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# Variability and genetics of spacer DNA sequences between the ribosomal-RNA genes of hexaploid wheat (*Triticum aestivum*)

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**Summary.** Using restriction enzyme digests of genomic DNA extracted from the leaves of 25 hexaploid wheat (*Triticum aestivum* L. em. Thell.) cultivars and their hybrids, restriction fragment length polymorphisms of the spacer DNA which separates the ribosomal-RNA genes have been examined. (From one to three thousand of these genes are borne on chromosomes 1B and 6B of hexaploid wheat). The data show that there are three distinct alleles of the 1B locus, designated *Nor-B1a, Nor-B1b,* and *Nor-B1c,* and at least five allelic variants of the 6B locus, designated *Nor-B2a, Nor-B2b, Nor-B2c, Nor-B2d,* and *Nor-B2e.* A further, previously reported allele on 6B has been named *Nor-B2f.* Chromosome 5D has only one allelic variant, *Nor-D3.* Whereas the major spacer variants of the 1B alleles apparently differ by the loss or gain of one or two of the 133 bp sub-repeat units within the spacer DNA, the 6B allelic variants show major differences in their compositions and lengths. This may be related to the greater number of rDNA repeat units at this locus. The practical implications of these differences and their application to wheat breeding are discussed.

**Key words:** *Triticum aestivum* – Ribosomal RNA – *Nor* alleles – Nucleolar organiser regions – Restriction fragment length polymorphism

## Introduction

Variability in the lengths of the non-transcribed spacer DNA sequences that separate the ribosomal-RNA (rRNA) genes of wheat was reported by Appels and Dvořák (1982) who used the restriction endonuclease TaqI to digest genomic DNA extracted from aneuploids of 'Chinese Spring' wheat. Using appropriate nullisomic-tetrasomic lines, they were able to link specific DNA spacer segments on to the 1B, 6B and 5D chromosomes of *T. aestivum.* In the cultivar 'Chinese Spring', chromosome 1B shows a single 3.1 kb spacer segment, 6B has two segments of ca. 2.7 and 2.8 kb, and the 5D rRNA locus contains a 1.7 kb spacer. From plants in which chromosomes 1B and 6B from three different cultivars were substituted into 'Chinese Spring' wheat, it was found that all four cultivars contained the 3.1 kb spacer of chromosome 1B and the 1.7 kb spacer of 5D. It was also found that the 6B spacer fragment of 'Timstein' was longer than the common 1B fragment whereas in 'Hope' and 'Cheyenne', the reverse was true. Subsequently, Dvořák and Appels (1986) estimated the lengths of the major 6B fragments. The 'Timstein', or T6B fragment was 3.9 kb, the 'Cheyenne' (C6B) fragment of 2.8 kb was of the same length as one of the 'Chinese Spring' 6B fragments, and the 'Hope' (H6B) fragment was estimated to be 2.4 kb.

Chromosomes 1B and 6B contain from one to three thousand copies (repeat units) of the rRNA genes at their nucleolar organiser regions, *Nor-B1* and *Nor-B2* (McIntosh 1986) respectively, whereas 5D (*Nor-D3*) has from one to four hundred. This was shown by filter and in situ hybridization studies in which radioactively-labelled rDNA sequence probes were either applied to purified DNA or to chromosomal spreads of various aneuploids and ditelocentrics of wheat (Flavell and O'Dell 1976; Appels et al. 1980; Payne et al. 1984).

From plasmids originally isolated by Gerlach and Bedbrook (1979), who inserted entire rDNA repeat units (as defined by EcoR1) into the vector pACYC184, a number of rDNA gene sequences have been isolated for use as molecular probes. For example, the plasmid pTa250 includes a 9.0 kb length of wheat DNA comprised of both the transcribed 26S-5.8S-18S rDNA gene sequences and the non-transcribed spacer DNA which

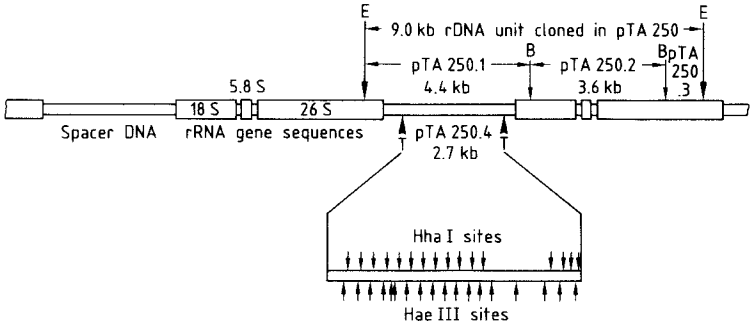


**Fig. 1.** Diagrammatic representation of ribosomal DNA sequences in wheat. Various cloned fragments are labelled and the positions of some restriction enzyme sites are indicated (E = EcoRI; B = BamHI; T = TaqI)

separates the repeated genes (Fig. 1). Using other restriction enzymes, wheat rDNA sequences present in pTa250 were further sub-cloned into the vector pBR322. A 2.7 kb fragment isolated by TaqI digestion was cloned into pBR322 to give the plasmid pTa250.4 (Fig. 1; Appels and Dvořák 1982). This fragment was shown to comprise the bulk of the spacer DNA in that when pTa250.4 is used as a molecular probe, it pairs only with chromosomal DNA or DNA fragments that contain the specific spacer DNA sequences and not at all with the 26S, 18S or 5.8S rDNA sequences. Further digestion of the 2.7 kb spacer fragment with the restriction enzymes HhaI and HaeIII showed that much of the spacer is composed of 133 bp tandemly repeated subunits (Fig. 1) and it was accordingly suggested that much of the variation in length of these sequences is due to changes in the number of sub-units within the spacer region (Appels and Dvořák 1982; Dvořák and Appels 1986).

Variability in length of specific DNA sequences can be detected as restriction fragment length polymorphism or RFLP. We decided to study RFLP within the spacer DNA from a range of wheat cultivars to determine the degree of variability, to investigate the potential of the variant sequences as genetic markers for the different nucleolar organiser regions (and for genes closely linked to these regions), and their possible use in cultivar identification and pedigree analysis.

## Materials and methods

*Plant material*

Leaves were obtained from individual plants grown in the field (1985) and glasshouse (1986). Twenty-four Australian wheat cultivars and lines; 'Banks', 'Condor', 'Condor'S'P44' ('CSP44'), 'Cook', 'Corella', 'Hartog', 'Kite', 'Ml843', 'Millewa', 'Olympic', 'Osprey', 'Oxley', 'Quarrion', 'Robin', 'Rosella', 'Sun89D', 'Sun92A', 'Suneca', 'Shortim', 'Skua', 'Takari', 'Teal', 'Vasco', and 'Warigal'; one cultivar of rye (*Secale cereale* L. cv. 'Rosen') and one of durum (*T. turgidum* L. cv. 'Kamilaroi') were investigated. The wheat line 'Sun89D' is disomic for a 1RS/1BL translocation chromosome (D. R. Marshall, personal communication): that is, the short arm of wheat chromosome 1B has been replaced by the short arm of rye chromosome 1R. After an initial study, various of these plants were intercrossed and subsequent progenies were grown in the glasshouse.

*DNA isolation and analysis*

From 1–2 g of fresh leaves were homogenized in liquid nitrogen using an "Ultra Turrax" homogenizer. The thawed homogenate was thoroughly mixed with 4 ml of extraction buffer (0.05 M Tris-HCl pH 7.5, 0.1 M NaCl, 0.2 M EDTA), 0.5 ml of 5% SDS, and 0.5 ml Proteinase K (0.5 mg/ml), and incubated at 37 °C for 1–2 h. After centrifugation at 8,000 rpm for 10 min, DNA was isolated following the methods of Appels and Moran (1984). RNAase treatment was omitted. Genomic DNA (10 µg) was digested with restriction enzyme (10 units of TaqI) for 1.5 h, electrophoresed through 1.0% w/v agarose gels in Tris-borate buffer, transferred to nitrocellulose filters (Southern 1975) and hybridized with the probe pTA250.4 labelled by ($\alpha^{32}$P)dCTP using a nick translation kit (BRESA). Autoradiography was performed at –80 °C using X-ray film and intensifying screens. A DNA extract of 'Chinese Spring' wheat (Appels and Dvořák 1982) was digested to provide spacer rDNA fragments of known length. These were used to calibrate the lengths of all other DNA fragments: estimates are ± 50 bp.

## Results

*Placement on chromosomes 1B, 6B and 5D*

Genomic DNA from the wheats, the durum and the rye were analysed. Both the rye and the durum lacked the short (1.7 kb) rDNA spacer sequence produced by the 5D rDNA locus (Appels and Dvořák 1982). 'Rosen' rye has two prominent fragments of 3.05 and 2.60 kb, and two minor fragments of 3.50 and 2.75 kb. The 2.60 kb fragment is also present in the wheat line 'Sun89D' (Fig. 2). Because this wheat has a 1RS/1BL translocation chromosome substituting for 1B entire, the 2.60 kb fragment must be derived from the rRNA locus on the short arm of chromosome 1R. Furthermore, as the wheat locus on the short arm of chromosome 1B is absent, the remaining DNA segments found in 'Sun89D', comprised of a major 3.90 kb fragment and a number of minor fragments, must be produced by the rRNA locus of chromosome 6B. As these fragments are found in 15 other wheats (Fig. 2; Table 1), it can be

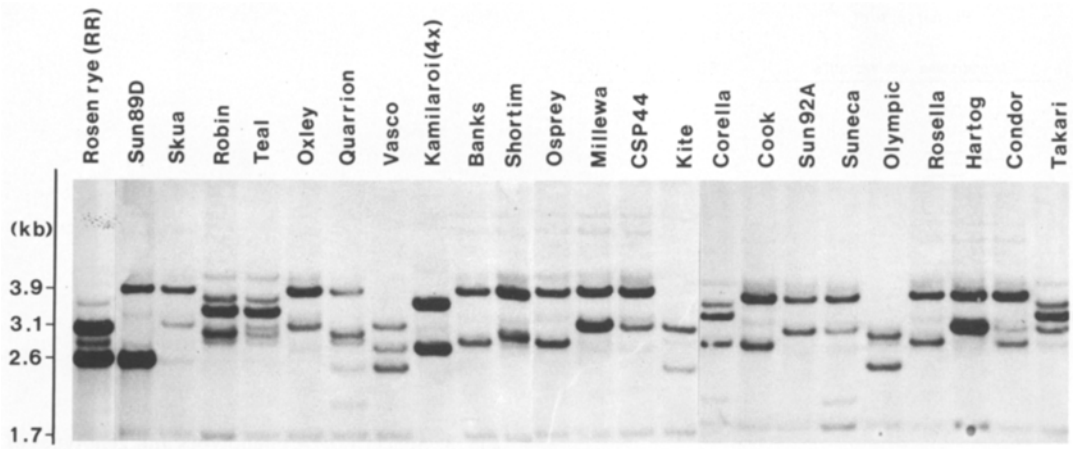


**Fig. 2.** Variable rDNA spacer sequences in a rye (RR), a durum (4x), and 22 hexaploid wheat cultivars

assumed that all of these wheats share the same rRNA locus on the short arm of 6B. Consistent with this conclusion, the major 3.9 kb fragment has previously been located on chromosome 6B of 'Timstein' wheat (Dvořák and Appels 1986) and, as we confirm below, the minor fragments are also components of this variant.

Secondly, with the exception of 'Sun89D', all 15 of the wheats containing the 3.9 kb fragment, and indeed, all of the hexaploid wheats investigated, contained prominent DNA fragments of 3.10, 2.95 or 2.80 kb (Fig. 2). Since the 3.10 kb fragment has been located on chromosome 1B of four wheat cultivars (Appels and Dvořák 1982) and as the two other fragments appeared to be alternates to the 3.10 kb fragment, this indicated that all three fragments are specified by the rRNA loci of chromosome 1BS.

Consequently, in the seven cultivars lacking the 3.90 kb fragment, all of which had either the 3.10, 2.95 or 2.80 kb fragments of 1B, the remaining spacer DNA fragments are likely to be components of rRNA loci on chromosome 6B. These fragments are as follows:

i) the single 2.45 kb fragment present in 'Kite' and 'Olympic' (and also in 'Warigal'), has previously been located on chromosome 6B of 'Hope' (Appels and Dvořák 1982);
ii) of the two fragments present in 'Vasco', one is identical in length to the 2.45 kb fragment of 'Hope', and the longer 2.70 kb fragment is identical in length to one of the components of the 'Chinese Spring' 6B locus (Fig. 3). Thus, both 'Vasco' fragments are found at the 6B locus of other wheats;
iii) in four of the wheats investigated, a three-fragment complex was found that included a lightly-labelled 4.15 kb fragment, a moderately-labelled 3.65 kb fragment, and a heavily-labelled 3.40 kb fragment. Other, longer and more faintly-labelled fragments were also present (Fig. 3). As none of the fragments of this complex have previously been located on to chromosome 6B, wheats containing this variant were intercrossed with other wheats and $F_1$ and $F_2$ progenies studied (as discussed below);
iv) a 2.85 kb fragment has peviously been located at the 6B locus of 'Cheyenne' and this fragment is found in conjunction with the 2.70 kb fragment in 'Chinese Spring' (Appel and Dvořák 1982).

These fragments, therefore, appear to be specific components of the *Nor* loci of chromosome 6B and differing combinations of these fragments are found in different variants of this locus. Similarly, the fragments present within the *Nor* loci of chromosome 1B are found in differing combinations to give the variants of the 1B loci. The different 6B and 1B variants are depicted in Fig. 3.

Another lightly-labelled DNA fragment, ca. 2.00 kb in length, was found in 'Corella', 'Quarrion' and 'Suneca' (Fig. 2). This fragment has not been located to a particular chromosome. The tetraploid wheat, 'Kamilaroi', also contains two major fragments of some 3.65 and 2.70 kb and whilst the latter is similar in length to one of the 6B fragments present in 'Chinese Spring' and 'Vasco', this tetraploid has not been further investigated.

*Parental phenotypes*

Individual plants are expected to contain homologous pairs of chromosomes 1B and 6B. These homologues will be either homozygous or heterozygous for their rRNA loci. From Fig. 2, it can be seen that both 1B and 6B variants are present and the labelled DNA fragments represent the phenotype of the individual plant. Several of the cultivars show similar phenotypes. Thus, 'CSP44', 'Oxley', 'Skua' and 'Suneca' (excepting the 2.0 kb fragment) are similar, as are 'Hartog',

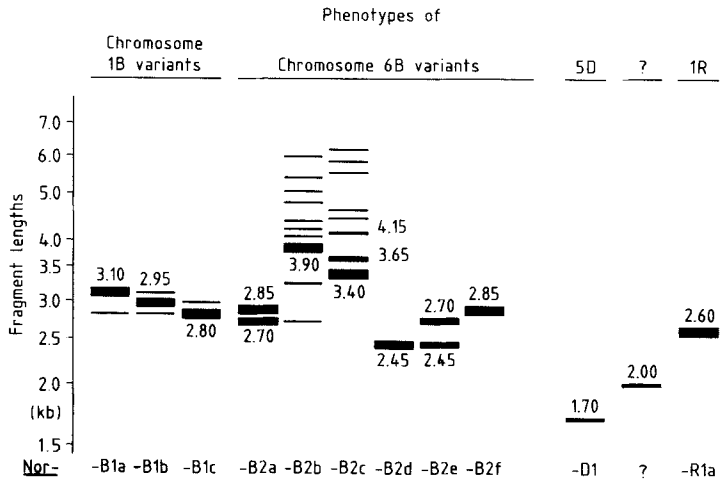


**Fig. 3.** Spacer rDNA phenotypes and genotypes of the chromosome 1B, 6B, 5D and 1R variants

'Millewa' and 'Sun92A'. In the latter three, however, the 3.1 kb 1B spacer is more heavily labelled than the 3.90 kb 6B spacer whereas in the former, the reverse is true. (In Table 1, these are designated 1B > 6B and 1B < 6B, respectively). The same 6B variant is also found in combination with the 2.95 kb 1B fragment in 'Shortim' and with the 2.80 kb 1B fragment in 'Banks', 'Cook', 'M1843', 'Osprey' and 'Rosella'. The 'Condor' sample also shares the 6B fragment but shows both the longer and shorter 1B fragments. The plant was assumed to be heterozygous for these variants but the original leaf sample may have been taken from more than one plant. The same is true for 'Teal', which showed the longer and intermediate length 1B fragments, and for the sample of 'Quarrion', which showed two 6B variants and two of the 1B variants also. The three-fragment complex present in 'Teal' was also present in 'Takari', in 'Robin' and in 'Corella', with the longer, intermediate, and shorter 1B fragments, respectively, and these 1B fragments were respectively found in association with the 2.40 kb 6B fragment in 'Kite', 'Olympic', and 'Warigal' (see Table 1).

As some of the phenotypes may have been due to heterozygosity, and as it was apparent that a number of other heterozygote combinations could be obtained, several of the plants from which the DNA samples were obtained were intercrossed. Occasionally, sib-plants were used for this purpose and if so, their selfed progenies were also studied in the next generation. Two cultivars containing apparently unique 6B variants ('Chinese Spring' and 'Vasco'), and 'Sun89D' with its 1B/1R translocation chromosome, were not further studied.

*First generation phenotypes*

The intercrosses effected were between cultivars containing the three 1B variants and three of the 6B variants. Of the 36 possible phenotypic combinations of 1B and 6B variants, 23 were obtained from the second generation parental and hybrid progenies. These are shown in Fig. 4. Whilst not all of the possible combinations between 1B and 6B were developed, all three 1B variants have been combined as heterozygotes as have the three 6B variants. With one exception ('Quarrion'), all of the parental plants bred true, their progenies being homozygous for one or other of the 1B and 6B genotypes. In all of the $F_1$ progenies investigated, the phenotypes were both additive and dosage dependent in that differences in the intensity of labelling could be used to distinguish between single and double doses of the 1B and 6B chromosomes. It was also observed that the comparative labelling intensities between 1B and 6B (i.e. 1B < 6B and 1B > 6B) were maintained when these variants were heterozygous, as found in the various crosses involving 'Suneca' and 'Hartog'.

Nine plants from the assumed 'Condor' parent were analysed and all were homogeneous, indicating that the original sample must have been obtained from two different plants. A mixed origin for the original sample of 'Quarrion' was also indicated in that when progeny from a single plant were analysed, all were homozygous for the 3.90 kb 6B variant and eight of the ten plants contained the 2.80 kb 1B variant. Two lacked any 1B variant whatsoever and presumably, therefore, also lack the nucleolar organiser regions of chromosome 1B. (Further progenies of these plants are being studied).

In general, when these results are combined with the results obtained by Appels and Dvořák (1982) and Dvořák and Appels (1986), it is clear that the 1B and 6B variants are alleles of genetic loci on chromosomes 1B and 6B. Proof that the three-fragment complex containing the 3.40, 3.65 and 4.15 kb fragments is an allele of the 6B locus was determined from the $F_2$ progeny of the cross between 'Corella' and 'Hartog'.

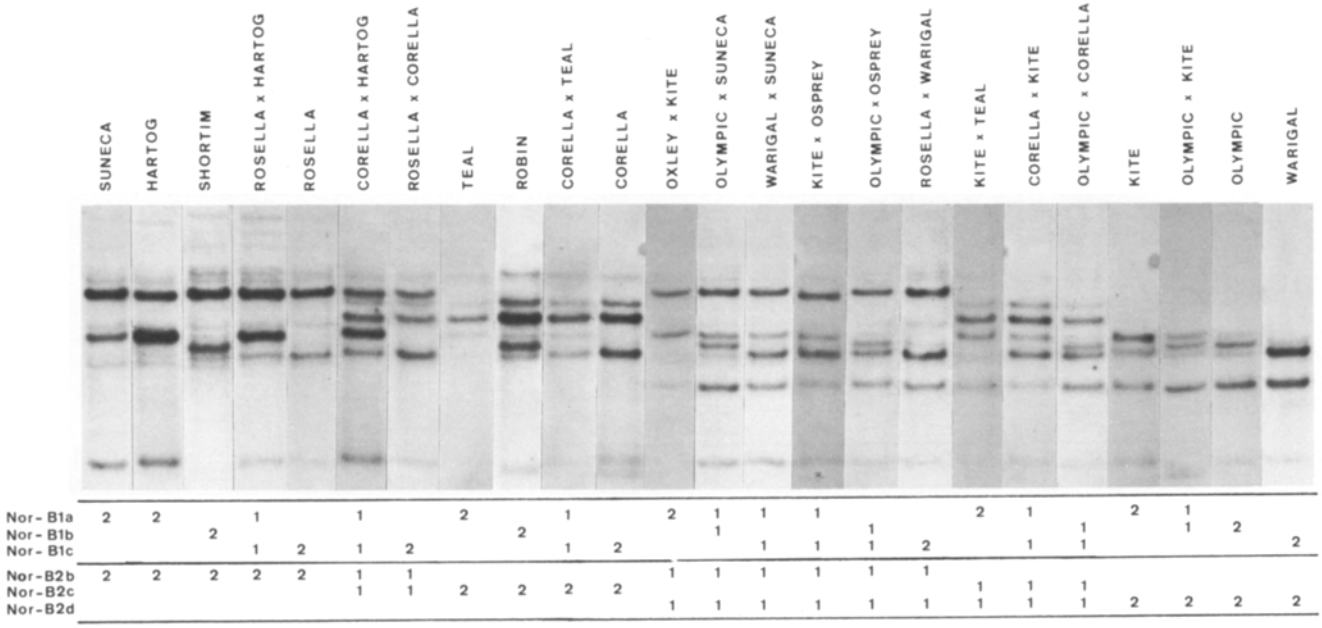


**Fig. 4.** Ribosomal DNA spacer phenotypes and genotypes of selected wheat cultivars and their $F_1$ progenies

*Phenotypes of a second generation*

'Corella' contains the shorter of the 1B variants (2.8 kb) and the three-fragment complex tentatively assigned to 6B. 'Hartog' has the 3.1 kb fragment of 1B, and on 6B, the major 3.9 kb fragment and associated minor fragments (Fig. 2). The $F_1$ hybrid between these two cultivars shows a summation of all of the major fragments (Fig. 4). Proof that the two 1B and the two 6B variants are allelic was provided by the analysis of 50 $F_2$ progeny from an $F_1$ plant of this cross. The different phenotypes and the numbers of plants of each phenotype are shown in Fig. 5. As expected, the two 1B allelic variants are present in a 1:2:1 ratio ($\chi^2 = 0.04$; $0.9 > P > 0.8$), the 3.9 kb fragment and the three-fragment variant are also present in a 1:2:1 ratio ($\chi^2 = 1.96$; $0.2 > P > 0.1$), and are therefore alleles of the *Nor* locus on chromosome 6B, and the two alleles at the two loci show the expected combination of nine different phenotypes in a 1:2:1:2:4:2:1:2:1 ratio ($\chi^2 = 5.68$; $0.7 > P > 0.5$). In addition, this cross demonstrates that the more lightly-labelled fragments found in conjunction with both the major 3.90 kb fragment of 6B and also with the three-fragment complex are integral parts of the two allelic variants.

## Discussion

*Allelic nomenclature*

The nucleolar organiser regions adjacent to the satellites of chromosomes 1B and 6B have been designated *Nor-B1* and *Nor-B2* (McIntosh 1986). As the rDNA spacer variants that we have found indicate variation within the highly repeated rRNA genes that comprise the *Nor* loci, we have designated the alleles by the same symbols. Thus, the 1B alleles are designated *Nor-B1a, Nor-B1B* and *Nor-B1c,* and the 6B alleles are designated *Nor-B2a, Nor-B2b, Nor-B2c, Nor-B2d* and *Nor-B2e.* The 5D allele is designated *Nor-D3* (Fig. 3). The 2.60 kb rye spacer found in 'Sun89D' is only one of the components of the cereal rye phenotype (Fig. 2). Although 'Rosen' rye itself is not the actual source of the 1R chromosome present in 'Sun89D', this spacer has been found in all triticales that have been studied (Brettell et al. 1986; May and Appels, unpublished) and we have tentatively designated this rye allele as *Nor-R1a.*

Some of these allelic variants have been described previously and other 6B variants are known. One of these is found in 'Cheyenne' wheat and consists of a single fragment, ca. 2.85 kb in length, that is identical to one of the fragments present in *Nor-B2a* of 'Chinese Spring' (Dvořák and Appels 1986). This allele is designated *Nor-B2f.*

Other variants may also be present in other hexaploid wheats. For example, when genomic DNA's were doubly digested with BamHI/EcoRI and then probed with the plasmid pTA71 (which contains an entire 9.5 kb wheat rRNA gene; Gerlach and Bedbrook 1979), Snape et al. (1985) located three variants of rRNA gene spacer sequences on each of chromosomes 1B and 6B in four wheat cultivars. Unfortunately, these

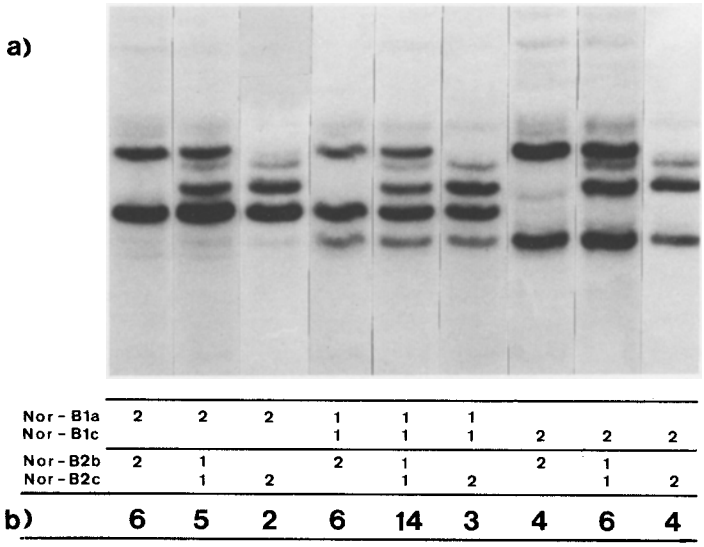


**Fig. 5.** Ribosomal DNA spacer phenotypes and genotypes (**a**) and the number of plants of each phenotype (**b**) in the 50 $F_2$ progeny of a cross between 'Corella' and 'Hartog'

restriction enzyme profiles are neither comparable nor easily related to the genotypes that we have described.

*Chromosome 1B variants*

Clearly, the 1B alleles are closely related in that there are only three spacer variants present. These show both qualitative and quantitative changes that are distinctive of the three *Nor-B1* genotypes. The major 3.10 kb spacer of *Nor-B1a* is associated with a minor amount of the 2.80 kb spacer. The 2.80 kb fragment is the major fragment of the *Nor-B1c* allele and is in turn associated with a minor amount of the 2.95 kb spacer segment. The 2.95 kb fragment is the major segment in *Nor-B1b* which also includes minor amounts of both the 3.10 and 2.80 kb segments (Fig. 3). These allelic variants can be simply explained. Within the accuracy of our length determinations, the major 1B spacer fragments differ in length by approximately 150 bp. We have consequently assumed that these differences are a function of the number of repeat sub-units within the spacer DNA region. Appels and Dvořák (1982) showed that these sub-units are 133 bp in length, as difined by HhaI and HaeIII digestion (Fig. 1). Thus, the differences in lengths of the major 1B spacer segments are likely to be caused by the addition or loss of one or two of these sub-repeats from the spacer DNA region. (Digests with BamHI show that the 1B fragments labelled with pTA250.4 show similar differences in length – data not shown).

*Chromosome 6B variants*

In contrast to the *Nor-B1* alleles of chromosome 1B, the *Nor-B2* alleles of chromosome 6B are much more complex in that there are at least six allelic variants, the number of major fragments defined by TaqI digestion range in length from 2.4–4.15 kb, three of the variants have unique fragment profiles, and only three of the fragments (2.85, 2.70 and 2.45 kb) are common to two of the genotypes (Fig. 3). Whilst it is possible to determine that the major fragments differ in length by multiples of the 133 bp sub-unit: for example, the two spacer fragments of *Nor-B2a* found in 'Chinese Spring' differ by one sub-unit (Appels and Dvořák 1982) and the 2.45 kb fragment of *Nor-B2d* and *e* differs from the 2.70 kb fragment found in *Nor-B2a* and *e* by the loss of two sub-units, the actual number of sub-units involved in the differences in length between the shorter and longer spacer fragments can only be estimated. These estimates are dependent upon the accuracy of the length measurements and for the longer fragments, the differences in lengths are of the order of 250 bp or two sub-units.

Differences between the 1B and 6B allelic variants are further seen when the actual lengths of the *Nor-B1* and *Nor-B2* spacer variants are compared. Whereas both the chromosome 1B and 6B variants differ in length by various multiples of 133 bp, the 1B and 6B variants differ from one another by about one half of this amount. Thus, the 6B spacer DNA differs from the 1B spacer in the position of at least one of the TaqI restriction sites.

*Evolution of variants*

This data suggests that in the evolution of the Triticeae, events have occurred which have resulted in the amplification of certain rDNA spacer variants within a locus. The mechanism responsible for such amplification is not clear although processes such as unequal crossover events have been discussed (Arnheim 1983). It is suggested that the 1B variants are closely related in that only three spacer variants differing in length by

**Table 1.** Genotypic combinations of parental wheat cultivars

| *Nor* alleles on chromosome 6B | *Nor* alleles on chromosome 1B | | | |
|---|---|---|---|---|
| | | *Nor-B1a* | *Nor-B1b* | *Nor-B1c* |
| *Nor-B2a* | 'Chinese Spring'[a] (1B < 6B) | (1B > 6B) | | |
| *Nor-B2b* | 'CSP44' 'Oxley' 'Skua' 'Suneca' 'Condor' 'Timstein'[a] | 'Hartog' 'Millewa' 'Sun92A' | 'Shortim' | 'Banks' 'Cook' 'M1843' 'Osprey' 'Rosella' 'Quarrion' |
| *Nor-B2c* | 'Takari' 'Teal' | | 'Robin' | 'Corella' |
| *Nor-B2d* | 'Kite' 'Hope'[a] | | 'Olympic' | 'Warigal' |
| *Nor-B2e* | 'Vasco' | | | |
| *Nor-B2f* | 'Cheyenne'[a] | | | |

[a] Appels and Dvořák; Dvořák and Appels 1986

one or two sub-repeats have been found. These variants are common, although differing in proportion, to at least two of the *Nor-B1* alleles. The greater number of variants at the 6B locus may be related to the greater amount of rDNA generally found at this locus (Flavell and O'Dell 1976; Appels et al. 1980) for this could allow the 'fixation' of a greater range of variants during the evolution of hexaploid wheat.

*Cultivar genotypes and homozygosity*

As expected of a self-fertile species such as wheat, individual plants within a cultivar are largely homozygous. In this study, the progeny from representative plants of each cultivar were generally homozygous for their *Nor* loci on chromosomes 1B and 6B and identical to their parental plants. Thus, the cultivars can be divided into sub-groups according to their shared alleles (Table 1). However, some heterogeneity within cultivars must be present as was seen in the original samples taken from plants of 'Condor', 'Quarrion' and 'Teal'.

*Implications for plant breeding*

Whether or not any of these *Nor* alleles have different effects on the phenotype of the plant is unknown; indeed, it may be unlikely that there are different phenotypic effects given the importance of these loci to the plant. Even if there has been no selection for these allelic variants, it is possible that during the selection process for the cultivars, agronomic phenotypes that are linked to the various combinations of *Nor* homozygotes have been selected. If any characters can be found that link the cultivars placed in the sub-groups of Table 1, selection for the agronomic character could be effected by selection for homozygosity of the particular *Nor* alleles. Of course, this may already have been carried out in that various of these cultivars are genetically related (e.g. Fitzsimmons et al. 1985) but, as yet, no specific agronomic effects have been found to be associated with the different sub-groups.

The ability to recognise allelic variants of the *Nor* loci is of significance, however, in that the positions of a number of genes have been mapped with respect to nucleolar organiser regions generally. These include various protein and enzyme loci on chromosomes 1B (Payne et al. 1984; Snape et al. 1985), 6B (Dvořák and Chen 1984; Dvořák and Appels 1986), and 1R (Lawrence and Appels 1986). Consequently, the presence of RFLP in the spacer DNA separating the rRNA genes of wheat is of immediate benefit to the recognition of new agronomic linkages and further variants at loci linked to these individual *Nor* variants. Whilst no other variants have yet been directly linked, such genetic studies are of immediate application and the markers found will be of importance in the future. As these could be present in the progenies of the present plants, seed from those plants that are homozygous for the different variants of the two loci have been deposited with: The Curator, Australian Winter Cereals Collection, R.M.B.944, Tamworth, N.S.W. 2340, Australia.

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