PATTERNS OF FLOWER MICROSTRUCTURAL VARIATION WITHIN THE GENUS *BROMUS*

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ABSTRACT

Twenty five species from four sections of the genus *Bromus* were evaluated by means of numerical analyses of characters of flower microstructures (lodicule, lemma, palea). Special development of lodicules (lobe and hairiness) and both glumellae (lemma and palea) similar in grasses evolutionarily close (Bromeae, Brachypodieae, Triticeae) was discovered. For most of the characters the original interspecific and intersectional relations were observed in a space of minimum spanning tree (MST). The evolutionary old section *Pnigma* occupies the largest space. Species of *Pnigma* having small chromosomes can be distinguished from those having large ones. The section *Ceratochloa* is scattered through the smallest space. The above points to the endemic evolution of high polyploids of the section. The sections *Bromus* and *Genea* are evolutionarily close and they distinctly overlap each other in the MST space. *Bromus sterilis*, *B. catharticus* and *B. riparius* are extremes in their own sections.

KEY WORDS: *Bromus*, flower microstructure, variation, numerical taxonomy.

INTRODUCTION

Studies of plant microstructures are significant for consideration of relations between units of the same or different taxa. They supplement the taxonomic description based on macro-morphology. Observation of microstructural ontogeny, especially in natural or induced mutants, is important for concluding about the evolution of a given character and its state (plesio- or apomorphy). Microstructures of the flower such as lodicules, glumes and glumellae were analysed in some grasses, however, it has not been done for the genus *Bromus*. Baum (1971) considered the evolution of lodicule in cultivars of *Avena sativa* and *A. byzantina*. He used also the characters of lodicule for phylogenetic analysis of the tribe Triticeae (Baum 1983). A more detailed study of lodicule morphology was made for the genus *Triticum* (Fritsch et al. 1977; Kosina 1999). Harlan (1945) found changes in lodicule morphology in *Bromus carinatus* depending on breeding behaviour of the flowers. Morphology of epidermis of inflorescence bracts was analysed in various grasses (Clark, Gould 1975; Hayward, Parry 1980), but it has not been done for the genus *Bromus*, especially by means of numerical methods. The characters of epidermis were applied in a study of fossil material (Tubb et al. 1993; Kosina 1996a) and their taxonomic value was considered for the North American species of *Festuca* (Consaul, Aiken 1993). The goal of the present paper is to describe the interrelations between species and between sections of *Bromus* making use of microstructural characters. The analyses made with application of methods of numerical taxonomy (phenetics) are basic for the advanced cladistic analysis de-

scribing the phylogenetic status of the tribe. The evolution of the tribe Bromeae progressed differently in the Old and New World (Armstrong 1991) and correlated with genome amplification. One ought to put the question – What is the relation between micromorphological variation and data of other kinds? Previously some correlations between the data from morphology, biochemistry and cytogenetics were found in the genus *Bromus* (Kosina 1996b).

MATERIAL AND METHODS

Three groups of morphological characters describing flower microstructures (lodicules, lemma, palea) were studied in random samples ($n = 30$) of 25 species of the genus *Bromus*. The species belonged to four sections of the genus:

A. Section *Genea* – *Bromus rubens* L. (Bru), *B. sterilis* L. (Bst), *B. madritensis* L. (Bma), *B. diandrus* Roth (Bdi), *B. tectorum* L. (Bt);

B. Section *Bromus* – *Bromus arvensis* L. (Bar), *B. secalinus* L. (Bse), *B. japonicus* Thunb. (Bj), *B. commutatus* Schrader (Bco), *B. squarrosus* L. (Bsq), *B. scoparius* L. (Bsc), *B. lan- ceolatus* Roth (Bl), *B. danthoniae* Trin. (Bda);

C. Section *Pnigma* – *Bromus purgans* Hook. (Bpr), *B. ramosus* Hudson (Bra), *B. riparius* Rehmman (Bri), *B. erectus* Hudson (Be), *B. benekenii* (Lange) Trimen (Bbe), *B. pumpel- lianus* Scribn. (Bpu), *B. ciliatus* L. (Bci);

D. Section *Ceratochloa* – *Bromus brevis* Steud. (Bbr), *B. catharticus* Vahl. (Bca), *B. sitchensis* Trin. (Bsi), *B. aleuten- sis* Trin. (Bal), *B. marginatus* Ness (Bmr).

Taxonomic treatment of the accessions was followed after Smith (1980) and supplemented according to Muntz, Keck (1959), Hultén (1968), Tzvelev (1976) and Stebbins (1981).

Accessions were obtained from collections in Karlsruhe, Konstanz, Leipzig, Gatersleben, Genova, Liegé, Christchurch, Palmerston North, Jalta, Stavropol and Buenos Aires, and some of them were also gathered from natural localities in Poland (*B. sterilis*, *B. tectorum*, *B. secalinus*, *B. erectus*, *B. marginatus*). The material was cultivated in equal environmental conditions in the author's grass collection in the Botanical Garden of Wrocław University. Taxonomic identification of accessions was checked personally by the author and consulted in part with Professor G.L. Stebbins. The experimental design was a one-way, completely randomized classification. Mature spikelets were fixed in FAA. Lodicules and glumellae were excised from the first two flowers and were observed under a light microscope, often with use of polarized light. The following characters were studied:

- lodicales: width (W), length (L), shape (S), hairiness (H);
- morphology of abaxial epidermis in lemma (l) and palea (p): amplitude of sinusoid wall (ASWl, ASWp), diameter of papilla (DPl, DPp), width of long cells (WLCl, WLCp), number of meristemoid events (NMI, NMP);
- meristemoid pattern of abaxial epidermis in lemma (l) and palea (p) and taken in the intercostal region: number of single short cells (Sl, Sp), number of cell diads – cork cell + silica cell or another arrangement (DI, Dp), number of cell triads – papilla + cork cell + silica cell or another arrangement (TI, Tp), number of all meristemoid events – the events mentioned above and others (NMI, NMP).

Fig. 1. Dendrograms for three sets of characters based on Pearson's coefficient of correlation. **a** – morphological characters of lodicule; **b** – morphological characters of abaxial epidermis of lemma (l) and palea (p) as well as sums of meristemoid events in each bract; **c** – characters of meristemoid activity in abaxial epidermis in lemma (l) and palea (p).

All the abbreviations given in parentheses were used in the text and diagrams. Data were analysed with application of the software NT-SYS v.1.8 (Rohlf F.J. 1994). Character correlations were presented in the form of dendrogram. Numerical data for taxa were evaluated with the use of different similarity measures and methods of clustering. The best clustering was established by means of cophenetic correlation.

RESULTS

Correlations between characters

A dendrogram of lodicule characters (Fig. 1a) is composed of the two weakly correlated clusters, i.e. dimensions of the organ and its shape plus hairiness. Width of lodicule was rejected from the set of characters describing the species. A correlation analysis of epidermal characters in lemma and palea (Fig. 1b) presents strong interrelations of morphological characters within each bract as well as the independence between morphology and meristemoid activity of the epidermis. Characters highly correlated with the amplitude of sinusoid wall as well as with indices of meristemoid activity were removed to consider the similarities between species. An analysis of meristemoid states in abaxial epidermis of the inflorescence bracts showed that in lemma the development of single cells (papillae, hooks or hairs) was the main event, while in palea the cell triads were most frequent (Fig. 1c). The lemma triads and single cells in palea are weakly correlated with all the meristemoid events. Diads of each bract create separate clusters.

Interrelations between species or sections

In a study of taxa matrix based on lodicule morphology the highest cophenetic correlation ($r_{\text{coph}} = 0.97$) was obtained for the Canberra metric. Connections between species are presented in a space of the minimum spanning tree, MST (Fig. 2). The sections *Genea* and *Bromus* are separated from *Ceratochloa*. *Bromus brevis* (Bbr) is connected with *B. sterilis* (Bst), however, if the character of "hairiness" is rejected, this species joins again *B. aleutensis* (Bal) and in a dendrogram (UPGMA, unweighted pair-group method, arithmetic average + Canberra metric) creates one distinct cluster of *Ceratochloa* (Bbr, Bsi, Bal, Bmr). The annual species of this section, *B. catharticus* (Bca), is distant from the cluster and is close to *B. ramosus* (Bra). All the sections, excluding *Ceratochloa*, occupy large parts of the tree. The variations of *Bromus* and *Genea* sections overlap in part. Most of the annual species have short and narrow lodicules while the perennials have a little longer and rather round ones. There are some exceptions from this picture, i.e. *B. riparius*, *B. diandrus*, *B. pumpellianus*, *B. commutatus*, *B. catharticus* (Bri, Bdi, Bpu, Bco, Bca).

When morphological characters of epidermis were applied for the ordination of taxa the highest cophenetic correlation ($r_{\text{coph}} = 0.87$) was obtained for the set "UPGMA + average taxonomic distance". In MST (Fig. 3) there are clear links between species of *Genea*. The very distant position of *B. catharticus* (Bca) is a result of the very large amplitude of sinusoid wall and diameter of papilla. The above characters are less useful for taxonomy of *Bromus* (compare cophenetic correlations).

Application of meristemoid events gives the highest cophenetic correlation ($r_{\text{coph}} = 0.87$) for the UPGMA and average Manhattan distance. The section *Ceratochloa* is distinguished in MST as a compact group (Fig. 4). The species of *Genea* have immediate links but they occupy a large space of

the tree. *B. diandrus* (Bdi) and *B. catharticus* (Bca) are exceptions in both sections. Some species of the *Bromus* section have also such links, e.g. *B. commutatus*, *B. danthoniae*, *B. secalinus*, *B. scoparius* and *B. arvensis*, *B. squarrosus* (Bco, Bda, Bse, Bsc, Bar, Bsq). The section *Pnigma* has the largest variation. Its species are scattered, sometimes extremely.

DISCUSSION

Relations between characters

A description of the microstructures analysed above was often given in the world literature, but not for brome-grasses. Especially, it was not done by means of the numerical approach considering characters and taxa relationships separately. Relations between characters of the lodicule are similar to those in the tribe Triticeae (Kosina 1996c). This shows the two-stage development of lodicule, i.e. creation of lobe (or lobes) and development of hairs and stomata. These two developmental events would be independent. Correlations of the morphological characters of inflorescence bract epidermis are similar to those in the genus *Brachypodium* (Kosina 1996d). The meristemoid pattern of abaxial epidermis is characteristic for species of *Bromus* and differs from, e.g. the pattern in *Brachypodium*. For both genera, *Bromus* and *Brachypodium*, there are various interrelations of characters of this pattern, but generally the correlations are rather weak in both groups. This proves that information in each character is important for description and discrimination of species. Frequency of meristemoid events is different through the surface of lemma

or palea and depends probably on distribution of vascular tissue and phytohormones in the organ. One should expect that the meristemoid pattern for the bract as a whole is different in various species or sections. Such a situation may result from the occurrence of a given type of short cells only within a limited area as well as from the change of the organ, e.g. reduction of palea into a papery structure with low meristemoid activity as in *Ceratochloa*. The interrelations of characters presented here show the common developmental way in grasses treated as a large evolutionary unit. This concerns at least the tribes evolutionarily close, e.g. Bromeae, Triticeae, Brachypodieae, and proves the special development of organs: lodicule lobe – its hairiness, developmental independence of both glumellae (morphology of epidermis), originality of meristemoid pattern (frequency of events, types of cells, pattern of their distribution).

Interrelations of species and sections

Distribution of species in the MST space, when three groups of characters are used, is partly similar. In the *Genea* section, *Bromus rubens*, *B. madritensis* and *B. tectorum* are especially close and have immediate links. *B. sterilis* is distant from this group when described by characters of epidermis. Similar interrelations between taxa were found for the embryo morphology (Kosina 1996b) and brome-grass karyotypes (Naganowska 1993). *B. diandrus* has an intermediate link with the above group and such a picture exists also for the embryo morphology (Kosina 1996b). The section *Ceratochloa* forms a compact set often with immediate links between species, however, the annual *B. catharticus* is always

Fig. 2. Minimum spanning tree (MST) for 25 species of the genus *Bromus* described by lodicule morphology with use of the Canberra metric. For Figs 2, 3, 4: a, b, r are the parameters of arrangement of the diagram in the 3-D space.

Fig. 3. Minimum spanning tree for 25 species of *Bromus* described by morphology of abaxial epidermis in lemma and palea (with exclusion of sums of-meristemoid events) using the average taxonomic distance.

Fig. 4. Minimum spanning tree for 25 species of *Bromus* described by characters of meristemoid activity of abaxial epidermis in lemma and palea using the Manhattan distance.

distant from other relatives. This is confirmed by the anatomy of caryopsis (Kosina, unpublished). When the brome-grasses are described by two characters (shape and length of lodicule) then they are separated well enough in two groups, annuals versus perennials. This behaviour depends probably on the biology of flowering (self- or cross-pollination). Differences in lodicule morphology in cleisto- and chasmogamous flowers are well known in *Bromus carinatus* (Harlan 1945). The above is also well expressed in the dimorphism of lodicules in *B. brevis* from the same section. Easy discrimination of *B. catharticus* described by a set of characters of the flower organs proves the correlated development of "the flower cavity" noted by Millet (1988) in wheat. Stebbins (1981) and Armstrong (1991) suggest that the section Pnigma may be divided in two groups of species, evolutionarily older with small chromosomes from Eurasia and younger with larger ones from America. The complex *B. benekenii*-*B. ramosus* from Eurasia with large chromosomes may here be an exception and a link between species from the Old and New World (Armstrong 1991). Both these groups *B. benekenii*, *B. ramosus*, *B. purgans*, *B. ciliatus* versus *B. erectus*, *B. riparius*, *B. pumpellianus* (Bbe, Bra, Bpr, Bci vs. Be, Bri, Bpu) are well separated by the meristemoid pattern of epidermis. Species having large chromosomes (Bci, Bra, Bpr, Bbe) are relatively uniform in relation to the shape and length of lodicule and are linked directly, which may point to similarity in their flowering behaviour. However, *Bromus erectus* is also close them and shows more complex relations. The section Pnigma, considered as an old evolutionary unit, for the lodicule morphology and epidermal meristemoids occupies a large space in MST. This space overlaps considerably with other sections which may depend on the creative evolutionary role of Pnigma (Stebbins 1981). The section Ceratochloa is located in the smallest MST space (see lodicules and meristemoids) which may result from the endemic evolution of its highly polyploidized members. Distinct overlapping of areas of the section Genea and Bromus and discrimination from Ceratochloa point to their similar evolution. A set of characters describing the morphology of epidermis does not distinguish the sections enough, however, in the given diagram immediate links between members of Genea are distinct. These characters correlate probably with the xeric nature of the plant. To consider properly the evolution of the tribe one should study the variation of all the types of characters in relics and endemics seen as relatives of the brome-grasses. This should be done for the endemic genera, *Megalachne* from the Juan Fernández Islands, *Littledalea* from the mountains in Central Asia, *Bromus trinii* from the Pacific coast of both Americas, *Pseudodanthonia*, *Metcalfia*, *Sinochasea* and also for *Brachypodium* and the tribe Aveneae (Stebbins 1981).

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WZORY MIKROSTRUKTURALNEJ ZMIENNOŚCI KWIATU W RODZAJU *BROMUS*

STRESZCZENIE

Relacje między gatunkami czterech sekcji rodzaju *Bromus* opisanych cechami mikrostruktur kwiatu (łuszczyki, plewki) analizowano metodami taksonomii numerycznej. Odkryto charakterystyczny rozwój łuszczyków oraz plewek w plemionach traw ewolucyjnie bliskich (Bromeae, Brachypodieae, Triticeae). Oryginalne stosunki między gatunkami oraz sekcjami obserwowano w przestrzeni drzewa minimalnych odległości (minimum spanning tree). Stara ewolucyjnie sekcja *Phyma* zajmuje w drzewie największą przestrzeń. Gatunki tej sekcji z małymi chromosomami są oddzielone od tych z dużymi chromosomami, w szczególności, gdy opisane są cechami merystemoidów odosiowych epiderm plewek. Sekcja *Ceratochloa* zajmuje najmniejszą przestrzeń, co potwierdza endemiczną ewolucję wysokich poliploidów tej sekcji. Sekcje *Genea* i *Bromus* są sobie ewolucyjnie bliskie i ich zasięgi w analizowanej przestrzeni znacznie się pokrywają. *Bromus sterilis*, *B. catharticus* i *B. riparius* są skrajnymi taksonami w ich sekcjach.

SŁOWA KLUCZOWE: *Bromus*, mikrostruktury kwiatu, zmienność, taksonomia numeryczna.