nevertheless, these lines are generally unsuitable for making bread because they have been found to produce poor-quality flour. For example, the 1RS chromosome arm carries genes for secalins (Miller 1984), such as Sec-R1 in place of gliadine gene gli-B1. Then, the knowledge of the Nor gene location, of the ω-gliadin patterns, and of the chromosome structure in rye (Henry and de Buyser 1985) allows for the presence of the 1RS chromosome arm and the characters associated to that structure to be revealed.

Androgenesis, when applied to advanced breeding lines, can enable the plant material to be efficiently stabilized, while conserving the potentials of the line used (de Buyser et al. 1981). This seems to be the case in the breeding scheme described in this work, especially since first-cycle doubled haploid lines arise from a F9 population. Androgenetic doubled haploid lines are theoretically characterized by an "instant" homozygosity consecutive to haploidization and subsequent artificial or spontaneous doubling. Our results are consistent with the fact that rDNA appears to be a suitable tool for following the segregation through androgenesis and ascertaining the homozygous state. De Buyser et al. (1985) have emphasized that the use of doubled haploids in plant breeding somewhat modifies the conventional breeding schemes and, consequently, should induce plant breeders to design new tests in order to evaluate the material furnished by androgenesis. In this way, we propose the use of nuclear rDNA as a molecular marker to evaluate the quality of a doubled haploid line, keeping in mind that the simultaneous use of other molecular markers would improve the accuracy of the test.

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