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NOTES

Variation in the restriction fragments of 18S-26S rRNA loci in South American Elymus (Triticeae)

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The repeat units of the 18S-5.8S-26S ribosomal genes (rDNA) of 15 South American and 6 Asiatic and North American species of Elymus were shown to vary between 8.7 and 11.3 kb among species. Interspecific variation in the EcoRI and BamHI cleavage sites was observed. BamHI restriction sites were present in the subrepeats in the intergenic spacers in rDNA from diploid Hordeum (H genome) and absent in the diploid Pseudoroegneria (S genome) and in nearly all the tetraploid species of Elymus (SH genomes). Tetraploid Elymus species have only two pairs of chromosomes with secondary constrictions similar to those found in Pseudoroegneria and a maximum of two different repeat unit length classes. These results suggest that the rDNA in the H genome was lost during the evolution of the tetraploid Elymus. However, the additional H genome present in the hexaploid Elymus with genome formula SSSHH'H' still show active ribosomal genes. Nucleolar organizer activity was also observed in rDNA from both the S and H genomes in artificial SS × HH amphiploids, suggesting no complete nucleolar dominance (amphiplasty) between the S and H genomes.

Key words: Elymus, ribosomal genes, RFLP, nucleolus.


Les unités répétitives des gènes ribosomiques (ADNr) de 18S-5.8S-26S de 15 espèces d'Elymus sud-américaines et de 6 espèces d'Elymus asiatiques et nord-américaines ont présenté, parmi ces espèces, des variations de 8,7 à 11,3 kb. Des variations interspécifiques ont été observées dans les sites de clivage d'EcoRI et de BamHI. Les sites de restriction de BamHI ont été présents dans les sous-répétitions au niveau des espaces intergéniques chez les ADNr d'Hordeum (génome H), mais ils furent absents chez les Pseudoroegneria diploïdes (génome S) et chez presque toutes les espèces d'Elymus tétraploïdes (génome SH). Les espèces tétraploïdes d'Elymus n'ont que deux paires de chromosomes qui possèdent des constrictions secondaires semblables à celles qui ont été observées chez les Pseudoroegneria et un maximum de deux classes différentes d'unités de longueur répétitives. Ces résultats suggèrent que l'ADNr du génome H a été perdu au cours de l'évolution des Elymus tétraploïdes. Toutefois, le génome H additionnel qui est présent chez les Elymus hexaploïdes de formule génomique SSSHH'H' montre, néanmoins, des gènes ribosomiques actifs. L'activité de l'organisateur nucléolaire a aussi été observée dans l'ADNr à la fois des génomes S et H chez des amphiploïdes SS × HH, ce qui suggère l'absence d'une dominance nucléolaire complète (amphiplastie) entre les génomes S et H.

Mots clés : Elymus, gènes ribosomiques, RFLP, nucléoles.

Introduction

The genus Elymus L. has a worldwide distribution and is the largest among the perennial Triticeae. The majority of species (75%) are tetraploids, but hexaploids and octoploids are also found. These allopolyploids show different combinations of the SS genomes from Pseudoroegneria, HH from Hordeum and YY from an unknown diploid (Dewey 1984).

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The SSSH tetraploids have four chromosomes with secondary constrictions and up to four nucleoli per nucleus (Hunziker 1966a; Dubcovsky et al. 1989; Morris and Gill 1987). This number is lower than that expected from the combination of SS and HH diploids, since four chromosomes with secondary constrictions are observed in diploid Pseudoroegneria and two or four are common in the diploid Hordeum (Hsiao et al. 1986).

Gill et al. (1988) assigned the two NOR loci in Elymus trachycaulus (SSHH) to the S genome. Chromosome morphologies (Dubcovsky et al. 1989) and C-banding patterns (Morris and Gill 1987) showed that the chromosomes with secondary constrictions from the tetraploid Elymus corre-
TABLE 1. Materials, chromosome number, maximum nucleoli per nucleus and ribosomal DNA repeat unit length variants in *Elymus* species

<table>
<thead>
<tr>
<th>Species</th>
<th>Country</th>
<th>Accession No.</th>
<th>2n</th>
<th>Maximum No. of nucleoli</th>
<th>rDNA repeat unit length (kb)</th>
<th>IGS BamHI sites</th>
<th>IGS EcoRI site</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>E. agropyroides</em> Presl.</td>
<td>Argentine</td>
<td>657</td>
<td>28</td>
<td>4</td>
<td>10.1, 10.5</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td><em>E. andinus</em> Trin.</td>
<td>Chile</td>
<td>825</td>
<td>28</td>
<td>4</td>
<td>9.7, 10.3</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td><em>E. angulatus</em> Presl.</td>
<td>Argentine</td>
<td>H-6419</td>
<td>28</td>
<td>4</td>
<td>10.2, 10.6</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td><em>E. antarcticus</em> Hook. f.</td>
<td>Argentine</td>
<td>O-230</td>
<td>28</td>
<td>4</td>
<td>9.6, 10.3</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td><em>E. araucanus</em> (Parodi) Löve</td>
<td>Argentine</td>
<td>655</td>
<td>28</td>
<td>4</td>
<td>9.8</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td><em>E. attenuatus</em> Löve</td>
<td>Peru</td>
<td>H-6486</td>
<td>28</td>
<td>4</td>
<td>9.0, 9.3</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td><em>E. breviaristatus</em> ssp. <em>scabrifolius</em> (Döll) Löve</td>
<td>Argentine</td>
<td>B-880011</td>
<td>28</td>
<td>2</td>
<td>9.5</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td><em>E. canadensis</em> L.</td>
<td>U.S.A.</td>
<td>PI-232249</td>
<td>28</td>
<td>4</td>
<td>9.8</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td><em>E. fibrosus</em> (Schrenk) Tzvelev</td>
<td>U.S.S.R.</td>
<td>PI-315493</td>
<td>28</td>
<td>4</td>
<td>9.0, 9.3</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td><em>E. glaucus</em> Buckl.</td>
<td>U.S.A.</td>
<td>PI-232263</td>
<td>28</td>
<td>4</td>
<td>9.6</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td><em>E. mutabilis</em> (Drobov) Tzvelev</td>
<td>U.S.S.R.</td>
<td>PI-314204</td>
<td>28</td>
<td>4</td>
<td>9.2, 9.6</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td><em>E. trachycaulus</em> (Link) Gould</td>
<td>U.S.A.</td>
<td>PI-232156</td>
<td>28</td>
<td>4</td>
<td>8.9, 9.2</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td><em>E. sibiricus</em> L.</td>
<td>P.R. China</td>
<td>PI-499619</td>
<td>28</td>
<td>4</td>
<td>8.7, 9.6</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td><em>P. libanotica</em> (Hackel) Dewey</td>
<td>Iran</td>
<td>PI-401325</td>
<td>14</td>
<td>4</td>
<td>9.4, 9.9, 10.2</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td><em>P. spicata</em> (Pursh) Löve</td>
<td>Canada</td>
<td>PI-236670</td>
<td>14</td>
<td>4</td>
<td>10.4</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td><em>H. violaceum</em> Boiss. &amp; Hohen.</td>
<td>Iran</td>
<td>PI-401390</td>
<td>14</td>
<td>4</td>
<td>9.4, 9.7</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Hybrid 1</td>
<td></td>
<td></td>
<td>28</td>
<td>7</td>
<td>9.8, 10.0, 10.2</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Hybrid 2</td>
<td></td>
<td></td>
<td>28</td>
<td>8</td>
<td>9.5, 10.0</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Hybrid 17</td>
<td></td>
<td></td>
<td>28</td>
<td>7</td>
<td>9.4, 10.0, 10.3</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Hybrid 14</td>
<td></td>
<td></td>
<td>27</td>
<td>7</td>
<td>9.4, 10.0, 10.4, 11.0</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Hybrid 3</td>
<td></td>
<td></td>
<td>28</td>
<td>6</td>
<td>9.2, 10.0</td>
<td>Yes</td>
<td>No</td>
</tr>
</tbody>
</table>

Ribosomal genes are well characterized in many cereals. They are organized as families of repeated genes in tandem arrays. Each repeat unit of rDNA contains a conserved coding region as well as variable intergenic spacer (IGS). This variation is primarily due to variation in the number of subrepeats in the IGS and to a lesser extent to the loss or gain of restriction sites within the IGS (for review see Appels and Honeycutt 1986).

The majority of the *Hordeum* species exhibit one or more BamHI sites in the IGS not found in other Triticeae (Appels et al. 1980; Molnar et al. 1989; Molnar and Fedak 1989). Among the five different patterns of the distribution of BamHI sites described for rDNA in *Hordeum*, the most widely distributed, particularly among the H genome, *Hordeum* was that with additional BamHI sites within the IGS subrepeats.

In the present paper we investigated the presence of these characteristic H-genome additional BamHI sites in the ribosomal genes of some artificial SS × HH amphiploids (Asay et al. 1987) and in natural tetraploid, hexaploid, and octoploid *Elymus*. We also examined the maximum number of nucleoli in these species to infer the number of transcriptionally active ribosomal loci.

### Materials and methods

#### Plant material

Species analyzed are displayed in Table 1, which also shows the collection country and the identification number.
Seeds with PI numbers were provided by Dr. Dewey and Dr. Jensen (U.S.A.), H numbers by Dr. Seberg (Denmark), B numbers by Ing. Duplancic (Experimental Agricultural Station, INTA Balcarce), and O numbers by Lic. Oliva (Experimental Agricultural Station, INTA Rio Gallegos). Identifications provided by the collectors were followed, except for E. cf. agropyroides PI-269646, which was included in E. scabriglumis. The other species were collected by the authors and identified using the keys of Nicora (1978). Lóve’s (1984) nomenclature was followed, but Hordeum was considered sensu Bothmer et al. (1991). Voucher specimens were deposited in the herbarium of the IRB–INTA Castelar (BAB).

The hybrid seeds Pseudoroegneria spicata PI-236670 × Hordeum violaceum PI-401390 sent by Dr. Dewey were obtained after open-pollination of the C1 amphiploids (Asay et al. 1987).

**Chromosome numbers**

Metaphase chromosomes were observed in root meristems pretreated in a saturated solution of p-dichlorobenzene at 4°C for 20 h and fixed in Carnoy’s solution (6:3:1 ethanol–chloroform–acetic acid). After hydrolysis in 1 M HCl at 60°C for 10 min, chromosomes were stained in basic fuchsin and squashed in 2% (w/v) acetic orcein.

**Nucleoli**

Nucleoli were stained with a solution of 1 g of silver nitrate dissolved in 1 mL 0.008% (w/v) sodium citrate in distilled water, in a wet chamber at 60°C. Three or more root tips per plant and young microspores were analyzed and the maximum number of nucleoli was scored. A detailed analysis of nucleolar frequencies, number of chromosomes with secondary constrictions, and karyotypes of the South American Elymus will be published elsewhere (S.M. Lewis, J. Dubcovsky, and A.J. Martinez, unpublished).

**DNA analyses**

Total cellular DNA was isolated from leaves of individual plants following the procedure of Dellaporta et al. (1983). DNA (3 μg) was digested with restriction endonuclease BamHI. Also, EcoRI and SstI digestions were analyzed to estimate the total length of the repetitive unit. Double digestions with EcoRI–BamHI were performed to map additional EcoRI or BamHI restriction sites within the IGS. After electrophoresis in 0.7–1% agarose gels, the DNA was transferred by blotting to Nytran nylon membranes according to supplied instructions.

Plasmid pTA71, with a 8.95-kb EcoRI fragment of wheat rDNA (Gerlach and Bedbrook 1979) was labelled by random priming (Pharmacia, N.J.). Hybridization was performed at 65°C overnight and the final wash was for 15 min at 65°C in 0.2× SSC and 0.1% SDS buffer. HindIII digested bacteriophage lambda DNA was used as the molecular size standard.

**Results**

Digestions of genomic DNA with SstI resulted in an invariant 3.9-kb fragment and variable 4.8- to 7.4-kb fragments. The invariant fragment spends the coding region of the 18S–5.8S–26S rRNA genes and the variant one includes the IGS in all the Triticeae analyzed so far (Appels et al. 1980).

Combinations of two or more different repeat until length classes occurred in 85% of the accessions (Table 1). Highly significant correlation (r = 0.84 p < 0.01) was observed between the maximum number of repeat unit length classes and the maximum number of nucleoli per species (Table 1).
suggesting that in most cases the different rDNA subunits are clustered at specific nucleolar organizer regions.

Only one EcoRI site per repeat unit was present in all species, except for *E. attenuatus* and *E. erianthus*. Though in both species this additional EcoRI site was within the IGS, double EcoRI-BamHI digestions showed differences in the distance between this additional site and the BamHI restriction site located in the 18S ribosomal gene (1.0 kb in *E. attenuatus* and 1.7 kb in *E. erianthus*).

Complex hybridization patterns were obtained with BamHI. All species show a conserved 3.9-kb fragment including the coding region (Fig. 1) and variable 4.8- to 7.4-kb fragments. The BamHI site in the 26S rDNA gene was not accessible to digestion in all repeating units (Appels and Honeycutt 1986) and this results in bands that correspond to the combined size of two adjacent BamHI fragments. Both species of *Pseudoroegneria* (Figs. 1A and 1B) and all tetraploid species of *Elymus*, except for *E. canadensis*, had only two BamHI sites. *Hordeum violaceum* (Fig. 1C) and *E. canadensis* had additional sites in the IGS. The observed BamHI restriction fragments did not add up to the length of the entire rDNA unit (Table 1).

The most plausible explanation is that there is a BamHI site within the IGS subrepeats as suggested by Molnar and Fedak (1989). This hypothesis explains the ladders with approximately 0.1 kb periodicity in *H. violaceum* and *E. canadensis* observed when autoradiographs were overexposed. Additional BamHI sites in the IGS and ladders with the same periodicity were found in all populations of hexaploids *E. patagonicus* and *E. scabriglumis* (Fig. 1, Q-V). Inter- and intra-specific differences were found in the position of the next BamHI site closest to the 26S BamHI (Fig. 1, Q-V).

No additional BamHI sites were found in the hexaploid *E. erianthus* and the octoploid *E. mendocinus* (Fig. 1, W and X).

Five tetraploid plants obtained from open-pollination of C1 artificial SSHH amphiploid between *H. violaceum* and *P. spicata* (Asay et al. 1987) showed the additional BamHI sites in the subrepeats of the IGS (Fig. 1, D-F). Chromosomes with secondary constrictions from *H. violaceum* and *P. spicata* are easy to differentiate (Hsiao et al. 1986) and both were observed in these plants. A maximum of eight nucleoli was found (Table 1).

### Discussion

Natural SSHH tetraploid *Elymus* showed two pairs of satellite chromosomes corresponding to those of the SS genome (Morris and Gill 1987; Gill et al. 1988; Dubcovsky et al. 1989), a maximum of four nucleoli, and no more than two rDNA repeat unit length classes. In natural tetraploids, except for *E. canadensis*, no BamHI restriction sites in the IGS could be detected. These results indicate that the rDNA genes from the H genome in these allotetraploids lost its nucleolar organizer capability and are no longer detectable by DNA hybridization.

A different pattern was observed in the offspring of the artificial amphiploid between *P. spicata* (S genome) and *H. violaceum* (H genome) (Asay et al. 1987). These plants showed up to four repeat unit length classes and additional BamHI sites in the IGS. Chromosomes with secondary constrictions in positions similar to those found in both parents and six to eight nucleoli were observed suggesting that H-genome rDNA genes were not only present but they were also active.

A similar situation was found in the natural tetraploid wheats (AABB genome pairs) where the number of rDNA repeat units from the A genome is so small that they are no longer active and are only detected with very sensitive in situ hybridization techniques (Mukai et al. 1991). On the other hand synthetic AABB tetraploids (Frankel et al. 1987) show no significant reduction of the A genome rDNA after 10 generations and, in addition, the A chromosome rDNA is transcriptionally active.

Genome analysis of the South American hexaploids *E. scabriglumis* and *E. patagonicus* suggested that these species had originated by hybridization of SSHHH tetraploids with diploid species of *Hordeum* (Hunziker 1966b, 1967; Dewey 1972; Seberg 1991). The proposed SSHHH′H′ genome formula is in accordance with the presence of additional BamHI restriction sites in the IGS subrepeats similar to those reported by Molnar et al. (1989) and Molnar and Fedak (1989) for nearly all the natural South American *Hordeum* species. The presence of four pairs of chromosomes with secondary constrictions and a maximum of eight nucleoli indicates that this additional H genome still possesses transcriptionally active ribosomal loci.

Nucleolar organizer activity of ribosomal loci from S and H genomes in the artificial SS × HH amphiploid and the SSHHH′H′ hexaploids suggests no complete nucleolar dominance (ampliploidy) between the S and H genomes.

### Acknowledgements

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Detection of polymorphic loci in Arachis germplasm using random amplified polymorphic DNAs

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The development of easily scoreable genetic markers in Arachis will facilitate the introgression of desirable traits from wild species into adapted germplasm. We have used random amplified polymorphic DNAs (RAPDs) to identify polymorphic molecular markers in a range of wild and cultivated Arachis species. From a total of sixty 10-mer oligonucleotide primers, 49 polymorphic loci were identified between a cultivated A. hypogaea type (TMV-2) and a synthetic amphidiploid (B x C) created from a A. batizocoi and A. chacoense cross. The inheritance of polymorphic markers, both in the amphidiploid and in the F1 progeny in a TMV-2 x (B x C) cross, has also been demonstrated. The potential exploitation of RAPD markers in groundnut improvement programs is discussed.

Key words: groundnut, Arachis species, RAPDs, amphidiploid.


Le développement de marqueurs génétiques pouvant être facilement inventoriés chez l’Arachis devrait faciliter l’introduction de traits désirables d’espèces indigènes dans le plasma germinal d’espèces cultivées. Des segments d’ADN amplifiés au hasard (RAPD) ont été utilisés pour identifier des marqueurs moléculaires polymorphes chez une gamme d’espèces indigènes et cultivées d’Arachis. Pour un total de 60 oligonucléotides promoteurs décamères, 49 locus polymorphes ont été identifiés chez des hybrides entre un type cultivé d’A. hypogaea (TMV-2) et un amphidiploïde synthétique (B x C), ce dernier étant issu d’un croisement entre l’A. batizocoi et l’A. chacoense. La transmission des marqueurs polymorphes, tant chez l’amphidiploïde que chez la descendance F1 d’un croisement TMV-2 x (B x C), a aussi été démontrée. Le potentiel d’exploitation des marqueurs RAPD dans les programmes d’amélioration de l’arachide est discuté.

Mots clés: arachide, espèces d’Arachis, RAPD, amphidiploïde.