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Tatjana Oja
Estonian Agricultural University, Tartu, Estonia

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PRELIMINARY ISOZYME EVIDENCE ON THE HYBRID ORIGIN AND DIPLOID PROGENITORS OF BROMUS PECTINATUS (POACEAE)

Tatjana Oja

Institute of Agricultural and Environmental Sciences, Estonian Agricultural University, 181 Riia, 51014 Tartu, Estonia (tatjana.oja@ut.ee)

ABSTRACT

Isozyme electrophoresis was used to study the origin of Bromus pectinatus (Poaceae, Pooidae). The morphological characteristics of B. pectinatus are intermediate between B. japonicus of sect. Bromus and B. tectorum of sect. Genea. Previous authors have suggested that sects. Bromus and Genea may be linked through B. pectinatus, a species that has been classified in sect. Bromus, though this placement has been questioned. Isozyme data support the allotetraploid nature of B. pectinatus and its position between sects. Bromus and Genea. Several heterozygotes are consistent with the view that B. pectinatus may have arisen through polyploidization of a hybrid between B. japonicus of sect. Bromus and B. tectorum of sect. Genea.

Key words: allopolyploids, Bromus pectinatus, diploid progenitors, isozymes.

INTRODUCTION

Section Genea Dum. of the genus Bromus L. (Poaceae, Pooidae) comprises weedy, annual grasses widely distributed in Mediterranean countries, southwest Asia, and extending to northern Europe (Stebbins 1981; Sales 1993). Stebbins (1981) hypothesized that the genus originated in western Eurasia. Many species of sect. Genea, except the diploid B. fasciculatus C. Presl, are widely introduced in North and South America, Australia, and New Zealand (Mack 1981; Upadhyaya et al. 1986). Some species, e.g., B. diandrus Roth, B. rigidus Roth, and B. tectorum L., have spread rapidly in new areas and become problematic weeds (Kon and Blacklow 1988; DiTomaso 2000; Novak and Mack 2001).

Among the characters provided by Smith (1985) in the description of sect. Genea are spikelets cuneiform at maturity, broader at the apex. Sales (1994) regarded the section as “the most highly evolved in the genus” and still in the process of further specialization with incomplete species differentiation.

Species of sect. Genea often display remarkable inter- and intraspecific variation in morphology. The section includes the following species: diploids (2n = 14) B. fasciculatus, B. sterilis L., and B. tectorum; tetraploids (2n = 28) B. madritensis L. and B. rubens L.; hexa- and octoploids B. diandrus and B. rigidus, each species with two ploidy cytotypes (2n = 42, 56) (Sánchez Anta et al. 1988; Devesa et al. 1990). Polyploidy has thus played a significant role in the evolution of sect. Genea, as well as in other sections of Bromus (Stebbins 1981). Isozymes have been used successfully (Oja and Jaaska 1996; Oja 2002b) to show the allopolyploid nature of the sect. Genea polyploids, and to identify some of their putative diploid progenitors. All three diploid species of sect. Genea have diverged from each other at a number of isozyme loci (Oja and Jaaska 1996). Only single-banded electrophoretic variants attributed to allozymes have been observed in all diploids examined, indicating that autogamy and self-fertilization are characteristic of them (Oja and Jaaska 1996; Oja 2002b). Each sect. Genea polyploid has revealed different fixed heterozygous phenotypes at several heterozygote loci, indicating their independent allopolloid origin from different diploid progenitors (Oja and Jaaska 1996; Oja 2002a; Oja and Laarmann 2002). Relationships among the Genea diploids and tetraploids as evidenced by patterns of isozyme variation (Oja 2002b) are summarized in Fig. 1. Tetraploid B. rubens may be derived from a hybrid of B. fasciculatus and B. tectorum, and tetraploid B. madritensis from a hybrid of B. fasciculatus and B. sterilis. The origin of the B. diandrus rigidus complex has been a matter of prolonged discussion. Many botanists have recognized the taxonomic problems in this complex (Ovadiahu-Yavin 1969; Tzvelev 1976; Böcker et al. 1990; Sales 1993). Previous isozyme investigations (Oja and Jaaska 1996; Oja and Laarmann 2002) support the view of Esnault and Huon (1987) and Sales (1993), that of a single species with different cytotypes. Cugnac (1933, 1937) suggested that B. diandrus is a hybrid between B. rigidus and B. sterilis. Isozymes indicate that of the three diploids only B. sterilis fits well as a genome donor for the B. diandrus/rigidus complex (Oja 2002b). The isozyme data also provide no evidence for the participation of tetraploids B. madritensis and B. rubens in the hybrid origin of the complex (Oja 2002b). The progenitor genotype of the complex may have been missed because of inadequate sampling of B. fasciculatus or, alternatively, because of an unknown or even extinct progenitor.

in sect. *Bromus* (Smith 1970). Sales (1993) further suggested that *B. pectinatus* is closely related to members of sect. *Genea*, and its placement in sect. *Bromus* is questionable owing to its cuneiform spikelets, characteristic of sect. *Genea*. In his study of the *B. pectinatus* complex, Scholz (1981) suggested a hybrid origin for the complex. The morphological characteristics of *B. pectinatus* are intermediate between *B. japonicus* Thunb. of sect. *Bromus* and *B. tectorum* of sect. *Genea*. *Bromus japonicus* is a morphologically highly variable diploid (2n = 14) species distributed in central Asia and the Mediterranean region, and is introduced to America and Australia (Smith 1985). The similarity of *B. pectinatus* to *B. japonicus* is reflected in a nomenclatural combination, *B. japonicus* var. *pectinatus* (Thunb.) Asch. & Graebn. As well, where the taxa are treated as conspecific. *Bromus pectinatus* resembles *B. tectorum* in general morphology and may have taken part in species formation in sect. *Genea*. Descriptions of *B. pectinatus* (Scholz 1981; Sales 1993; Phillips 1995) mention the vegetative vigor of the species, which may be due to its tetraploid nature (2n = 28; Mehra et al. 1968; Smith 1972). *Bromus pectinatus* is a weed species distributed throughout Asia, and introduced in South Africa and Australia (Smith 1972).

This paper summarizes the results of my six previous papers regarding sect. *Genea* allopolyploids and their putative diploid progenitors (Oja and Jaaska 1996; Oja 1999, 2002a, b; Oja and Laarmann 2002; Oja et al. 2003). In addition, new isozyme evidence concerning the allopolyploid *B. pectinatus* and its potential diploid progenitors is included.

### MATERIALS AND METHODS

#### Plant Material

It proved difficult to obtain seed samples of *B. pectinatus*. No botanical gardens provide this species, and even personal requests to researchers in South Africa were not fruitful. I was able to find only three seed accessions, all originating from Belgium, at the USDA Western Regional Plant Introduction Station at Washington State University (Pullman, Washington, USA). Information on the accessions and vouchers is given in Table 1.

#### Isozyme Analysis

Enzyme extracts were prepared from the shoots (primary leaf plus coleoptile) of 5–10-day-old etiolated seedlings, subjected to electrophoresis in vertical polyacrylamide gel slabs as described in Oja (1999) and Jaaska and Jaaska (1986, 1990), and stained for the following enzymes: malate dehydrogenase (MDH, EC 1.1.1.37), shikimate dehydrogenase (SKD, EC 1.1.1.25), 6-phosphogluconate dehydrogenase (PGD, EC 1.1.1.44), aspartate aminotransferase (AAT, EC 2.6.1.1.), superoxide dismutase (SOD, EC 1.15.1.1), peroxidase (PRX, EC 1.11.1.7), and phosphoglucoisomerase (PGI, EC 5.3.1.9). Preliminary analyses of seed progeny (up to ten seedlings per accession) revealed no allozyme variation within accessions, reflecting a selfing breeding system (Oja and Jaaska 1996; Oja et al. 2003). Therefore, 2–4 seedlings per accession were analyzed.

Isozyme results are described at the level of isozyme phenotypes, which correspond to respective genotypes. Therefore, isozyme symbols were used, e.g., AAT-B and AAT-C, instead of respective gene symbols in italics, e.g., Aat-2, Aat-3. Heterozygous phenotypes of dimeric enzymes were distinguished on the gels as symmetrical triplets with codominant isozyme variants as flanking bands, whereas monomeric enzymes showed two-banded heterozygous phenotypes (Wendel and Weeden 1989).

The isozyme nomenclature used, which distinguishes ge-

### Table 1. Seed accessions of *Bromus pectinatus* analyzed in this study from the USDA Western Regional Plant Introduction Station at Washington State University (Pullman, Washington, USA). Accession PI 442454 was misdetermined as *B. japonicus*. Detailed locality data for the accessions are unknown. Vouchers are deposited in TAA, Herbarium of the Institute of Agricultural and Environmental Sciences (Tartu, Estonia).

<table>
<thead>
<tr>
<th>Accession</th>
<th>Origin</th>
<th>Voucher</th>
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<tbody>
<tr>
<td>PI 202531</td>
<td>Belgium</td>
<td>Oja 475800</td>
</tr>
<tr>
<td>PI 442453</td>
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</tr>
<tr>
<td>PI 442454</td>
<td>Belgium</td>
<td>Oja 515800</td>
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</tbody>
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natically heterologous, homoeologous, and homologous isozymes (abbreviated to heterozyms, homoeozymes, and allozymes, respectively), follows Jaaska and Jaaska (1984). The use of different terms is needed to define more precisely the isozymes encoded by different types of genes in allopolyploids. These terms were used in my previous publications (Oja and Jaaska 1996; Oja 1998, 2002a; Oja and Laarmann 2002) describing isozymes in diploid and allopolyploid brome species.

RESULTS AND DISCUSSION

Isozyme variation in *B. pectinatus* and the related diploids *B. tectorum* of sect. *Genea* and *B. japonicus* of sect. *Genea* is summarized in Table 2. All three species share the same isozyme of MDH-B, SKD-A, and PGD-A, reflecting close genetic affinities among them and between sects. *Bromus* and *Genea*. *Bromus pectinatus* revealed fixed heterozygosities at three isozyme loci, AAT-B, AAT-C, and PRX-F, suggesting an allopolyploid origin. Fixed heterozygosity means that the same heterozygous isozyme phenotype is found in all plants without genetic segregation into homoeogamous allozyme phenotypes. Fixed heterozygosity of isozymes in allopolyploids has been frequently used as a criterion to distinguish them from autopoloids and to identify their diploid progenitors (e.g., Jaaska 1969, 1976; Barber 1970; Jaaska and Jaaska 1970, 1984; Roose and Gottlieb 1976; Watson et al. 1991). Fixed heterozygosities of allopolyploids combine additively electrophoretically divergent and codominantly expressed isozymes of diploid parents, allowing their identification. Allozymes of a heterozygote that differ electrophoretically in diploid parents become two codominant homoeozymes encoded by homoeologous loci (of composite genomes) of an allotetraploid (Jaaska and Jaaska 1984). Thus, the PRX-F/S, AAT-B/4, and AAT-C/3 heterozygosities indicate that *B. tectorum* of sect. *Genea* with species-specific PRX-F5, AAT-B5, and AAT-C1/3 heterozygosities analyzed in this study, this is only a preliminary hypothesis.

Three chromosome count reports point to two ploidy levels in *B. pectinatus*, 2n = 28 (Mehra et al. 1968; Spiess et al. 1999) and 2n = 14 (Moinuddin et al. 1994). All three accessions included in this study are allotetraploids based on the fixed heterozygous isozyme phenotypes.

Isozyme data thus evidence the allotetraploid nature of *B. pectinatus* and its intermediate position between sects, *Bromus* and *Genea*, confirming the suggestion of Scholz (1981) and Sales (1993) that the sections may be linked through *B. pectinatus*. Fixed heterozygosities of AAT-B, AAT-C, and PRX-F are consistent with the view that *B. pectinatus* may be the result of the hybridization between *B. japonicus* and *B. tectorum*, followed by the polyploidization of the hybrid. However, variation in allozymes PGD-A and PGI-A is not consistent with this hypothesis because PGD-A3 of *B. japonicus* and PGI-A6 of *B. pectinatus* were not found in *B. pectinatus*. There are several possible explanations for this result. First, it is possible that the parental diploids that contributed genomes have evolved since the polyploid formation with the appearance and distribution of new allozymes. Second, homozygous phenotypes of PGD-A and PGI-A of *B. pectinatus* may have arisen through a genome-specific silencing of PGD-A3 and PGI-A6 due to mutation to null-alleles during or shortly after the formation of the initial allopolyploid. Alternatively, PGD-A3 and PGI-A6 might remain undetected in *B. pectinatus* due to insufficient sampling. The putative phylogenetic position of *B. pectinatus* as evidenced from the isozyme data is summarized in Fig. 1. A wider isozyme survey of *B. pectinatus* is necessary for better understanding the origin of the species and the relationship between the two sections, including whether the sections are even valid, monophyletic sections.

ACKNOWLEDGMENTS

The research was supported by grant 5739 from the Estonian Science Foundation. Valuable comments by Dr. V. Jaaska are greatly appreciated. I am grateful to the USDA Western Regional Plant Introduction Station for the seed accessions used in this study.

LITERATURE CITED


