## BIOSYSTEMATIC STUDIES IN BRACHYPODIUM (POACEAE)

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by<br>Mir Ajab Khan<br>B.Sc., M.Sc.

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## ABSTRACT

## BIOSYSTEMATIC STUDIES IN BRACHYPODIUM (POACEAE)

Mir Ajab Khan

The present study on the genus Brachypodium has been carried out from a biosystematic view-point. The following taxa were used: B. sylvaticum, B. glaucovirens, B. pinnatum, B. rupestre, B. phoenicoides, B. retusum, B. mexicanum and B. distachyon. All the species are perennial and chasmogamous except B. distachyon, which is annual and only partially chasmogamous.
B. sylvaticum, which is the most widespread species of the genus, overlaps most of its characters with those of B. pinnatum and B. glaucovirens. This and other taxonomic problems were investigated by the study of morphology, anatomy, cytology, seed-protein band analysis and artificial hybridisation.

The main morphological variations which were analysed are the pubescence of plants; leaf-blade length, width and colour; leaf-blade rib shape; and lemma awn length. Special attention was paid to wild-collected plants intermediate in appearance between various pairs of species.

Species of the genus Brachypodium have been artificially hybridized in this study for the first time; most crosses were successful in producing $F_{1}$ hybrids. The degree of success was found to be a valuable new taxonomic character in the genus. Natural hybridization was shown to occur in some cases, but seems to be very rare.

A complex situation of intraspecific chromosome number variation exists in the genus. Chromosome counts with their locality and country of origin are given in Appendix 1. B. pinnatum with $2 \mathrm{n}=36$ was found for the first time. The evolution of chromosome numbers in the genus is discussed; the basic chromosome number seems to be 9 .

Meiosis is regular in the species but in some wild intermediates it is slightly irregular. Chiasma frequency is highest in B. sylvaticum and B. glaucovirens and lowest in B. phoenicoides. Synthetic hybrids could be divided into three groups according to their meiotic behaviour, from slightly irregular or almost regular to very irregular.

The evolution of Brachypodium and a summary classification of the representatives used in this study is presented in the Discussion. It is concluded that B. distachyon should remain in the genus Brachypodium in a separate section from all the other species. Brachypodium sylvaticum, B. glaucovirens, B. pinnatum, B. phoenicoides, B. retusum, and B. mexicanum are to be considered to be separate species, but $B$. rupestre should be relegated to varietal rank under B. pinnatum.

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### 1.1.1: Grasses

Grasses are the group of plants which inhabit more regions of the earth than any other group of plants. They are adapted to various habitats from the tropics to the tundra, from marshes and swamps to the deserts, and from mountain summits to the sea. Their widespread distribution on the earth is probably due to their several lines of parallel and convergent evolution, and to many trends of adaptation having been reversed. Grasses are the most beneficial group of all plants. Cereals have been vital to mankind since the very earliest civilizations.

1. Grasses provide food for living because they include such staple food producing plants as wheat, oats, barley, rye, rice, maize, sugar-cane, bamboo and the various millets. Moreover, they provide food indirectly in the form of fodder for herbivorous animals.
2. Most fertile and productive soils of the earth were developed under a cover of grasses. Because of their often stoloniferous or rhizomatous habit they prevent soil erosion. Grasslands provide one of the most important habitats for wild-life, forming the basis of many food-chains.
3. Grasses are used for many non-food purposes by man such as building (straw, bamboo), weaving (clothes,
baskets), ornaments (floral displays, beads) and perfume (lemon grass).
4. They provide aesthetic and recreational uses such as turf for lawns and sports grounds.

The saying, "All flesh is grass" is not only metaphorical but literal (Isaiah l000BC).

It is the need of the day to increase crop production for cereals and forage, and to improve the quality of turf grasses for lawns and sports. Plant breeders are interested to know the wild ancestors of grass crops since they may have useful characters to be introduced for vigour, disease resistance and high protein value, etc. Therefore a detailed study of the grasses is vital, and much remains to be discovered about this extremely adaptable family.

At the generic level, Compositae and Orchidaceae are larger than the Gramineae, while in number of species the Gramineae take fourth place after Leguminosae, Compositae and Orchidaceae. There are an estimated 620 genera and 10,000 species of grasses (Willis 1973). Taxonomically, the Gramineae are a difficult family. Stebbins (1972) pointed out that "grasses have a reputation for being taxonomically difficult. Biosystematic study is necessary to understand the Gramineae because morphologically they provide limited taxonomic information because of the reduced inflorescence and rather uniform vegetative parts".

temperate regions.
Palisot de Beauvois (1812) named and described
a large number of grass genera and recognized the grass family as the best known of the higher plants. Kunth (1833) distinguished 13 tribes but recognized no subfamilies. Fries (1846) recognized Clisanthées and Euryanthées as his two main groups of grasses. Bentham (1881) divided Brown's Paniceae (as Panicaceae) into 6 tribes and his Poaceae into 7 tribes, and further divided some of the tribes into sub-tribes. Harz (1880) recognized three main groups of grasses: Frumentaceae, Sacchariferae and Fragmitiformes.

A phylogenetic system of classification of the grasses was presented first by Avdulov (1931) and elaborated in various versions by Pilger (1954), Tateoka (1957), Prat (1960), Stebbins \& Crampton (1961), Parodi (1961), Jacques-Felix (1962), Potztal (1964), Gould (1968), Clayton (1978), and Hilu \& Wright (1982); as shown in Table 1.1/1. Most of these workers claimed their classifications to be phylogenetic systems but actually this is largely not so. Hubbard (1966) recognized 59 tribes which he placed in 19 'groups' which were not named or given a precise taxonomic rank.

### 1.1.3: Phylogeny of Grasses

Efforts have often been made to trace the descent of present-day grasses but the fossil record for grasses is so limited that it is almost negligible as

## TABLE 1.1/1

Major classifications of grasses since Avdulov

| Avdulov | Pilger | Tateoka | Prat |  |
| :--- | :---: | :---: | :---: | :---: |
| (1931) | $(1954)$ | (1957) | $(1960)$ | Crampton |
|  |  |  | (1961) |  |
|  |  |  | Parodi |  |
|  |  |  |  |  |
|  |  |  |  |  |


| Poatae | Panicoideae | Panicoideae | Festucoidées | Panicoideae |
| :---: | :--- | :--- | :--- | :--- |
| Phragmitiformes | Andropogonoideae | Eragrostoideae Panicoidées | Eragrostoideae |  |
| Festuciformes | Eragrostoideae | Pooideae | Chloridoidées | Festucoideae |
| Sacchariferae | Festucoideae | Arundinoideae | Bambusoidées | Arundinoideae |
|  | Bambusoideae | Pharoideae | Oryzoidées - | Bambusoideae |
|  | Olyroideae |  | Olyroidées | Oryzoideae |
|  | Oryzoideae |  | Phragmitiformes |  |


| Jacques- | Potztal | Gould | Clayton |  |
| :--- | :--- | :--- | :--- | :--- |
| Felix | $(1964)$ | $(1968)$ | $(1978)$ | Wright |
| $(1962)$ |  |  | $(1982)$ |  |


| Panicoide | Pooideae | Festucoideae | Bambusoideae | Festucoideae |
| :--- | :--- | :--- | :--- | :--- |
| Chloridoide | Micrairioideae | Panicoideae | Centothecoideae Nardoideae |  |
| Festucoide | Eragrostoideae | Eragrostoideae | Arundinoideae | Oryzoideae |
| Arundinoide | Oryzoideae | Bambusoideae | Chloridoideae | Arundinoideae |
| Bambusoide | Olyroideae | Oryzoideae | Panicoideae | Centothecoideae |
| Oryzoide | Panicoideae | Arundinoideae | Pooideae | Panicoideae |
| Stipoide | Andropogonoideae |  | Eragrostoideae |  |
| Streptogynoide | Bambusoideae |  | Bambusoideae |  |
| Ehrhartoide | Anomochlooideae |  |  |  |
| Olyroide |  |  |  |  |
| Zizanioide |  |  |  |  |

compared to their large number of genera and species in the world today. Grasses probably came into being in the Mesozoic, after flowering plants were well diversified. The first reported grass-like fossils are from the Upper Cretaceous, and the first definitely known grass fossils are from late Tertiary rocks of Europe (Gould 1968). Carbonized grass fruit from Tertiary deposits of the Florissant beds of Colorado were assigned to Stipa by Cockerell (1908). Hutchinson (1934) suggested that the grasses along with the sedges have been derived from Liliales through the Juncaceous stock. Lawrence (1969), however, did not regard the Gramineae and Cyperaceae as closely related. He noted that grasses have terminal flowers whose ovaries probably evolved from ancestral types with parietal placentation, whereas sedges have axillary flowers whose ovaries evolved from types with free central placentation. The Cyperaceae are more like the Juncaceae than the Gramineae. Ziegerspeck (1938) believed that the parental line from Liliales divided immediately into two branches, one giving rise to Commelinaceae and Gramineae and the other to Juncaceae and Cyperaceae. From the first line Commelinaceae branched off, leaving the ancestral stock of Gramineae, which after reduction and specialization produced the Gramineae in its present form. Stebbins (1956) suggested the relationship of grasses to be with the families Flagellariaceae and Restionaceae.

Sharma (1979) suggested that in response to the forces of evolution and natural selection grasses have added some parts and eliminated others to produce an extraordinary mosaic of characters. He suggested that progenitors of the present day grasses were probably herbaceous forest grasses which now seem extinct but lived in the Cretaceous, judging from the few fossil data that are arailable for grasses. The characters of those grasses are now found in Bambuseae, Phareae and some members of Oryzeae.

Avdulov (1931) suggested that the phylogenetic tendencies within the Gramineae have been from high basic number and small sized chromosomes to low basic number and large chromosomes. The series Phragmitiformes (including tribes Arundineae and Bambuseae) with high basic numbers is primitive, and genera with low basic numbers (many of his Festuciformes) are more advanced. Bews (1929), Prat (1936) and Stebbins (1956) regarded the Bambuseae as the most primitive tribe within the family Gramineae. The Bambuseae have three lodicules; six stamens; perfect, many flowered spikelets; woody, perennial stems; articulate leaves; awnless glumes and lemmas; and high numbers of chromosomes of small size. Beetle (1955) regarded Phareae as the most primitive of all tribes. The Phareae have well-developed lodicules, six stamens, perfect spikelets, a herbaceous perennial habit, petiolate leaves, and a tropical distribution. Tateoka (1957) placed Phareae and Oryzeae


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in his primitive group. The members of the Oryzeae have a one-flowered spikelet and some members like Hygroryza, Oryza and Leersia possess six stamens. The Oryzeae also possess high chromosome numbers of small size. Sharma (1979) suggested courses of evolution and phylogenetic alliances in the Gramineae based on cytological, anatomical and physiological characteristics, as shown in Fig.1.1/1.


### 1.1.4: Morphology

Morphological characters provide useful information for the recognition of all levels of taxonomic ranks (families, genera and species, etc.). Many taxa of flowering plants have been distinguished on the basis of only morphological characters. Inflorescence and flower characters are generally considered to be more reliable than vegetative characters in grass systematics. Floral characters, such as the type of inflorescence, number of spikelets per inflorescence, number of florets per spikelet, position of sterile or rudimentary florets, nature of callus, spikelet length, pedicel length, glume length, lemma length, number of veins on lemma and glumes, awn length, position of awn on lemma, glume/lemma length ratio, lemma/palea length ratio, number and length of anthers, size and shape of caryopsis, and nature of hilum have been used to recognize various grass taxa.

However, vegetative characteristics, such as plant habit (annual or perennial, nature of rhizome, etc.),


* $\mathrm{C}_{4}$ TYPE OF PHOTOSYNTHETIC PATHWAY. A SINGLE SHEATH SURROUNDING THE VASCULAR BUNDLES.
$* \mathrm{C}_{3}$ TYPE OF PHOTOSYNTHETIC PATHWAY. TWO SHEATHS SURROUNDING THE VASCULAR BUNDLES.
Figure l.l/l: Phylogenetic alliances in the Gramineae based on
characteristics, according to Sharma (1979)
culm height, number of nodes, leaf-blade length and width, pubescence of leaf, degree of fusion of leafsheath, and nature of ligule have been widely used in species differentiation. The use of floral and vegetative morphology collectively is an essential basis for a complete and natural plant classification.


### 1.1.5: Anatomy

Grass anatomy was first used for systematic work by Duval-Jouve (1875). While studying the transverse sections of grass leaves, he used the character of the position of bulliform cells in relation to the vascular bundle for identification purposes. Schwenderer (1890) studied the nature and arrangement of cells surrounding the vascular bundles. Pée-Laby (1898) surveyed the distribution of sclerenchyma, bulliform cells and chlorenchyma in grass leaves. In the twentieth century many anatomical characters have been utilized by grass taxonomists. Avdulov (1931) related several anatomical characters to his cytological data.

Variations in the leaf epidermal morphology are used extensively in the classification and identification of grasses and in studies on their phylogenetic relationships. Grob (1896) and Pée-Laby (1898) were the first to study epidermal anatomy. Prat (1932, 1936, 1948, 1951) stated that the epidermal cells of the Gramineae have a higher degree of specialization than in any other family. The most conspicuous features of grass leaf epidermis are
that the cells occur in longitudinal rows and exist in two main types, long-cells and short-cells. Prat described in detail many aspects of these two types of cells, including the differences in the shape of silicacells (one type of short cell), of the festucoid (pooid), panicoid, chloridoid and oryzoid grasses. He noted the absence of bicellular micro hairs in pooid grasses and the differences between typical panicoid and chloridoid micro-hairs. Metcalfe (1960) surveyed the accumulated literature on the systematic value of grass vegetative anatomy, with special reference to the epidermis, and presented many new data as well. This is the standard reference work on the subject, and Metcalfe's terminology of anatomical features is followed in this thesis.

In a longitudinal row of epidermal cells, it will be seen that short cells may be solitary, in pairs, in rows of $3-5$ cells or in a row of more than 5 cells. When short-cells occur in groups it is important to determine the relative arrangement and frequency of cork-cells and silica-cells in each row. In grasses silica cells may or may not be associated with cork-cells; in other siliceous plants (i.e. other than grasses) there are no cork-cells (Lawton 1980). The shapes of silica-cells and the enclosed silica bodies are important for taxonomic purposes. Sangster (1970) stated that the silica-body pattern may be affected by various environmental and ontogenetic factors, and the pH of the soil and the relative availability of silica can result in larger or smaller silica bodies. In Pooideae silica-cells are
usually rounded, elliptical, long and narrow or crescentshaped. In Panicoideae silica-cells are dumb-bell-shaped, cross-shaped or saddle-shaped. Silica-cells are mostly cross- or saddle-shaped in Eragrostoideae, and in Bambusoideae mostly cross-, saddle- or dumb-bell-shaped. In some cases abaxial epidermis characters have been found more helpful to separate species and genera.

Church (1949) used the character of intercostal
long-cells with smooth walls to distinguish Glyceria from Puccinellia and Torreyochloa, in which the corresponding cells have undulate walls. S申rensen (1953) also found epidermal features useful in identifying puccinellia. Borrill (1961a) recognized a new species, Dactylis marina Borrill, in which the epidermal cells differ in shape from those of other tetraploid members of the complex and bear conspicuous papillae. Stomal length and frequencies have also proved useful in separating diploid subspecies of Dactylis glomerata L. Claustres \& Huon (1965) and Huon \& Redon (1967) investigated the relationship between Festuca rubra $L$. var. arenaria (Osbeck) Fr. and F. juncifolia St. Amans, and suggested that quantitative and qualitative differences exist between them. Shortcells in F. juncifolia on costal and intercostal regions were found to be cork-cells whether they occurred singly or in pairs. Long cells of the intercostal region were measured as c. $120 \mu \mathrm{~m}$. F. rubra var. arenaria has pairs of short-cells in which one is a silica-cell and the other is a cork-cell, and long-cells c. $150 \mu \mathrm{~m}$ long. However, Auquier (1971) observed pairs of cork-cells and silica-cells
in basal leaves of F. juncifolia. Stace \& Cotton (1974) have also observed pairs of cork-cells and silicacells in F. juncifolia.

Tateoka et al. (1959) used epidermal characters to distinguish taxa at higher levels. He observed the shape of bicellular hairs in a large number of tribes and found differences to be taxonomically useful.

The stomata found in the Gramineae are different in shape from those in any other plant family, the guardcells being dumbell-shaped with thin-walled, bulbous ends and a thick-walled central region surrounding the stomatal orifice (Lawton 1980). Stomatal length, distribution and frequency can be important taxonomic characters.

The systematic value of the embryo and caryopsis was investigated by Bruns (1892), Van Tieghem (1897), Yakovlev (1950), Reeder (1953, 1957, 1962) and Kinges(1961). Brun (1892) observed about 60 grass embryos with special reference to the epiblast. Van Tieghem (1897) studied the embryos in longitudinal sagittal section and found two basic differences between the panicoid and pooid types. He concluded that many genera needed taxonomic rearrangements on the basis of embryo study. Reeder (1953) realised the importance of Tieghem's work and later (1957, 1961) examined 400 species of grasses belonging to more than 175 genera. He recognized six embryo types on the basis of four important embryo characters. However, embryo characters have been found to be of better value below the level
of genus.
Sinnott (1939), Sinnott \& Block (1939),
Reeder \& Von Maltzalen (1953) and Row \& Reeder (1957) investigated the differences in typical pooid and panicoid root epidermis cell divisions, the position of root hairs in the epidermal cell, and the angle at which the root hairs emerge.

Brown, Heimsch \& Emery (1957) concluded that two tunica layers in the shoot apex are most common in the sub-family Pooideae, whereas a single layer is most common in the sub-family Panicoideae. Brown, Harris \& Graham (1959) studied the stem internodes of 133 species of grass from 80 genera and 21 tribes. They reported that about 93 per cent of Pooideae had hollow internodes. The number of grasses with solid or semi-solid internodes in tribes of the Eragrostoideae and Panicoideae ranged from 49 to 100 per cent. Brown, Harris \& Graham (1959) concluded that grasses of hot, arid regions tend to have solid internodes. Brown, Pratt \& Mobley (1959) found that pooid stems with hollow internodes were found to lack culm pulvini, but have well-developed sheath pulvini. In contrast, panicoid and eragrostoid grasses with solid or semi-solid stems tend to have well-developed culm pulvini and no sheath pulvini.

In section, grass leaves mostly consist of mesophyll, vascular bundles, bundle-sheaths and sclerenchyma. Mesophyll is the ground tissue occupying all the space in the leaf not occupied by the three
other tissues. It is made up of thin walled assimilatory chlorenchyma cells and associated colourless parenchyma cells (Gould 1968), but is rarely differentiated into contrasting palisade and spongy layers. Most of the grasses from temperate regions have diffusely arranged cells of chlorenchyma, the 'festucoid' (pooid) pattern. In many grasses from tropical regions the chlorenchyma cells radiate out in a more or less regular way from the vascular bundles, the 'panicoid' pattern (Metcalfe 1960). In the chloridoid pattern, the chlorenchyma cells are very regularly radiately arranged around the bundle, and adjacent bundles with their associated mesophyll are separated by areas of large colourless cells (Gould 1968). Goossens (1938) stated that both conditions (radiate and non-radiate) of chlorenchyma exist in the genus Sporobolus; most species have regular radiately elongated cells but $S$. panicoides has irregularly arranged cells.

The correlation of non-radiate chlorenchyma with temperate regions and radiate assimilatory tissue with tropical grasses is actually not so strong as was claimed by earlier workers (Ellis 1976). Intercellular air spaces occur between the chlorenchyma cells of many grasses (Ellis 1976). Hydrophilous species usually have conspicuous air spaces (Arber 1934; Vickery 1935). Large 'fusoid' cells are present in the mesophyll region in bamboos and in a few other grasses (Ellis 1976). Arm cells are characteristic of the Bambuseae and Oryzeae
(Ellis 1976).
Many of the tropical grasses with the 'panicoid type leaf' and 'chlordoid type leaf' exhibit the C4 pathway (Hatch \& Slack 1966) in addition to the C3 pathway of photosynthesis. The $C 4$ pathway involves the fixing of carbon in the chlorenchyma by the utilization of the enzyme phosphoenol pyruvate carboxylase, and transferring it to the bundle sheath cells where it is released in the form of carbon dioxide to be fixed again by the C3 pathway.

The C4 pathway is an adaptation to the tropical environment to enable these grasses to exploit that environment more effectively than those in which the C4 pathway is absent (Renvoize, 1981). The existence of the distinctive anatomy (Kranz syndrome) associated with C4 photosynthesis is an important taxonomic character.

Vascular bundles consist of xylem and phloem elements. The smaller vascular bundles may be reduced to one or few tracheids and a similar number of sieve elements (Gould 1968). The vascular bundles are much more crowded in the leaves of some grasses than in others. The distribution pattern of vascular bundles of different orders in a leaf is variable and can be valuable taxonomically. The vascular bundles are circular, elliptical, or conspicuously angular in outline (Metcalfe 1960).

Vascular bundles are surrounded by one or two bundle-sheaths (inner and outer). Each sheath is made up of a single layer of cells. In some grasses it is difficult
to decide whether one or two sheaths are present, when it is better to recognize the existence of three categories: single, double or intermediate. The single or outer sheath usually consists of large, thin-walled cells (Metcalfe 1960). These cells either are translucent (without chloroplasts) or contain green plastids similar to or different from the chloroplasts of the chlorenchyma (Ellis 1976). The inner bundle sheath is usually made up of small cells with greatly thickened inner and radial walls (Gould 1968) and without chloroplasts (Ellis 1976). Festucoid grasses are characterized by a double sheath (Metcalfe 1960) and panicoid and eragrostoid grasses lack an inner sheath (Gould 1968).

Sclerenchyma fibres provide mechanical support for softer tissues. Sclerenchyma is present in the form of strands or girders between the epidermis and the outer bundle sheath. Rarely in some species sclerenchyma lies between the vascular bundles (Metcalfe 1960). Sclerenchyma may penetrate the bundle sheath on one or both sides to connect with the sclerenchyma of the vascular bundles (Gould 1968). If the sclerenchyma lies in a discrete bundle it is called a 'strand', but if it extends from the epidermis to the bundle sheath it is termed a 'girder' (Metcalfe 1960) or an I-beam construction (Gould 1968).

Grasses from arid areas have well-developed sclerenchyma tissue while many tropical grasses often have a high proportion of the smaller bundles not accompanied by sclerenchyma (Ellis 1976). Where it is well-developed,


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the sclerenchyma often forms a continuous arc between the epidermis and the row of vascular bundles.


### 1.1.6: Cytology

Grasses, like other plants, show variations in their cytological characters, i.e. the number and morphology of chromosomes and their behaviour at meiosis.

Cytological studies of grasses were first carried out by Kumada (1919) on Zea mays, Bremer (1923, 1924, 1925) on Saccharum, Longley (1924) on Zea mays and hybrids involving that species, and Evans (1926) on Festuca.

Avdulov (1931) realised the great significance of chromosome number and size in grass taxonomy, especially at the sub-family and tribal levels. He reported chromosome counts for 232 species. Tischler (1950) published 107 reports, Carnahan \& Hill (1961) gave counts for more than 1,550 species, and Bolkhavskikh et al. (1969) listed approximately 3,989 species. Gould (1968) reported the typical basic chromosome number (x) for the six sub-families of grasses as follows: Pooideae $x=7$, Panicoideae $x=5,9,10$, Eragrostoideae $x=9,10$, Bambusoideae $x=12$, Oryzoideae $x=12$, and Arundinoideae $\mathrm{x}=6$, 12. Exceptions to the general rule are numerous, as reported by Carnahan \& Hill (1961), basic numbers of $x=4,8,11,13,17,19$, and 23 being found in addition to the above.

Polyploidy is of very common occurrence in the grasses. Carnahan \& Hill (1961) calculated that 80 per cent of the species for which chromosome numbers
were then known were polyploid, of which 7 per cent were aneuploid.

Several investigations have reported the occurrence of chromosome fragments or accessory, supernumerary or B-chromosomes in grasses. Apart from their effect on meiosis or fertility of plants, they rarely are of value as a taxonomic character.
1.1.7: Hybridization

Hybridization is relatively common in the plant kingdom. It introduces new genes into a population and consequently increases the probability of genetic change. It acts as a factor that increases variability. Interspecific hybridization is an important factor for the evolution and the variation of plants (Anderson 1949, 1953; Grant 1971; Stebbins 1950, 1959; Stace 1975). It is found commonly that hybrids become established in disturbed areas such as woodland, clearings, gullies and cultivated ground. In such places the conditions required by the parental species are not met in their entirety, but the somewhat modified and intermediate requirements of the hybrids are often better satisfied.

Interspecific and intergeneric hybrids have been produced and studied to provide information on the systematic and phylogenetic relationships within the Gramineae and also to provide fundamental data of possible value in the improvement of grasses by breeding.

In the Gramineae, both natural and artificial hybrids have been reported considerably more often than in any other plant family except the Orchidaceae. Ullmann (1936) reported 74 naturally occurring and 64 artificial hybrids. Myers (1947) recorded 229 interspecific and intergeneric hybrids known in forage grasses; of these 93 were naturally occurring and others were produced artificially. Carnahan \& Hill (1961) listed 256 interspecific and 95 intergeneric hybrids, mostly artificial. Knobloch (1968) listed 2,400 grass hybrids, both interspecific and intergeneric, but this list does not differentiate between naturally occurring and artificial hybrids. Jenkin (1933) reported on the intergeneric hybrids between Lolium L. and Festuca L. synthesized at the Welsh Plant Breeding Station, Aberystwyth. Camus (1957) found that in Bromus it was easier to cross diploid $x$ diploid or tetraploid $x$ tetraploid than to cross diploid $x$ tetraploid. Likewise, the diploid and tetraploid species of Bromus have not been successfully crossed with the octoploid species. Fagerlind (1937) stated that it was easier to produce hybrids by crossing polyploids than by crossing diploids. He found that both Galium mollugo $L$. and G. verum $L$. were either diploid or tetraploid; at the diploid level they were intersterile yet at the tetraploid level fertile hybrids were formed.

Artificial and spontaneous intergeneric hybrids of Elymus and Hordeum species have been reported by various
research workers (e.g. Lepage 1952, 1953; Bowden 1958; Morrison \& Rajhathy 1959). Crossability studies of wheat and rye have been widely reported, particularly that of hexaploid wheat and cereal rye, Secale cereale L. (e.g. Kiss \& Rajhathy 1956, Riley \& Chapman 1967, Lange \& Wajeieckowska 1976). Riley \& Chapman (1967) located two genes in wheat for crossability with rye, on chromosomes5A \& 5B, and discussed their evolutionary significance. It is also known that species which do not produce hybrids in nature, might do so under garden conditions. Natural hybrids in grasses have not been found between taxa in different tribes, but several intertribal crosses (e.g. Bromus $x$ Festuca) have been successfully synthesized (Stace 1975).

Knobloch (1972) claimed that natural hybridization has played a much greater part in speciation than has mutation. Hybridization followed by polyploidy may give rise to fertility in the hybrid. For example, Nilsson (1935) hybridized Festuca arundinacea Schreb. with F. gigantea (L.) Vill. and found that the amphiploid produced 91 per cent normal pollen grains by the third year. As already stated, about 80 per cent of grasses are polyploids, presumably of this type. Carroll \& Borrill (1965) found that in Dactylis L. crosses between diploids and tetraploids produced tetraploid offspring with a frequency not less than that of triploids, this being caused, presumably, by unreduced gametes.

Breeding systems can have a profound effect on the genetic structure of the plant population. Saunders \& Hamrick (1980) studied the breeding system in 15 populations of the prairie grass, Elymus canadensis L. They found that, although E. canadensis is a predominently self fertilizing species, out-crossing rates vary from population to population. Kannenberg \& Allard (1967) and Allard \& Kannenberg (1968) found that favourable environmental conditions produce higher out-crossing rates in the Festuca microstachys complex. The predominant trend in the evolution of the genus Poa L. has been from out-breeding species to polyploid apomictic complexes (Stebbins 1971). The annual habit is thought to have evolved more recently than perenniality in most cases, including Poa. It is most. likely that in Poa annua L., with annual and perennial, and diploid and tetraploid ecotypes, the trend of evolution is toward inbreeding tetraploids (Ellis 1973). Many polyploid species are predominantly inbreeding; the genus Vulpia is a good example (Barker \& Stace 1982).

Different sorts of isolating mechanisms have been classified by many research workers (cf. Stace 1975). Isolating mechanisms can be said to be of two main sorts, external and internal. External mechanisms are influenced by factors such as ecology, geography, flowering season, the time of flowering within the day, type of pollination and breeding behaviour, etc.

Internal mechanisms include pollen tube failure, seed
inviability, and hybrid inviability or non-fitness, etc. A detailed study of these mechanisms is essential in understanding the population structure and evolution of species in the wild.

The phenomenon of unilateral incompatibility has been discussed by Harrison \& Darby (1955), Lewis \& Crowe (1958), and Nettancourt (1977), among others.

### 1.1.8: Chemical characters

Chemotaxonomic work is useful for improving classifications as well as in finding relationships between plant species. In plants many chemical substances are present but not all of them give useful information to taxonomists. For example, in grasses seed proteins have been found to be particularly useful, although grasses also possess many secondary plant products such as alkaloids.

Serology, electrophoresis and chromatography have proved to be very useful techniques for taxonomic surveys. Hall (1959) used immuno-electrophoretic analysis of seed proteins of allopolyploid rye-wheat and its parental species to trace the relationships of these plants. Fairbrothers \& Johnson (1961) found serological differences between grasses of Pooideae and Eragrostoideae. At a lower level they distinguished between species of the pooid genera Festuca, Bromus, Lolium, Dactylis, Melica and Briza and also between eragrostoid grasses of the genera Eragrostis, Tridens, Triplasis, Distichlis and Spartina. Smith (1972)


#### Abstract

studied the inter-relationships of annual species of Bromus. He found that the degree of similarity of immuno-electrophoretic data was correlated with that of morphological, cytological and cytogenetical data. He (Smith $1969,1971,1976$ ) also studied a range of grass genera and generally found useful serological differences between them.


Johnson \& Hall (1965) used seed protein extracts of wheat and wheat relatives for electrophoresis. They found that homology of fractions in the resulting spectra was well correlated with the genetic affinity among the species and among their genomes. Johnson (1972) used the seed protein profiles to find the origin of hexaploid wheat (Triticum aestivum L.). Bebyakin \& Kumakov (1981) carried out an electrophoretic analysis of gliadin in $F_{f}$ hybrids of Triticum durum.

With the advent of paper and thin-layer chromatography, phenolic compounds have been investigated extensively in taxonomic studies, and such compounds have been useful in many taxonomic and phylogenetic problems. Investigations concerning flavonoid compounds of a number of species of the family Gramineae have been carried out by several workers. Kaneta \& Sugiyama (1973) identified flavone compounds in 13 species of Gramineae. They found that Brachypodium sylvaticum had the compound isoorientin. Starch grains in the endosperm of grass caryopses have been used as taxonomic characters. The
species of Brachypodium studied by Tateoka (1962) had simple starch grains.

Cugnac (1931) found that he could divide
grasses according to whether the sugars they possess were characterized by levulosides (levulifères) or not (sacchariferes). Whereas various pooid genera, such as Agrostis, Arrhenatherum, Dactylis, Bromus, Festuca, Lolium, Agropyron and Hordeum, fell in the former group, the two species of Brachypodium examined (B. pinnatum and B. sylvaticum) were unique among pooids in falling into the latter group, along with Phragmites, Cynodon, Spartina, Saccharum, Sorghum and Zea.
1.2.1: Taxonomic history of Brachypodium P. Beauv. Linnaeus (1753), in Species Plantarum, included only one species (Bromus pinnatus L. Brachypodium pinnatum (L) P. Beauv.) which would be placed in Brachypodium today. However, he later described Bromus distachyos L. (1756) (= Brachypodium distachyon (L.) Beauv.) and Festuca phoenicoides L. (1767) (= B. phoenicoides (L.) Roem. \& Schultes).

Beauvois (1812) described the genus Brachypodium, and included twenty species (plus two others with a question mark) in it. Many of these are now placed in other genera, but among the true species of Brachypodium were B. sylvaticum (Hudson) Beauv., B. pinnatum (L.) Beauv., B. gracile Beauv., B. retusum (Pers.) Beauv. and B. distachyon (L.) Beauv.

Niles \& Chase (1925) chose B. pinnatum as the lectotype species of the genus.

Dumortier (1824) divided the genus into three sections: Dryopyron Dumort., including Brachypodium proper: Distachys Dumort., including B. distachyon and one other species; and Apalochloa Dumort., including the single species B. nardus (DC.) Beauv., which is now known as Nardurus maritimus (L.) Murb., Vulpia unilateralis (L.) Stace or V. hispanica (Reichard) Kerguélen.

Link (1827) described a new genus, Trachynia Link, with two species, T. distachya (L.) Link and
T. rigida (Roth) Link. The former is here selected as the lectotype of the genus Trachynia, as it is the correct epithet for the species to which both of Link's species belong. Modern opinion is divided as to whether Trachynia deserves generic status; it is nowadays more usually included in Brachypodium (e.g. Smith 1980). Bluff, Nees \& Schauer (1836), like Dumortier, separated Brachypodium proper (section Genuini) from species nowadays placed in other genera at the sectional level (sections Nardurus (Reichenb.) Bluff, Nees \& Schauer, and Catapodium (Link) Bluff, Nees \& Schauer). Nyman (1855) divided Brachypodium into two main groups of uncertain rank: Perennia, including Brachypodium proper, and Annua, including three lesser taxa, one of which was called Trachynia (Link) Nyman (but is of uncertain rank) and contained B. distachyon. The other annual groups included species not nowadays included in Brachypodium.

Ascherson \& Graebner (1901) included only three species in the genus, all of which are considered members of Brachypodium in modern works. They divided the genus into two groups (sections or subgenera?), Eubrachypodium Ascherson \& Graebner for the perennial species (B. pinnatum, B. sylvaticum and B. ramosum Roemer \& Schultes, each with a complex system of infraspecific categories); and Trachynia (Link) Ascherson \& Graebner for B. distachyon.

Nevski (1934) separated Ascherson \& Craebner's two groups as genera. Brachypodium proper was divided
into two sections: Leptorachis Nevski, for B. sylvaticum; and Eubrachypodium Nevski, for B. pinnatum and two other species.

Maire \& Weiller (1955) included Trachynia in Brachypodium, which was divided into section Eubrachypodium (should be Brachypodium) and Trachynia (Link) Maire \& Weiller (given as section Trachynia (Link) Nyman, but Nyman's taxa were of uncertain rank). Maire \& Weiller subdivided the type section into subsection Leptorachis (Nevski) Maire \& Weiller for B. sylvaticum, and subsection Neobrachypodium Maire \& Weiller (should be Brachypodium) for B. phoenicoides, B. pinnatum and B. ramosum. B. sylvaticum was divided into subsp. eu-sylvaticum Maire \& Weiller (should be subsp. sylvaticum) and subsp. glaucovirens Murb.

St. Yves (1934) followed Ascherson \& Graebner and Maire \& Weiller in recognising two sections: Eubrachypodium and Trachynia. St. Yves' work was a detailed monograph of the genus in Europe and the Mediterranean region. He recognized only four species, each with a complex arrangement of subspecies, varieties and subvarieties: B. pinnatum (including B. sylvaticum, B. glaucovirens (Murbeck) Fritsch and B. rupestre (Host) Roemer \& Schultes; B. phoenicoides; B. ramosum ( $=\mathrm{B}$. retusum) ; and B. distachyon. St. Yves also described supposed hybrids between B. ramosum and B. distachyon ( $=$ B. $\times$ paui Sennen). Camus (1931, 1958) had described B. $x$ cugnacii as a hybrid between B. sylvaticum and B. pinnatum. St. Yves (1934)
considered this to be the same as his B. pinnatum var. glaucovirens (Murb.) St. Yves.

Lőve \& Lőve (1961), emphasizing chromosome number differences, raised Brachypodium section Leptorachis Nevski to a genus, as Brevipodium Löve \& Löve, including the single species B. sylvaticum (Hudson) Löve \& Löve.

Smith (1980), in Flora Europaea, included the genera Brevipodium and Trachynia in Brachypodium, which was divided into sections Brachypodium and Trachynia. Section Trachynia contained only B. distachyon. Section Brachypodium contained four species: B. sylvaticum, with subsp. sylvaticum and subsp. glaucovirens; B. pinnatum, with subsp. pinnatum and subsp. rupestre (Host) Schübler \& Martens; B. retusum; and B. phoenicoides.

Hubbard (1935) erected a new section, Festucopsis Hubbard, for two very different species, B. sanctum (Janka) Janka and B. serpentini Hubbard. However, these differ markedly from the rest of Brachypodium, and Melderis justifiably raised Hubbard's section to the genus Festucopsis (Hubbard) Melderis in the tribe Triticeae. The two species are F. sancta (Janka) Melderis and F. serpentini (Hubbard)(Melderis 1978).

In the present study, the genus Brachypodium is taken in the sense of Flora Europaea (Smith 1980), but for ease of reference the two non-typical subspecies recognized by Smith will be referred to as full species.

The species involved are therefore:
B. sylvaticum (Hudson) Beauv.
B. glaucovirens (Murbeck) Fritsch
B. pinnatum (L.) Beauv.
B. rupestre (Host) Roemer \& Schultes
B. retusum (Pers.) Beauv.
B. phoenicoides (L.) Roemer \& Schultes
B. distachyon (L.) Beauv.

My own views on their status will be given in the
Discussion. Other species will be mentioned occasionally. There are probably about 14 species in the world.

### 1.2.2: Morphology of Brachypodium

All the species of the genus Brachypodium are perennial, except B. distachyon which is annual. The stem is hollow and usually wholly herbaceous, but in B. retusum and some close relatives it is woody below. The stem of $B$. retusum and its close relatives is often branched, whereas in most species it is simple. The number of nodes and their hairyness are useful characters in the genus Brachypodium. For example B. sylvaticum is densely hairy at the nodes while B. pinnatum is pubescent and B. phoenicoides is almost glabrous. Rhizomes in Brachypodium are often extensively branched, but B. sylvaticum and some other species are relatively shortly rhizomatous.

Leaf-blades are linear, and acuminate or obtuse at the apex. They are flat when moist but convolute when dry in most species, but are only rarely tightly convolute in B. sylvaticum and B. distachyon. B. retusum and B. mexicanum have particularly short leaf-blades
(less than 10 cm ) and B. pinnatum and B. phoenicoides particularly long ones (up to 45 cm ). This can be a useful taxonomic character; in B. sylvaticum the leaf-blades are rarely over 35 cm long. The leafblades are conspicuously patent in B. glaucovirens and B. retusum (including its close relatives), but they are not patent in other species. Leaf colour is also an important character in some species. The leaves of B. phoenicoides are dark green, those of B. glaucovirens and B. rupestre are glaucous-green, those of B. sylvaticum are usually mid-green, and those of B. pinnatum are light green or yellowish green. Leaf-blades in "B. ramosum subsp. boissieri (Nyman) St. Yves" are covered with a deciduous white bloom.

The adaxial surface of the leaf-blades is characterized by many parallel ribs. The ribs of B. phoenicoides are prominent and strongly elevated, occupying two-thirds of the leaf thickness (St. Yves 1934). The ribs of "B. ramosum var. arbuscula St. Yves" are a little less elevated than in B. phoenicoides, whereas they are not prominent in B. sylvaticum, B. glaucovirens or B. pinnatum. However, ribs in B. sylvaticum are slightly prominent on the lower surface of the leaf-blade.

Pubescence of leaf-blades varies. B.
sylvaticum is usually hairy, while B. phoenicoides is glabrous. B. pinnatum is usually glabrous but sometimes hairy. Leaf-blades in B. glaucovirens are glabrous
and in "B. ramosum subsp. boissieri" are densely covered with long pilose hairs on the adaxial surface. In B. distachyon leaf-blades are sparsely hairy.

The ligule is membranous and usually blunt, and varies in length in different species. For example in B. pinnatum it is up to 2 mm long, in B. kawakamii Hayata about 0.5 mm long, and in B. sylvaticum $1-6 \mathrm{~mm}$ long.

The leaf-sheath has no auricles. It is
rounded on the back and overlapping on the front. It is ribbed and its pubescence varies in different species. For example it is densely hairy in B. sylvaticum, glabrous in B. phoenicoides and B. retusum, and sparsely hairy in B. distachyon.

The inflorescence in Brachypodium is a raceme of distichous, alternate, shortly pedicellate spikelets inserted with the back of the lemma to the rachis. In B. sylvaticum it is nodding but in other species it is erect. The inflorescence of B. sylvaticum, B. pinnatum and B. phoenicoides has a larger number of spikelets and is longer than in the rest of the species. B. distachyon, B. kawakamii and a few other non-European species have only l-3(4) spikelets. B. sylvaticum has (3-)8-12 spikelets, B. pinnatum up to 15 , B. phoenicoides $6-9(-13)$ and B. retusum 1-5(-7).
B. glaucovirens is the only species within the genus which has obviously patent spikelets. In the genus Brachypodium there is one spikelet at each node except for abnormalities. Spikelets are terete and
tapering towards the apex, except in B. distachyon which has laterally compressed spikelets. Spikelets usually have many florets; B. sylvaticum has 8-16, B. pinnatum 8-22, B. rupestre has $5-10$ and B. retusum has (6-)l0-15. B. mexicanum has the lowest number of florets within the genus (up to 6).

The florets are bisexual but the uppermost florets are often reduced or imperfect. The rachilla disarticulates above the glumes and between the florets. The glumes are sub-equal, persistent and acuminate with thin veins; lower glumes have 1-5 and upper glumes 3-7 veins. The glumes are herbaceous to coriacious, convex and rounded on the back, and fall short of the lowest floret.

The lemmas are lanceolate, scarious on the margins and convex on the back. They are usually 5-7(9)veined and acuminate or abruptly narrowed into a slender, straight, scabrid awn. Awn length varies in different species in the genus. In B. sylvaticum the awn equals or exceeds the lemma and is probably the longest in the genus. B. phoenicoides is characterized by having shorter awns, and sometimes the lower florets are almost awnless. B. kawakamii also has short awns. The palea is as long as the lemma or a little shorter, hyaline, emarginate or truncate, 2 -veined, and 2 -keeled with ciliate and scabrid keels.

The lodicules are two, small, oblong, attenuate towards the apex, and hairy at the apex or ciliate
on the margins. In B. kawakamii the lodicules have a swollen base and are fleshy. Stamens are usually three but B. distachyon sometimes has only two stamens. B. distachyon has the smallest anthers (about 1 mm ) within the genus; in B. sylvaticum they are $3.5-4.0 \mathrm{~mm}$, in B. pinnatum $3.5-4.5 \mathrm{~mm}$, and in B. phoenicoides $3.8-5.0 \mathrm{~mm}$. The ovary is hairy at the apex and the stigmas are plumose and sub-sessile. The caryopsis is tightly enclosed by the lemma and palea. It is oblong and rather flattened with a short hairy appendage at the apex. The hilum is elongate and linear, and the embryo small and basal.

### 1.2.3: Anatomy of Brachypodium

The leaf anatomy of Brachypodium species has been described by Pée Laby (1898), Duval-Jouve (1875), Lewton-Brain (1904), Lohauss (1905), Strecker (1913), Gunzel (1912), Burr \& Turner (1933), Prat (1932, 1933, 1936), Metcalfe (1960), Scholz (1968), Borsos (1974) and Sustar (1976).

Brachypodium is characterized by the festucoid leaf anatomy. On the abaxial and adaxial surface of the leaf macro-hairs are found, but micro-hairs are always absent. Burr \& Turner (1933) mentioned macro-hairs on the adaxial surface of B. pinnatum and 'asperities' (hooks) on both surfaces, whereas hooks and hairs are found on both surfaces of B. sylvaticum. Macro-hairs are absent from the abaxial epidermis of B. pinnatum but
prickle hairs and hooks are occasionally present between the ribs (Metcalfe 1960). The leaf lamina of B. rupestre is sparsely covered with minute hairs; there are no or very few long hairs (Borsos 1974). Borsos described 'setiform hairs' (bristles) in the sclerenchymatous zone of B. rupestre. Metcalfe (1960) found bristles and soft macro-hairs on the adaxial surface of B. retusum. Metcalfe (1960) mentioned that in B. sylvaticum long macro-hairs with swollen bases were fairly numerous, prickle hairs were fairly frequent on the ribs, and hooks were abundant between the ribs. Metcalfe found two types of macrohairs on the ribs of $B$. sylvaticum: long hairs with smaller bases, and short rigid and thick-walled hairs. In B. distachyon macro-hairs are of considerable length on both surfaces (Metcalfe 1960). St. Yves (1934) found that in B. retusum the whole abaxial surface of the leaf is granulose, and the adaxial surface has fairly short rigid hairs all over.

Lohauss (1905) divided Brachypodium into two groups on the basis of leaf anatomy. One group, which he called the "pinnatum; sylvaticum group", was characterized by a relatively thin leaf with relatively few non prominent adaxial ribs. The other group, which he called the "mucronatum, ramosum group", possessed leaves with more abundant and conspicuous adaxial ribs.

St. Yves (1934) stated that the form of
long-cells of the adaxial epidermis, whether they are rectangular, slightly undulate or strongly sinuous ("engrenees"), differ in the major groups of the genus.

Also the nature and distribution of the long-cells of the abaxial surface have a certain taxonomic value. Abaxial long-cells between the ribs in B. sylvaticum have thin sinuous walls (Metcalfe 1960), while in B. pinnatum they have thin but non-sinuous or slightly sinuous walls. In B. pinnatum the long-cells on the ribs are narrower and have thicker walls than between the ribs. St. Yves (1934) described the abaxial epidermal long-cells of "B. pinnatum var. silvaticum (Hudson) St. Yves sub-var, villosum (Lej. \& Court) St. Yves" as slightly sinuous- or nearly straight-walled. Those of B. phoenicoides are with scarcely sinuous walls and are granulose, while those of $B$. retusum are with strongly sinuous walls. Those of $B$. distachyon possess very slightly and laxly sinuous or nearly straight walls. Metcalfe (1960) reported that the long-cells in B. retusum are rectangular with sinuous walls. Borsos (1974) said that the epidermal cells between the ribs in $B$. rupestre are smaller and narrower than in B. pinnatum, and their walls are almost straight or only slightly sinuous; on the ribs the long cells have slightly sinuous walls. The long cells of $B$. pinnatum on the ribs zone are smaller and narrower than between the ribs, and their walls are strongly sinuous and thickened. Unfortunately many of Borsos' data are difficult to utilise, because she misidentified or mis-named (i.e. confused) the abaxial and adaxial surfaces of the leaf. Moreover, she claimed that the differences she found between B. pinnatum and B. rupestre are the
reverse of those found by Scholz (1968).
In Brachypodium the silica-bodies over the ribs are variable (Metcalfe 1960), ranging from tall, narrow types fitting into the concavities in adjacent cork-cells, to horizontally elongated types with sinuous out-lines; they are saddle-shaped in some species.

Metcalfe (1960) stated that the short-cells on the abaxial surface in B. pinnatum are mostly paired over and between the ribs or are occasionally in rows of about 3 cells over the ribs. The silica-bodies in B. pinnatum are rather variable in shape. Those between the ribs and over the small ribs are tall, narrow or rounded, and each fitting into the concavity of an adjacent cork-cell, whereas those over the larger ribs are more oblong. Gunzel (1921) described the silica-bodies in B. distachyon as being dumb-bell-shaped over the ribs and those in the leaf-sheath as elliptical. Lohauss (1905) described the silica-bodies on the adaxial surface of B. sylvaticum as saddle-shaped or dumb-bell-shaped, rarely rounded. Metcalfe (1960) described the shortcells on the adaxial surface of B. sylvaticum as mostly solitary between the ribs, and in rows of more than 5 over or on the margins of the ribs. The latter are horizontally elongated with sinuous outlines.

In B. mucronatum the silica-bodies on the ribs are saddle-shaped. Metcalfe (1960) described kidneyshaped short-cells bearing bristles in B. retusum. Borsos (1974) found that the cork-cells on the ribs of B. rupestre
are small, thin and about $2-3$ times smaller than the silica-bodies, and their sides adjacent to the silicabodies are concave. She described the silica-bodies as tetragonal, with rounded corners, medially constricted, or elongated with multiple constrictions. In B. pinnatum the short-cell row consists of cork-cells and silicabodies. The cork-cells are about half as big as the silica-bodies and their side adjacent to the silica-bodies is concave.

The silica-bodies are flatly circular, oval or rounded-tetragonal (Borsos 1974). Scholz (1968) stated that the silica-bodies in B. pinnatum are circular or rounded-quadrangular. Stomata in the genus Brachypodium are commonly with parallel-sided subsidiary cells. St. Yves (1934) stated that the distribution of stomata appeared to be variable in different parts of the leafblades. Metcalfe (1960) stated that stomata were absent on the abaxial surface of B. pinnatum. Borsos (1974) found on the abaxial surface of B. pinnatum a single row of stomata in the intercostal zone near the ribs, whereas in $B$. rupestre no stomata occur on the abaxial surface. In B. pinnatum and B. sylvaticum the bulliform cells are in several rows and fill the whole of the sinus between the ribs, whereas in B. phoenicoides the bulliform cells are in $3-5$ rows and occupy only the central part between the ribs. In B. retusum bulliform cells are in 5 or more rows which can encroach up the sides of the ribs, and in B. distachyon the pattern is similar (St.

Yves 1934). Borsos (1974) stated that there are 6-8 rows of bulliform cells in B. rupestre and 6-10 rows in B. pinnatum.

Vascular bundles in the genus Brachypodium are not conspicuously angular in outline. The mesophyll in the chlorenchyma is not radiate. The bundle sheaths are double, i.e. with an inner and an outer bundle sheath, but the outer bundle sheath is inconspicuous in some species (Metcalfe 1960). Transverse sections of the leaf lamina of $B$. pinnatum reveal low, rounded ribs and fairly wide shallow furrows on the adaxial surface, while in B. sylvaticum the leaf surface is flat.

In "B. ramosum subsp. boissieri (Nyman)
St. Yves" the sclerenchymatous tissue develops at the ribs in a continuous or sub-continuous band on the abaxial side (St. Yves 1934). Metcalfe (1960) described the position of sclerenchyma in B. pinnatum as forming a sheath around each vascular bundle and as extending on both adaxial and abaxial sides as a girder which reaches the true epidermises. The narrowest girders are about 1-4 cells wide, and the widest ones (associated with larger vascular bundles) occupy the whole of the leaf rib area. Borsos (1974) stated that the sclerenchyma cells in B. pinnatum have strongly thickened walls which are best developed around the larger vascular bundles. St. Yves (1934) found in B. phoenicoides several leaf ribs with I-shaped sclerenchyma girders which are composed of colourless cells towards the leaf surfaces. Prat
(1936) concluded that the anatomy of Brachypodium as a whole is festucoid, but the occurrence of dumb-belland saddle-shaped silica-bodies, which have been found in certain species, is a character which is not typically festucoid.
1.2.4: Cytology of Brachypodium

The genus Brachypodium is characterized by having more than one basic chromosome number. For example, the four best-known species of the genus usually have $2 \mathrm{n}=18$ (B. sylvaticum), $2 \mathrm{n}=28$ (B. pinnatum), $2 \mathrm{n}=30$ (B. distachyon) and $2 \mathrm{n}=36$ (B. retusum). But Kozuharov et al. (1974) claimed that the basic chromosome number of the genus seems to be $x=7$, which has given rise to increasing polyploidy as well as to increases in the base number (to $x=8$ or 9 ) in the perennial species, and to decreases in base number ( $\mathrm{to} \mathrm{x}=5$ ) in the annual species.

A summary of chromosome counts reported by previous workers is given in Table 1.2/1. This table is based on the literature survey made by Robertson (1981), with Robertson's own counts and any more recent counts found in the literature. Robertson (1981) found no intraspecific variations in chromosome number in any of the species he examined. However, some previous workers have reported extensive intraspecific variation for B. pinnatum, B. sylvaticum and B. distachyon.

In B. Sylvaticum Kozuharov et al. (1974)
reported three new chromosome numbers $(2 n=28,42+2 B$, 56 ) in addition to the normal $2 \mathrm{n}=18$, and gave the karyotype of each. Mehra \& Sunder (1969) reported numbers of

TABLE 1.2/1

Brachypodium chromosome counts made by previous workers on material of known wild origin

| Taxon | Count | Locality | No. of reports |
| :---: | :---: | :---: | :---: |
| $\frac{\text { B. arbuscula }}{\text { Knoche }} \text { Gay ex }$ | $2 \mathrm{n}=18$ | Canary Islands | 2 |
| B. boissieri Nyman | $2 \mathrm{n}=36$ | Spain | 2 |
| B. distachyon (L.) | $2 \mathrm{n}=10$ | Bulgaria, U.S.S.R. | 2 |
|  | $2 \mathrm{n}=20$ | Spain | 1 |
|  | $2 \mathrm{n}=28$ | Tunisia, France, U.S.S.R. | 3 |
|  | $2 \mathrm{n}=30$ | S. and C. Europe, Portugal, U.S.S.R., Morocco | 9 |
| var. genuinum Guss. | $2 \mathrm{n}=10$ | France | 1 |
| $\frac{\text { B. flexum }}{\text { K. Larsen }} \text { (Nees) }$ | $2 \mathrm{n}=18$ | S. and E. Africa | 2 |
| $\frac{\text { B. glaucovirens }}{\text { (Murbeck) Fritsch }}$ | $2 \mathrm{n}=16$ | Bulgaria | 1 |
| $\frac{\text { B. mexicanum }}{\substack{\text { (Roemer \& Schultes) } \\ \text { Link }}}$ | $2 \mathrm{n}=38$ | Costa Rica, Mexico | 5 |
| $\frac{\text { B. phoenicoides }}{\text { Roemer \& Schultes }}\left(L_{.}\right)$ | $2 \mathrm{n}=28$ | France, Portugal | 7 |
|  | $2 \mathrm{n}=36$ | France | 1 |
| var. mucronatum <br> (Willk.) Henriq. | $2 \mathrm{n}=28$ | Portugal | 3 |
| B. pinnatum (L.) Beauv. | $2 \mathrm{n}=14$ | Bulgaria | 1 |
|  | $2 \mathrm{n}=16$ | Bulgaria | 2 |
|  | $2 \mathrm{n}=18$ | Greece | 1 |
|  | $2 \mathrm{n}=20$ | U.S.S.R. | 1 |
|  | $2 \mathrm{n}=28$ | Germany, Holland, Sweden, France, Portugal, Bulgaria, Italy, Czechoslovakia, England, Greece | 33 |

var. rupestre (Host) $2 \mathrm{n}=14$ Bulgaria ..... 1
Roemer \& Schultes
B. retusum (Pers.) 2n=27 France ..... 1
Beauv.
Beauv.
2n=36 France ..... 2
B. sylvaticum (Hudson) $2 n=14$ India ..... 2
Beauv.
$2 \mathrm{n}=18 \quad$ Japan, Portugal, Canary ..... 66
Islands, Bulgaria, England, Germany, Czechoslovakia, India, France, Italy, Sweden, Belgium, Poland, Switzerland, Denmark, Hungary, Greece, U.S.S.R.
2n=28 Bulgaria ..... 1

- $2 \mathrm{n}=28+1 \mathrm{~B}$ India ..... 1
$2 n=42 \quad$ India ..... 1
$2 n=42+2 B \quad$ Bulgaria ..... 1
$2 \mathrm{n}=56$ Bulgaria ..... 1
subsp. kurilense $2 \mathrm{n}=18$ U.S.S.R. ..... 1Probat.
var. luzoniense $2 \mathrm{n}=14$ Taiwan ..... 1
Hackel
var. miserum (Thunb.) $2 \mathrm{n}=18$ Japan ..... 1
var. multiflorum Willk. $2 \mathrm{n}=18$ No location ..... 1
$2 \mathrm{n}=14$, 18 and 42, but, as pointed out by Robertson (1981), the photographs they provided of the $2 \mathrm{n}=14$ and 42 plants are clearly not of Brachypodium. However, $2 n=14$ has been also counted for $B$. sylvaticum var. luzoniense from Taiwan by Hsu (1971).

In B. pinnatum, Kozuharov et al. (1974) reported the numbers $2 \mathrm{n}=14,16$ and 28 , and again gave karyotype analyses. The plants with $2 \mathrm{n}=14$ and 16 differed somewhat in appearance from the normal B. pinnatum with $2 \mathrm{n}=28$, and the anthers suggested that the former might be referable to B. tenerum Velenovsky. Unfortunately, in the case of both B. sylvaticum and B. pinnatum, the karyotype analyses threw little light on the evolutionary relationships between the chromosome number races. There are also two more recent counts of $2 \mathrm{n}=20$ and $2 \mathrm{n}=18$ for B. pinnatum from U.S.S.R. (Sokolovskaya \& Probatova 1978) and from Greece (Strid 1983) respectively.
B. retusum appears usually to have $2 n=36$
according to Robertson (1981) but there is a single count of $2 n=27$ (Natarjan 1978). In addition the very closely related B. boissieri also has $2 \mathrm{n}=36$ (Robertson 1981). However, the closely related B. arbuscula Gay ex Knoche from the Canary Islands has $2 \mathrm{n}=18$, which strongly points to it being a patroendemic.
B. phoenicoides usually has $2 \mathrm{n}=28$, but there is a report of $2 \mathrm{n}=36$ (Natarjan 1978).
B. distachyon has a base number of $x=5$, with chromosome races of $2 \mathrm{n}=10,20$ and 30 , of which the last
is the most common, but in addition there are three counts of $2 \mathrm{n}=28$.

All other taxa have had only one chromosome number reported: B. flexum $(2 n=18)$, B. glaucovirens $(2 n=16)$, B. mexicanum $(2 n=38)$ and B. rupestre $(2 n=14)$. It is possible that some of the anomalous counts referred to above, in addition to those of Mehra \& Sunder (1969), are based upon mis-identifications.

Tateoka (1962) stated that the chromosomes of B. mexicanum, which were then studied for the first time, were similar in size and morphology to those of other species of Brachypodium.

Over all, the size of chromosomes of the species of Brachypodium is small (Avdulov 1931, Tateoka 1956, Kozuharov et al. 1974). Kattermann (1931) observed meiosis of $B$. pinnatum and stated that the chromosomes were very small and difficult to study. The evolution of chromosome numbers in Brachypodium is clearly complex and remains a major problem.

### 1.2.5: Hybridization in Brachypodium

There is very little known about hybridization in the genus Brachypodium. All the knowledge so far accumulated is based solely upon morphological studies of wild plants.

Sennen (1911) reported B. paui Sennen as a hybrid of B. ramosum $\times$ B. distachyon. Hansen (1959) mentioned that Anderson (1931) reported a hybrid between
B. pinnatum and B. sylvaticum, and Camus (1958) also reported natural hybrids between these two species. Camus described the morphology of the hybrid as differing from B. pinnatum in its wider, less rough leaf-blades, sparsely pubescent lemma and longer awns; and from B. sylvaticum by its narrower, inrolled, less hairy leaf-blades, glabrous leaf-sheaths, less acuminate glumes, slightly less hairy lemmas and shorter awns. Stace (1975) stated that putative hybrids of B. pinnatum $\times$ B. sylvaticum had been recorded from Denmark, Czechoslovakia, France and Ireland. The hybrids were of intermediate character and had been found in the proximity of parent populations. Kozuharov (1974) believed that B. glaucovirens is intermediate between B. sylvaticum and B. pinnatum and regarded its chromosome number as possible evidence of hybridization between B. pinnatum and B. sylvaticum at the diploid level. But St. Yves (1934) said that the pollen and seed of B. glaucovirens is good and that the plant occurs where there is no B. pinnatum. Nevski (1934) considered B. villosum Drab. to be possibly a hybrid between B. pinnatum and Agropyron repens (L.) Beauv., although B. pinnatum is not known in the region concerned (Lena Kalyma district). Jenkin $(1955,1959)$ was unsuccessful in crossing B. sylvaticum with Festuca gigantea (L.) Vill., Lolium perenne L. and Bromus asper Murray (B. ramosus Hudson).


#### Abstract

1.2.6: Distribution and Ecology of Brachypodium

Like most of other genera of the Pooideae, Brachypodium is mainly found in temperate and warm temperate parts of the world. However it is very widespread and often very disjunct, and this fact, together with the absence of any close relatives, led Tateoka (1968) to suggest that Brachypodium had an early origin (early Tertiary or even late Cretaceous) and that its present-day distribution is largely relict.


The distribution of the species has been surveyed by Maekawa (1964) and Tateoka (1968), and Meusel (1965) has provided distribution maps for three species. Figs. 1.2/1 to $1.2 / 6$ show the distribution of the six main species used in this project.

More than half of the species are distributed in the Mediterranean and neighbouring regions, from which they extend sparingly to eastern Asia (as far as Japan) and to the tropical African mountains. Besides these are several extra species distributed in various areas distant from the Mediterranean: B. bolusii Stapf and B. flexum (Nees) K. Larsen in southern Africa; B. kawakamii Hayata in Taiwan; B, longisetum Hitchcock in Guinea; and B. pringlei Scribn. and B. mexicanum (Roem. \& Schultes) Link in southern and central America. Some species like B. sylvaticum and B. pinnatum are very widespread, whilst others, e.g. B. kawakamii, are narrow endemics, interpreted as paleoendemics by Tateoka
(1968). Both the species B. pinnatum and B. sylvaticum have a similar wide distribution in Europe. There are some differences in detail, especially in the north and in the south, in both of which B. sylvaticum extends further (Fig. 1.2/2). In Britain, B. sylvaticum occurs more or less over the country, but B. pinnatum is very rare in Wales, Scotland, Ireland, East Anglia and south-west England.
B. pinnatum (Fig. 1.2/3) is absent from some parts of southern Europe and perhaps entirely from North Africa. It extends eastwards to central Asia, and has been introduced into some other parts of the world (e.g. Australia, S. America). B. pinnatum is more thermophilous; therefore it generally occurs in more open situations, and in the north it occurs mainly on south-facing slopes. It is fairly unpalatable to animals, and therefore an undesirable weed of many grasslands. It is calcicolous, but not confined to calcareous soils. Cenci (1974) actually selected B. pinnatum to improve Italian pastures in places which were difficult for other grasses to colonise. It is resistant to fire penetration (Zimmermann 1979).
B. sylvaticum is the most widespread species of the genus. It extends into North Africa and eastwards into central Asia. Closely related species, or infraspecific variants, occur right across Asia as far as Japan and Taiwan. Moreover, the species of Brachypodium in America, south and tropical Africa, and eastern Asia are all more
similar to B. sylvaticum than to any other European species. This suggests that B. sylvaticum is part of a world-wide species complex. B. sylvaticum is found on a wide range of soils, both heavy and light, as well as basic and acidic. It is particularly characteristic of woodland, but also occurs in completely unshaded habitats. The two species often grow together in mixed communities.
B. phoenicoides (Fig. 1.2/4) occurs in dry and usually open habitats in the Mediterranean region. It largely replaces B. pinnatum in North Africa, in much of Spain and in the warmest parts of the European Mediterranean zone, where it often occurs on sand dunes.
B. retusum (Fig. 1.2/5) occurs in dry rocky places in the open or in open woodland in the Mediterranean region. The very closely related subsp. boissieri is endemic to the Sierra Nevada in Spain. B. retusum is represented in the Canary Islands by the endemic B. arbuscula, which occurs on the four main western islands.
B. distachyon (Fig. 1.2/6) is another Mediterranean species, and is additionally found as an introduced weed in South Africa, Australia, America and many parts of Asia. It is a weed of many disturbed habitats in the Mediterranean region. The natural habitat appears to be sandy or gravelly ground, mostly on calcareous soils but also in acid and gypsaceous areas.
smensal/







#### Abstract

1.2.7: Relationship of Brachypodium with other genera Brachypodium belongs to the sub-family Pooideae of the Poaceae. Nowadays it is usually placed in the unigeneric tribe Brachypodieae, but previously it has been associated with various genera in other tribes. The relationships of Brachypodium are not obvious, but some of the genera which have been suggested as close to it are Bromus L., Agropyron Gaertner and Festucopsis (Hubbard) Melderis.


Many of the earlier classifications placed Brachypodium in a broadly defined tribe Festuceae (Poeae). For example, Stebbins \& Crampton (1961) included the following genera in the tribe: Brachypodium, Bromus, Festuca, Lolium, Scleropoa, Puccinellia, Torreyochloa, Sclerochloa, Scolochloa, Hesperochloa, Poa, Briza, Catabrosa, Dupontia, Phippsia, Coleanthus, Dactylis, Cynosurus, Lamarckia and Arctagrostis. Clifford \& Goodall (1967), using a numerical study of 52 characters, recognized the following genera in their cluster $B$ : Agrostis, Ampelodesmos, Avena, Brachyelytrum, Brachypodium, Bromus, Brylkinia, Danthonia, Glyceria, Melica, Milium, Phalaris, Poa, Sesleria, Stipa and Triticum.

However, many workers have defined tribes on much narrower limits. Harz (1880) first described the Brachypodieae as a separate tribe, including in it the genera Brachypodium, Bromus and Ceratochloa. Avdulov (1931) similarly included only Bromus, Boissiera and Brachypodium in his sub-tribe Brachypodieae of the tribe

Festucaceae. Hubbard (1948) suggested that Bromus and Brachypodium should not be included in the Poeae, but might be closer to the Triticeae, and Tutin (1952) also included only Bromus and segregates and Brachypodium in the tribe Brachypodieae. Hubbard (1954) restricted the Brachypodieae to Brachypodium only, separating Bromus into the tribe Bromeae. This had earlier been done by Melderis (1950) and Hylander (1950).

The characters most used to link Brachypodium to Bromus are the hairy terminal appendages to the ovary and the simple starch grains. In addition, these two genera both possess the usual feature of thick-walled cells in the peripheral cell layer of the nucellus, differing from both Poeae and Triticeae in this respect (Smith 1969). Similarly, relationship to Agropyron and other Triticeae is suggested by the simple spicate or racemose inflorescence and the simple starch grains. The Poeae have compound starch grains and lack a hairy ovary appendage, and no recent authors have seriously suggested a close relationship in that direction.

Smith (1969) found that there were no serological
affinities between Brachypodium and Bromus, the Poeae or the Triticeae. Cugnac (1931) examined the carbohydrate compounds of various grass genera. Brachypodium was clearly distinguished from Agropyron, Bromus and Festuca. Brachypodium rhizomes contain starch but no laevulose sugars, while the other three genera have no starch but possess laevulose sugars. In fact Brachypodium more
closely resembles Phragmites, Molinia, Spartina and Cynodon in these characters than it does its more probable relatives on morphological grounds.

Macleod \& McCorquodale (1958) worked with the soluble sugars and oligosaccharides of grass seeds. Bromus and Brachypodium were found to be quite different; Brachypodium contains the trisaccharide raffinose and the tetrasaccharide stachyose, both of which are absent from Bromus, and the latter of which is absent from Agropyron and Festuca as well. Fructosans were found in Bromus and Agropyron, but not in Brachypodium or Festuca.

Brachypodium differs markedly from Festuca,
Bromus and Agropyron in having small chromosomes and base numbers of $x=5,7$ and 9 , whereas in the latter three genera the chromosomes are larger and $x=7$.

Tateoka (1955) examined the striking similarity on the basis of morphological characters between some species of Brachypodium and Agropyron. The only morphological difference was the presence of a pedicel in the former genus and its absence in the latter. There is a particularly close resemblance between B. sylvaticum and A. yezoense. However, the chromosomes of these two species are markedly different in number, size and morphology, which led Tateoka to conclude that the similarity between them is through convergent evolution.

The genus Festucopsis (Hubbard) Melderis shows a close similarity to Brachypodium in the racemose inflorescence structure, but not in the vegetative parts.

Previously Festucopsis was considered a section of the genus Brachypodium (Hubbard 1948), but Melderis (1978) raised this section to generic level. He based this decision on various micro-morphological characters (leaf anatomy, lemma and glume structure). In addition, Jones (1955) reported considerably larger chromosomes based on $x=7$ in Festucopsis, and this difference has also been confirmed by J. P. Bailey (pers. comm.).

Sharma (1979) suggested a close relationship between festucoid genera and the tribe Stipeae, since both have a festucoid embryo and leaf anatomy. Also both are susceptible to the weed-killer isopropyl-N-phenyl carbamate (Stebbins 1956). Since the Stipeae, like the Brachypodieae, have simple starch grains, whereas most other members of the festucoid tribes have compound grains, Sharma suggested that Brachypodium might be particularly close to the Stipeae.

Stebbins (1972) speculated that the most primitive grass inflorescence probably consisted of a small number of spikelets arranged in a short raceme, such as that now found in the genus Brachypodium. The lack of any wild hybrids and the failure in all attempts to produce artificial hybrids between Brachypodium and any other genus is further evidence of its isolation. Nevski (1933) suggested that Brachypodium arose from a group he called Protohordeae, but also thought that the Bromeae were an earlier development from it. Tateoka (1968), Smith (1969) and others have considered that Brachypodium is an ancient genus which arose from the
basal Poeae-Triticeae stock.
1.2.8: The aims of the present study
B. sylvaticum and B. pinnatum show much variation in their morphological characters, which overlap to such an extent that they create a problem for identification. This led me to investigate other aspects of their variation, such as anatomy, cytology, seed-proteins and hybridization, to try to clarify their phenetic and phylogenetic relationships. In order to do this, certain closely related taxa, such as B. glaucovirens and B. phoenicoides, were also studied. The two other European species, B. retusum and B. distachyon, were also studied from a comparative point of view, in order to ascertain their relationships with the $B$. sylvaticum- $B$. pinnatum complex. The above species were studied as far as material allowed from across their European range. In addition, B. mexicanum was used in some electrophoretic and breeding work.

## SECTION 2

MATERIALS AND METHODS

Living specimens from the University of Leicester University Botanic Garden and herbarium material from the Leicester University Herbarium (LTR), were used in this research.

Caryopses of Brachypodium were obtained through seed exchange or collecting trips. About 5-6 caryopses per 4-inch pot were sown under green-house conditions in 16 hr light/8hr dark with the temperature $15-19^{\circ} \mathrm{C}$. A mixture of Levington Compost:clay:grit in the ratio of 3:2:1 was used.

As most of the species of the genus Brachypodium do not flower under continuous green-house conditions, chill treatment during the winter was given to induce flowering in the spring. Plants were grown in 8-inch pots sunk in the field in the Botanic Gardens. The frost-sensitive species of B. retusum and B. mexicanum, and the annual B. distachyon, were kept in the greenhouse.

In May and June a representative plant from each sample was collected in flower and pressed to provide a permanent voucher specimen. Living plants were also transferred from the field to the green-house still in their 8-inch pots for the hybridization experiments.

A list of all the living material used (coded Bl to B366) is in Appendix 1.

Six to eighty specimens per species were used for morphological characters; 3-8 values were measured for each character of a representative plant.

The following characters were measured:

1. Height of plant
2. Nature of rhizome
3. Pubescence of culm
4. Number of internodes
5. Length of internodes
6. Pubescence of nodes
7. Length and width of leaf-blades
8. Pubescence of leaf-blades
9. Colour of leaf-blades
10. Pubescence of leaf-sheaths
11. Length of ligule
12. Length of raceme
13. Distance from uppermost leaf-sheath to base of inflorescence
14. Distance between spikelets on rachis
15. Length of pedicel
16. Length of spikelet
17. No. of spikelets
18. No. of florets (fertile and sterile)
19. Length of lower glume
20. No. of veins on lower glume
21. Length of upper glume
22. Number of veins on upper glume
23. Pubescence of glumes
24. Length of 2nd, 3rd, and 4th lemmas of 3rd spikelet
25. Number of veins on lemma
26. Width of lemma
27. Pubescence of lemma
28. Length of palea
29. Pubescence of palea margin
30. Length of lemma awn
31. Number and length of anthers
32. Length and width of caryopsis

Morphological results are given for B. sylvaticum,
B. glaucovirens, B. pinnatum, B. rupestre, B. phoenicoides,
B. retusum and B. distachyon, as well as for a number of intermediate or problematical plants which appear under their code-number.

CHAPTER 2.3: LEAF ANATOMY


#### Abstract

2.3.1: Preparation of leaf material

Leaves from living or dried plants were used for anatomical studies. Dried leaves were placed in boiling water for a little while to soften the leaf until it had unfolded and was ready for epidermal scraping and section cutting. Fresh leaves were used direct. Lower culm leaves were used for anatomical studies as suggested by Burr \& Turner (1933). Culm flag leaves were initially used for epidermal studies but they were less satisfactory as diagnostic characters (especially in the degree of long-cell wall undulation) were less well developed.


### 2.3.2: Epidermal anatomy

Clarke's (1960) technique as modified by Cotton (1974) was used for epidermal preparations. The fresh or softened dry leaves were placed in a tube filled with $88 \%$ lactic acid kept hot in a boiling water bath for about $50-60$ minutes.

When the abaxial epidermis was to be prepared the leaf was placed on a tile adaxial surface uppermost and flooded with cold lactic acid. Using a sharp scalpel blade the adaxial epidermis was cut across the leaf and, staring at the cut, was scraped away together with the mesophyll cells, until just the abaxial epidermis of the leaf remained on the tile. The epidermis was placed outside uppermost and mounted in clean $88 \%$
lactic acid.
When a preparation of the adaxial epidermis was to be made the leaf was placed abaxial side uppermost and flooded with cold lactic acid. It was then scraped as above. In the case of B. phoenicoides it was more difficult to obtain good preparations of the adaxial epidermis, because of the very tough prominent ribs and the delicate cells between them. This difficulty was overcome by scraping very gently for a long time with a sharp scalpel.

Preparations of the abaxial and adaxial epidermises were photographed using a 35 mm camera mounted on the microscope.

Epidermal preparations were treated with a saturated solution of phenol to stain the silica-bodies.

### 2.3.3: Internal anatomy

A segment of leaf-blade was fixed in embedding material on a freezing microtome. The sections were cut usually $25-30 \mu \mathrm{~m}$ or sometimes up to $40 \mu \mathrm{~m}$ thick. The sections were collected from the microtome knife with a soft paint brush and placed in $70 \%$ alcohol and stained by the following procedure:

1. Stained in $1 \%$ safranin in $70 \%$ alcohol for 30 minutes;
2. Washed with $70 \%$ alcohol and then with I.M.S.;
3. Stained in $1 \%$ Fast Green in I.M.S. for 30 seconds;
4. Washed in I.M.S. and then absolute alcohol;
5. Mounted in euparal.

The sections were photographed as above.

## CHAPTER 2.4: CYTOLOGY

### 2.4.1: Mitotic preparations

Root tips were collected from plants growing in pots as well as from germinating caryopses.

The root tips from pots were collected between 12.00 and 14.00 hr . Caryopses were grown on moist filter papers in petri-dishes, the margins of which were wrapped with nescofilm to avoid evaporation, and placed in an incubator at $23^{\circ} \mathrm{C}$. Caryopses of all the species were ready for treatment within 4-6 days, except B. distachyon which germinated within 3 days. Caryopses were pretreated when the roots were about 7 -10mm long. To avoid fungal infection, the caryopses were treated with $10 \%$ Domestos and 2-3 washes with sterilized distilled water before putting on the sterilized filter paper.

To obtain quick-growing roots from the pots, the older roots were cut off and new soil was put into the pots to encourage fresh growth.

Aqueous solutions of different chemicals as suggested by Sharma \& Sharma (1965) and Darlington \& LaCour (1969) were used as pretreatment agents, under a wide range of conditions:

1. $0.05 \%$ and $0.5 \%$ aqueous colchicine
2. Saturated aqueous hexachlorocyclohexane
(gammexane)
3. 0.002M 8-hydroxy-quinoline
4. Saturated $\alpha$-bromo-naphthalene.

Saturated $\alpha-$ bromo-naphthalene proved to be the most successful pretreatment agent in this study.

The following method was used for the pretreatment of root tips:

1. The roots were pretreated in a saturated $\alpha$-bromo-naphthalene solution at $4^{\circ} \mathrm{C}$ for $20-21 \mathrm{hr}$ in the dark;
2. The roots were rinsed with distilled water;
3. The material was fixed in $3: 1$ mixture of absolute alcohol: glacial acetic acid for 24 hr and stored in $70 \%$ alcohol at $4^{\circ} \mathrm{C}$;
4. The root tips were hydrolysed in $1 N$ hydrochloric acid for $32-35 \mathrm{~min}$ at $60^{\circ} \mathrm{C}$;
5. The root tips were stained in feulgen reagent for $2-3 \mathrm{hr}$;
6. The root tips were macerated in $2.5 \%$
solution of sigma P 4625 pectinase in $5 \%$ MES solution buffered at pH 5 at $25^{\circ} \mathrm{C}$ for $25-30 \mathrm{~min}$;
7. Excised meristems were placed on a clean slide and teased out in a drop of $45 \%$ acetic acid;
8. A cover slip was placed over the teased tissue, tapped to separate the cells, and then squashed to flatten them;
9. The cover-slip was sealed with rubber solution;
10. Good preparations were photographed as above.

Two things were found very important: firstly
the roots must be left for at least 24 hours in fixative; and secondly the roots must not be left more than 48 hours in fixative. If these points were not observed the roots would not stain properly.

### 2.4.2: Meiotic preparations

Inflorescences were fixed when they were fully emerged from the upper leaf-sheath. A precise time for collecting the inflorescences was vital. This was found to be very early in the morning, about 2 hours after sunrise. All the fixed material was kept in 3:1 absolute alcohol:glacial acetic acid and stored at $4^{\circ} \mathrm{C}$ until required. After trying different stains, $2 \%$ acetoorcein (LaCour 1941) proved to be the best.

The following procedure was used for pollen mother cell meiosis:

1. Fix inflorescence in 3:1 absolute alcohol: glacial acetic acid;
2. Dissect out anthers in $45 \%$ acetic acid on a slide under binocular microscope;
3. Remove the anther wall material;
4. Add a drop of $2 \%$ aceto-orcein to the material and add cover slip;
5. Tap cover slip to separate pollen mother cells and squash to flatten them;
6. Seal with rubber solution;
7. Photograph good cells.


#### Abstract

It was also found satisfactory to dissect the anthers for meiosis directly from the plants on to a slide in $45 \%$ acetic acid without previous fixing. The frequency of various chromosome configurations was scored from $20-40$ or more pollen mother cells per plant. Minimum chiasma frequency was calculated at diakinesis.


### 2.4.3: Pollen fertility

Inflorescences were collected before anther dehiscence, placed in $70 \%$ alcohol, and stored at $4^{c} \mathrm{C}$ until required. Anthers were dissected in a drop of Muntzing's aceto-carmine/glycerine solution (mixed equal amounts of $1 \%$ aceto-carmine and neutral glycerine). Anther wall material was removed before the cover slip was placed on the preparation. At least 200 pollen grains were scored for each plant and the percentage of stained, full grains was calculated. This was taken to represent pollen fertility, although there is no direct evidence that fully formed and stained pollen is in fact always viable.

CHAPTER 2.5: HYBRIDIZATION


#### Abstract

2.5.1: Preparation for hybridization

Perennial plants to be used for hybridization were taken from open field conditions and placed in an unheated green-house, where they were kept until after seed harvest. The annual B. distachyon flowers within about $32-40$ days of sowing and seeds were sown at weekly intervals in April and May.

Crossing experiments were carried out from the middle of June to the end of September 1980, and from the end of May to the end of September 1981.


### 2.5.2: Self fertility experiments

To determine the degree of self fertility, two types of experiments were carried out:

1. Some of the inflorescences from the required taxa were bagged before anthesis until inflorescences were ready to harvest.
2. Some of the inflorescences of the required taxa were emasculated and bagged while other (unemasculated) inflorescences were bagged for pollen collection. The emasculated inflorescences were hand pollinated with pollen from the same plant. The degree of self fertility was determined as the seed-set expressed as a percentage of the total number of fertile florets.
2.5.3: Hybridization experiments

Seven species and two unknown taxa (B144 and B249) of Brachypodium were used for artificial hybrization: B. sylvaticum, B. glaucovirens, B. pinnatum, B. phoenicoides, B. retusum, B. distachyon and B. mexicanum. One of the two unknown taxa (Bl44) was originally labelled as B. rupestre, but it is not this species. Many crosspollinations in all combinations, both intraspecific and interspecific, were made using samples of B. sylvaticum, B. pinnatum, and B. phoenicoides. More limited crosses were made using the other four species.

Emasculation technique: Inflorescences were chosen in which a good number of florets had large, yellowish-green anthers a few days before anthesis would have taken place in the most mature plants. All the florets of each inflorescence were emasculated. The culm was carefully bent and placed under the binocular microscope, care being taken not to break the culm by holding the inflorescence under the microscope by means of a suitable weight on the culm below the raceme. Clean fine forceps were used for emasculation. Emasculation in all the species was difficult because the lemmas were rounded on the back and the paleas tightly enclosed the stamens within the incurved margins. Emasculation took a long time because care was taken not to damage the paleas and lemmas. The lemmas and paleas were closed again to prevent the stigmas from desiccation. The emasculated inflorescences were covered by a pollen proof
bag as shown in Plate $2.5 / 1$.
Collection of pollen: Inflorescences were bagged (a few days before anthesis was expected) with transparent envelopes made of thin glazed paper. The inside of the bag appeared yellow after anthesis. Upon tapping, the pollen accumulated in the bottom of the bag.

Pollination technique: Pollination was carried out in the morning for all the species except B. phoenicoides. Pollen was used on its day of dispersal. In the case of B. phoenicoides pollination was carried out in the morning and also in the evening. Inflorescences were pollinated successively as theymatured. In most cases this took place over 6-8 days, whereas in normal (i.e. unemasculated) florets the period was 10-13 days or more. In emasculated B. distachyon the period was 4-5 days, and in B. mexicanum 5-6 days. In the case of B. distachyon the stigmas exserted either partially or not at all. In this case pollination was carried out under the binocular microscope by opening the florets with the help of forceps and transferring the pollen with the help of a fine paint brush. Except in one case of B. glaucovirens and a few other cases all the florets of one inflorescence were pollinated with pollen from the same male plant, to avoid contamination and to facilitate recording.

A soft paint brush was used to transfer the pollen to the ripe stigma. The brush was held above the stigma and gently tapped so that the pollen fell freely on to the mature stigma. After finishing the
cross pollination of one plant, the brush was washed with $70 \%$ alcohol and dried before use in other crosses; the process of pollination was carried out in a separate room to avoid contamination by air-borne pollen. The inflorescences were bagged again after pollination. The stigmas soon lost their freshness after effective pollination.

The following precautions were found useful to get good healthy seeds:

1. The plants should be watered regularly at suitable intervals;
2. Watering should be avoided on the surface of pollen proof bag;
3. Plants should not be kept in humid conditions;
4. The bags of pollinated inflorescences should be changed at intervals of 10-15 days until seed harvest.

### 2.5.4: Embryo culture

The caryopses obtained from crossing experiments mostly had little endosperm. In order to avoid the risk of losing viable embryos by growing caryopses directly in pots, the embryo culture procedure used by Barker (1980) for Festuca and Vulpia hybrids was adopted.

In a volumetric flask 20.0 g sucrose and 3.87 g Gamborg's B5 medium was made up to one litre with distilled water. The pH of the medium was adjusted to 5.8 and
then 6.0 gm of technical grade agar was added to the solution and the medium heated in a steamer for about 45-50 minutes. The hot nutrient agar was transferred to glass vials using an automatic 10 ml pipette. About 100 vials were needed for 1 litre of nutrient agar solution. Metal caps were put on the vials. The vials were autoclaved at $121^{\circ} \mathrm{C}$ at 1 atmosphere. After sterilization the culture vials were placed at an angle of 45 degrees until the agar was set. The margins of the caps on the vials were wrapped with nescofilm and stored at $4^{\circ} \mathrm{C}$ until required.

The processes for the surface sterilization of caryopses and removal of embryos from the caryopses was carried out under sterile conditions in a laminar flow cabinet. Caryopses were sterilized in 10\% Domestos for 7-9 minutes and rinsed in three changes of sterile water. Then the caryopses were sown on moist filter paper in petri-dishes and put in an incubator at $23^{\circ} \mathrm{C}$. After 24 hours the imbibed caryopses were placed in a refrigerator at $4^{\circ} \mathrm{C}$ for seven days.

Dissection of the caryopses to remove the embryo was carried out under a binocular microscope. With the help of two sterilized needles the seed-coat was removed near the embryo so that the embryo could be isolated without any injury. The embryo was disinfected in a $10 \%$ solution of Domestos for 3-4 minutes and rinsed in three changes of sterile water. The embryos of those caryopses which had no infection were directly transferred
to vials after 3 changes of sterile distilled water. The caps of the vials were wrapped with nescofilm and the vials transferred to an incubator at $25^{\circ} \mathrm{C}$. After 3-4 weeks, when the seedlings had 2-3 leaves and several roots (Plate $2.5 / 2$ ), they were carefully removed from the agar, the roots were washed with distilled water, and the seedlings were potted up individually in compost. An inverted glass tube or beaker was placed over each seedling to prevent desiccation during the first few days. The pots were placed in the green-house at $19^{\circ} \mathrm{C}$. The seedlings grew successfully in the green-house, and were taken to the cold green-house in the botanic garden and finally to the field before winter.

Plate 2.5/1: Emasculated inflorescence of B. pinnatum bagged for crossing


Plate 2.5/2: Seedling germinated following embryo culture

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## CHAPTER 2.6: ELECTROPHORESIS

The following stock solutions were made up: Acrylamide stock: 30 g acrylamide and 0.2 g bisacrylamide in 100 ml water filtered through Watman No. 50 filter paper. Stored at $4^{\circ} \mathrm{C}$. Tris buffer pH 6.8: 36.3 g Trizma in 250 ml water, adjusted to pH 6.8 with 5 N HCl and made up to 500 ml . Stored at $4^{\circ} \mathrm{C}$.

Tris buffer pH 8.8: 113.5 g Trizma in 25 Cml water, adjusted to pH 8.8 with 1 N HCl and made up to 500 ml . Stored at $4^{\circ} \mathrm{C}$. Running buffer (Tris-glycine): 6 g Trizma, 28.8 g glycine and $\lg$ SDS, dissolved in water and made up to 1l. Stored at room temperature.

Sodium dodecyl sulphate (SDS): 100 gSDS dissolved in water and made up to $1 \ell$. Stored at room temperature. Ammonium persulphate: 1 g in 10 ml water (made fresh each day).
$\mathrm{N} \mathrm{N}^{\prime} \mathrm{N}^{\prime} \mathrm{N}^{\prime}$ - Tetramethyl-ethylenediamine (Temed): Ex
stock ( $99 \%$ pure). Stored at $4^{\circ} \mathrm{C}$.
Farrel Extract: 10.4 ml Tris pH 6.8 buffer, 10.0 ml glycerol, 5.0 ml mercaptoethanol, and 2.3 g SDS made up to 100 ml with water.

Kenacid blue: 50 ml methanol, 5 ml acetic acid, 45 ml water, and 0.2 g kenacid blue heated at $50^{\circ} \mathrm{C}$ for lhr .

Each sample consisted of thirty five caryopses ground up with fine sand (about 40-100 mesh) purified by acid. 3 ml of Farrel extract was added to each sample and the mixture was left at $4^{\circ} \mathrm{C}$ for $30-45 \mathrm{~min}$. The solution was then poured into microtubes which were then marked according to their sample. The solution was centrifuged for 2 min at 10000 G . The supernatant was retained and a drop of $10 \%$ sodium azide was added to prevent bacterial infection. Then the supernatant was collected in small tubes and stored at $4^{\circ} \mathrm{C}$ until required.

The glass mould was smeared with soft yellow paraffin and the separating gel (made as shown in Table 2.6/1) was poured in. The comb was put on top of the mould and a smáll layer of degassed distilled water was poured over the separating gel and left for half an hour to set the gel. The the comb was taken off and the water was sucked out with the help of filter paper, and the comb was replaced. A layer of stacking gel (made as shown in Table $2.6 / 1$ ) was then poured on the top of the separating gel. A layer of degassed water was poured over the stacking gel, and covered with a polythene bag to prevent evaporation. The mould was put in the cold room $\left(4^{\circ} \mathrm{C}\right)$ for 3 hr or more, after which the comb and the lower spacer were removed. The slots at the top of the gel were marked on the mould to locate the position for loading extracts, as slots disappear after pouring running buffer at the top of the tank.

## TABLE 2.6/1

Composition of separating and stacking gels

| Stock solution | Separating gel | Stacking gel |
| :--- | :---: | :---: |
| $30 \%$ acrylamide | 15.0 ml | 2.3 ml |
| 1.875 M Tris pH 8.8 | 6.0 ml |  |
| 0.6 M Tris pH 6.8 |  | 1.0 ml |
| Distilled water | 8.4 ml | 6.6 ml |
|  | Degas | Degas |
| $10 \%$ SDS | 1.0 ml | 0.5 ml |
| Temed | $15 \mu 1$ | $12 \mu \mathrm{l}$ |
| $10 \%$ ammonium |  |  |
| persulphate | $170 \mu \mathrm{l}$ |  |

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The mould was placed in the gel tank in such a way that the margin of the tank and the mould were at the same level, and bulldog clips were placed round the mould and the tank to hold the mould in the correct position. Compartments of the tank were filled with Tris-glycine buffer, avoiding the formation of bubbles at the base of the gel. By carefully underlayering, the eight slots were loaded with $30 \mu l$ extract in each slot, using a microsyringe. The marker sample contained 0.5\% bromophenol blue. A constant voltage of 60 mV with minimum current was supplied until bromophenol blue entered the stacking gel.

## Electrophoresis was continued at 120 mV

(constant voltage) until the dye moved near the bottom of the gel. The apparatus was switched off. The mould was taken out of the gel tank and then the spacers on the sides were removed. With the help of a scalpel the mould was lifted up and the gel was carefully placed in the Kenacid blue stain and left overnight. The gel was destained in a mixture of 50 ml of distilled water, 40 ml of methanol and 7 ml of acetic acid. The gel was then recorded and photographed.

Drying the gel: When the gel was ready to be dried, it was washed for 20 min in running tap water and then immersed in a mixture of 1 ml glycerol, 10 ml acetic acid, and 89 ml water for $15-20 \mathrm{~min}$. Then the gel was placed on a piece of absorbent paper on the dryer screen and covered with a polythene sheet and a cellophane sheet, ensuring that all the air bubbles were removed. A
mylar sheet was then placed over the cellophane and wrinkles were removed. The dryer and sucker were switched on until the gel was dried (about 2 hr ).

## CHAPTER 3.1: MORPHOLOGY

3.1.1: $\frac{\text { Growth habit and vegetative morphology }}{\text { Among the perennial species, B. pinnatum, }}$
B. rupestre, B. phoenicoides and B. retusum are characterized by well developed, long, creeping rhizomes, whereas B. sylvaticum and B. glaucovirens have comparatively short rhizomes which acquire a fibrous, bunched appearance. The annual B. distachyon produces axillary fertile branches from the stem. B. retusum also has branched stems, but the other perennial species have not. Table 3.1/1 summarizes the main vegetative characters of the taxa. Plate 3.1/l illustrates five of the above species and a variant of $B$. pinnatum with $2 n=18$.

The culms in B. pinnatum (Plate $3.1 / 1 \mathrm{C}$ ) are glabrous. The number of internodes is lower and the length of internodes is longer than in B. sylvaticum. The leaf-blades are usually stiff, flat or somewhat convolute, and glabrous to slightly pubescent; in the majority of cases they are thinly hairy on the upper surface and glabrous on the lower, but sometimes they are glabrous on both surfaces. It is usually found that plants with narrower leaf-blades have more glabrous leaves. There is no abaxial tuft of hairs at the junction of leaf-blade and leaf-sheath in B. pinnatum. The leaf-blades are usually narrower and longer in B. pinnatum than in B. sylvaticum. Leaf-blade ribs in B. pinnatum are usually equally spaced and some are
more prominent than others. The leaf-sheath in B. pinnatum is glabrous to hairy and the margins of the leaf-sheath may be non-ciliated or ciliated.

Morphologically, variation in B. pinnatum is not very marked, but variants of B. pinnatum with $2 \mathrm{n}=18$ show some differences from normal (Table 3.1/1). The plants B228, B229 and B283 from Iran, B 242 and B281 from U.S.S.R., and B236 and B237 from Yugoslavia produce fewer culms. Culms in these variants are mostly glabrous but sometimes pubescent. The number of internodes is usually higher and the internodes shorter than in normal B. pinnatum. Leaf-blades in these variants are slightly pubescent on the upper side and glabrous or sometimes shortly pubescent on the lower side. All these variants have no abaxial tuft of hairs at the junction of leaf-blade and leaf-sheath, except in the case of B 229 from Iran. The leaf-sheaths in B228 are glabrous with non-ciliated to slightly ciliated margins, but some of the Iranian and Yugoslavian plants have pubescent leaf-sheaths with either nonciliated or ciliated margins. Bl44 from E. Germany (also $2 \mathrm{n}=18$ ) (Plate $3.1 / \mathrm{l}$ ) has some different characters from the other plants with $2 \mathrm{n}=18$. The leaf-blades are glabrous to minutely pubescent on the upper side and glabrous on the lower side. It is a vigorous plant and produces many more culms than the $2 \mathrm{n}=18$ plants from Iran, U.S.S.R. and Yugoslavia.

The two variants of $B$. pinnatum with $2 n=36$,
specimens B115 from Italy and B248 from U.S.S.R., show some differences, as shown in Table 3.1/1. Bll5 has an abaxial tuft of hairs at the junction of the leaf-sheath and leaf-blade, whereas B248 has not.

Culms in B. sylvaticum (Plate 3.1/1A) are mostly hairy but sometimes glabrous. B239, B259, B284, B289, B291, B292 and B293 from Iran have glabrous culms. Nodes are mostly hairy but sometimes pubescent. The internodes are shorter than in B. pinnatum and B. phoenicoides. The leaf-blades are green but B239, B259, B284, B289, B291, B292 and B293 have light green leaves. The leafblades are usually soft and flat, but sometimes they are somewhat convolute. In most cases they are more hairy on the upper surface than on the lower; sometimes the upper surface is slightly hairy and the lower surface is glabrous, as in B259, B284, B289, B291, B292 and B293. However B239, from a sand-dune in Iran, has completely glabrous leaf-blades. The leaves of all the specimens of B. sylvaticum except $B 239$ above, have a tuft of hairs on the abaxial side at the junction of leaf-sheath and leaf-blade. Leaf-blades in B. sylvaticum are usually broader than in B. pinnatum, B. phoenicoides, B. glaucovirens or B. distachyon. The ribs on the leaf-blades of B. sylvaticum are more prominent on the lower surface than on the upper surface. The glabrous members of $B$. sylvaticum cannot be distinguished from the glabrous members of $B$. pinnatum on the basis of aerial vegetative morphology.

## The B. pinnatum/sylvaticum intermediates

exhibit their intermediacy mainly in leaf-blade width and texture and leaf pubescence. The culms are glabrous to hairy. Some of the intermediates have shorter internodes than glabrous specimens of B. pinnatum. B63 from Czechoslovakia and B78 from Hungary have green leaves. B 51 and B 52 from England and B53 from E. Germany have densely hairy nodes like those of $B$. sylvaticum. B63 from Czechoslovakia and B78 from Hungary have leaf-blades shortly pubescent on the upper surface and glabrous on the lower, but B53 from E. Germany has leaf-blades more hairy on the lower surface. B5 from Fairlight, England, and $B 42$ from France are characterized by having leaf-blades glabrous on both sides. B351 and B352 from Cambridgeshire, England, have leaf-blades shortly pubescent to pubescent on the upper surface and glabrous on the lower. B51 and B52 from Milton Keynes, England, and B53 have an abaxial tuft of hairs at the junction of the leaf-sheath and leafblade, whereas B78, B63, B351 and B352 have not. B5 has a slight abaxial tuft of hairs at the junction of leaf-sheath and leaf-blade. The length and width of leaf-blade also varies in intermediates. They are comparatively broad ( $6.5-9.8 \mathrm{~mm}$ ) in B 63 and B 78 .

The internodes of the culms in B. phoenicoides (Plate 3.1/1E) are always glabrous but the nodes are shortly pubescent. The leaf-blades are dark green and usually long and narrow. They are shortly pubescent
on the margin of the ribs on the upper surface and glabrous below. B. phoenicoides is easily distinguished from B. pinnatum, B. sylvaticum, B. glaucovirens and B. distachyon on the basis of this character. The ribs in B. phoenicoides are also very prominent and are flat on the face (especially the large ones). This species is not very variable, but its var. mucronatum differs in having shorter leaf-blades with hairy abaxial surfaces.

B31 from Portugal and two herbarium specimens from Greece and Spain are intermediate between B. phoenicoides and B. pinnatum. In the Portuguese plant the leaf-blades are shortly and densely pubescent on the margins of the ribs and the faces of the ribs rarely have spreading hairs. All the leaf-sheaths are hairy and have ciliated margins. In the specimen from Greece, the leaf-blades are shortly pubescent on the margins on the ribs but slightly less so than in normal B. phoenicoides. The ribs are prominent but less so than in normal B. phoenicoides. Only the lower leaf-sheaths are hairy and have ciliated margins. The specimen from Spain more closely resembles the Portuguese plant, especially in pubescence, but has slightly more prominent ribs.

The culms in all the specimens of B. glaucovirens (Plate 3.1/1B) are glabrous, as are the leaf-blades. Usually they have no abaxial tuft of hairs at the junction of leaf-sheath and leaf-blade, but Bl52 from

Sicily has this character. The ligules are usually longer than in the other species. The leaf-sheaths are mostly glabrous but sometimes slightly pubescent. The margins of the leaf-sheaths are usually not ciliated but sometimes they are slightly ciliated.

B 230 ( $2 \mathrm{n}=18$ ) from Greece and B249 ( $2 \mathrm{n}=17$ )
from Turkey are treated as B. glaucovirens/sylvaticum intermediates. Leaf-blades in B249 are glabrous to sparsely pubescent on the upper surface and glabrous on the lower, whereas B230 has leaf-blades hairy on both surfaces. The lower surface of basal leaves is more hairy than that of the culm leaves, whereas in B. pinnatum, B. phoenicoides and B. sylvaticum the opposite is the case. In B. glaucovirens all the leaf-blades are glabrous. B230 has an abaxial tuft of hairs at the junction of the leaf-sheath and leaf-blade, whereas it is absent in B249.
B. rupestre has glabrous culms but the nodes are shortly pubescent. The leaf-blades are glabrous. The leaf-sheaths are glabrous and the margins are ciliated. B. rupestre can be distinguished from B. pinnatum by its glaucous leaves and glabrous leaf-sheaths with ciliated margins. Glabrous plants of B. pinnatum have more ciliated leaf-sheath margins.

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\text { B. distachyon (Plate } 3.1 / 1 F \text { ) usually has }
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glabrous culms but they are sometimes pubescent near the nodes. Pubescence of the leaf-blades varies but usually it enables B. distachyon to be distinguished from
all the other taxa. The leaf-blades are usually pubescent to densely pubescent on both surfaces. The abaxial surface has long spreading hairs only. The ribs of the adaxial surface have long spreading hairs on the face; the margins of the ribs may be glabrous or have dense short hairs. The ribs are not or are only slightly prominent. The leaf-sheaths are usually glabrous and the margins not ciliated, but in some cases they are pubescent and the margins are ciliated.

The culms in B. retusum (including B. boissieri) are usually glabrous but may be densely and shortly pubescent. The culms are somewhat woody at the base. This species is usually recognizable by its thin, branched culms, large number of internodes, and short, narrow leafblades, which are patent and whitish green in colour. The leaf-blades are shortly pubescent on the margins and slightly so on the face of ribs on the adaxial surface. The abaxial surface of the leaf-blades is usually glabrous but sometimes pubescent. The ribs are fairly prominent and have an almost rounded apex. The leaf-sheaths may be glabrous with usually ciliated or sometimes nonciliated margins, or pubescent with a ciliated margin. There is no abaxial tuft of hairs at the junction of leaf-sheath and leaf-blade.

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3.1.2: Floral morphology
    Table 3.1/2 summarises the floral morphology
of the taxa. The inflorescence is nodding in B.
sylvaticum (Plate 3.1/lA) but erect in the other species.
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B. glaucovirens (Plate 3.1/1B) can be easily distinguished from all the other species because its spikelets are narrowly tapering at the end and are strongly patent. In B. sylvaticum the spikelets usually overlap to a greater degree than in the other species. The spikelets in B. phoenicoides (Plate $3.1 / 1 E$ ) are usually larger than in the other species, and are characteristically falcate. The spikelets in B. retusum are also slightly falcate in some cases. The spikelets are more or less terete in all the species except B. distachyon, which has laterally compressed spikelets, which are few in number (1-3(5)) and crowded at the tip of the culm. Spikelets are dark green in colour in B. phoenicoides, green in B. sylvaticum and B. distachyon, bluish-green in B. glaucovirens, light green B. pinnatum and B. rupestre, and light green to almost whitish green in B. retusum.

The degree of pubescence varies in the spikelets of B. pinnatum, B. sylvaticum and B. distachyon, but in B. phoenicoides, B. glaucovirens, and B. rupestre they are always glabrous. In B. pinnatum the spikelets are glabrous to shortly pubescent. Spikelets in B. sylvaticum are mostly pubescent but sometimes glabrous, as in B239, B259, B284, B289, B291, B292, and B293 from Iran. The spikelets in B. distachyon are usually thinly pubescent to pubescent but are sometimes glabrous. In B. retusum they are usually glabrous but are sometimes pubescent. This pubescence is very characteristic; it appears as shining pearls with a pointed end. This type of pubescence
is also found in some specimens of B. distachyon. Three quantitative characters have been useful to distinguish different taxa and to identify problematical specimens. These are the length of pedicel, the length of glumes and the length of lemma awn. B. phoenicoides is characterized by the longest pedicels, but the length is variable (1.1-3.4mm). Most of the plants which exhibit anthesis in the morning have longer pedicels (up to 3.4 mm ) than the ones which exhibit anthesis in the evening ( $1.1-2.7 \mathrm{~mm}$ ), but there is overlap. B. distachyon always has very short pedicels. The length of pedicel in B. pinnatum and B. retusum is usually less than in B. phoenicoides, but greater than in B. sylvaticum, B. glaucovirens and B. rupestre. The pedicel lengths in B. pinnatum and B. phoenicoides overlap with each other, and B. pinnatum and B. sylvaticum also overlap to a little extent with each other.

In B. distachyon the top-most spikelet has more unequal sized glumes than the lateral spikelets (top-most spikelet: lower $3.5-5.9 \mathrm{~mm}$, upper $6.3-8.2 \mathrm{~mm}$; lateral spikelets: lower $5.1-6.3 \mathrm{~mm}$, upper $5.9-7.9 \mathrm{~mm}$ ). This sort of situation does not exist in any other species. Lemmas in B. phoenicoides var. mucronatum are always characteristically cuspidate to mucronate. In B. phoenicoides var. phoenicoides and B. retusum the lemmas are usually somewhat cuspidate but vary towards acuminate. Lemmas in B. pinnatum are mostly acuminate but sometimes slightly cuspidate. In B. sylvaticum,
B. glaucovirens, B. rupestre and B. distachyon the lemmas are always acuminate.
B. phoenicoides and B. retusum have the shortest awns, and sometimes lower florets in a spikelet are almost awnless. B. sylvaticum and B. distachyon have comparatively long awns. G1abrous members of B. sylvaticum (B239, B259, B284, B289, B291, B292, B293 from Iran) can be distinguished from B. pinnatum mostly on the basis of their greater awn length. The awn length of B. glaucovirens usually overlaps with that of B. sylvaticum. B. pinnatum has shorter awns than B. sylvaticum, B. glaucovirens and B. distachyon. The awn length of B. rupestre is not significantly different from that of B. pinnatum.

The palea in all the taxa is two-keeled and the margins of the palea are ciliated. The ciliations are especially conspicuous in B. distachyon.
B. phoenicoides, B. pinnatum and B. retusum
have the largest anthers among the species studied. Anther length in B. phoenicoides is variable (4.9-9.6mm). The plants which undergo anthesis in the morning have longer anthers ( $6.2-9.6 \mathrm{~mm}$ ) than those which undergo anthesis in the evening ( $4.9-6.6 \mathrm{~mm}$ ), with very little overlap. B. distachyon has the smallest anthers ( $0.3-1 \mathrm{~mm}$ ) among all the species. In B. distachyon anther shape after dehiscence is also different from that in the rest of the species because of their relatively long anther-lobes. Combined anther-lobe length in
B. distachyon is greater than half of the total length of anther, whereas in all the other species the opposite is true. Anther length in B. sylvaticum, B. glaucovirens, B. rupestre and B. retusum is usually less than in B. pinnatum and B. phoenicoides. It has been observed that the species with long anthers have longer pedicel lengths than the species with small anthers, as shown in Table 3.1/2. Anther number in all the taxa is 3 except in one specimen of B. distachyon (B67 from Portugal) with 2 anthers.

Among the variants of B. pinnatum with $2 \mathrm{n}=36$, B248 from U.S.S.R. has glabrous spikelets while Bll5 from Italy has pubescent spikelets. 8248 has shorter pedicels and glumes than Bll5, which resembles normal B. pinnatum in this respect. The awns in B 248 are shorter than in Bll5, but both fall within the range of normal B. pinnatum. The lemma in both B115 and B248 is cuspidate. The anthers in Bll5 are shorter than in normal B. pinnatum; anther length in B 248 was not recorded.

Among the variants of B. pinnatum with $2 n=18$,
Bl44 from E. Germany has more spikelets (7-10) than B228, B229 and B283 from Iran, B242 and B281 from U.S.S.R., B236 and B237 from Yugoslavia (2-8 spikelets). Normal B. pinnatum embraces both of these ranges. Bl44 from E. Germany has somewhat patent spikelets (Plate 3.1/1D) while normal B. pinnatum and the other variants do not. Spikelets in variants from E. Germany, Iran and U.S.S.R. and in B237 from Yugoslavia are glabrous, while in B236 from Yugoslavia they are slightly pubescent. Pedicel
length and glume length in B144 are like those in normal B. pinnatum, but in the other variants with $2 \mathrm{n}=18$ they are less. Awn length in Bl44 is not significantly different from that in the other variants with $2 \mathrm{n}=18$, except that B 236 has slightly longer awns (4.3mm). The lemmas in all these variants are somewhat cuspidate at the apex. The anther length of Bl44 is unknown but in the other $2 \mathrm{n}=18$ variants it is slightly less than in normal B. pinnatum.

Therefore, in B. pinnatum, there seems to be a stronger correlation between morphological characters and geographical origin than between the former and chromosome number. The German and Italian variants with $2 \mathrm{n}=18$ and 36 resemble normal B. pinnatum ( $2 \mathrm{n}=28$ ) more than they do the variants with $2 \mathrm{n}=18$ and 36 from Iran, Yugoslavia and U.S.S.R.

There is variation in the pubescence of the spikelets of the B. pinnatum/sylvaticum intermediates. Of the two plants from Cambridgeshire, England, B351 has glabrous spikelets while B352 has pubescent ones. B51 and B52 from Milton Keynes, England, are characterized by pubescent to densely pubescent spikelets; B5 from Fairlight, England, have slightly pubescent to densely pubescent spikelets; B53 from E. Germany has glabrous spikelets; and B 63 from Czechoslovakia, B78 from Hungary and B42 from France have shortly pubescent spikelets.

Pedicel length in B. pinnatum/sylvaticum
intermediates is usually greater than in B. sylvaticum, i.e. close to that in B. pinnatum. Similarly, glume length in intermediates is usually more like that in B. pinnatum, but in B352 it is like that in B. sylvaticum. Awn length is either like B. pinnatum or intermediate. Anther length is usually closer to that of B. sylvaticum but overlaps with that of $B$. pinnatum to some extent, and in B5, B78 and B63 it is exactly like that in B. pinnatum.

In B. phoenicoides/pinnatum intermediates the spikelets are glabrous. Pedicel length in intermediates is variable. The herbarium specimen from Greece has shorter pedicels than B. pinnatum or B. phoenicoides, while in the other two specimens they range between the lengths of B. pinnatum and B. phoenicoides. B3l has longer glumes than the other two intermediates, but all three fall within the ranges of B. phoenicoides and B. pinnatum in this character. Awn length in all three plants is intermediate between that of the two species. Anther length in the intermediates is smaller than in either of the two species.

The spikelets are glabrous in the B. glaucovirens/
sylvaticum intermediates. In B230 the spikelets are patent (as in B. glaucovirens), while in B249 they are erect (as in B. sylvaticum). Both of the intermediate plants have shorter anthers than in either species but in B230 the length overlaps with that of B. sylvaticum.
3.1.3: Key to species studied based on morphological

## characters

1. Annual; spikelets laterally compressed; anthers not more than 1 mm long, with lobes more than half total length B. distachyon
2. Perennial; spikelet terete; anthers more than 3 mm long, with lobes less than half total length
3. 
4. Leaves glaucous; spikelets patent B. glaucovirens
5. Leaves not glaucous; spikelets not patent 3.
6. Adaxial leaf surface with prominent ribs with pubescent sides 4.
7. Adaxial leaf surface with inconspicuous ribs with glabrous sides
8. 
9. Face of ribs flat and glabrous; internodes not more than 6; stems not branched B. phoenicoides
10. Face of ribs rounded and pubescent; internodes at least 7; stems branched B. retusum
11. Inflorescence nodding; rhizomes poorly developed; lemma awn c. $7-14 \mathrm{~mm}$ B. sylvaticum
12. Inflorescence erect; rhizomes well developed;
lemma awn c. $2.5-4 \mathrm{~mm}$
B. pinnatum

$\begin{gathered}\text { No．of } \\ \text { florets }\end{gathered}$
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$7-13$
$7-20$
$18-19$
$13-18$
$7-20$



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Plate 3.1/1: Living plants of Brachypodium
A - B. sylvaticum
B - B. glaucovirens
C - B. pinnatum ( $2 \mathrm{n}=28$ )
D - B. pinnatum variant Bl44 ( $2 \mathrm{n}=18$ )
E - B. phoenicoides
F-B. distachyon



## $\pi$




#### Abstract

3.2.1: Epidermal anatomy

On the abaxial leaf epidermis two major zones, the costal and intercostal zones, can be recognized, but on the adaxial leaf epidermis the intercostal zone can be divided into a marginal stomatal region and a central bulliform cell region.


Usually in grass taxonomy the abaxial epidermis is more useful than the adaxial surface, but in Brachypodium the opposite is more usually the case.

The characters of the abaxial and adaxial epidermises of the different taxa studied are given in Table 3.2/1 and in the following descriptions under four headings: short-cells, hairs, stomata, and long-cells.
B. sylvaticum: Abaxial side (Plate 3.2/1A) Short-cells: On the ribs short-cells are mostly paired or in a row of 3-4 cells or sometimes more together. Paired cells are usually a cork-cell and a silica-cell together. When the short-cells are in a group of more than two, there is a mixture of cork-cells, silica-cells and hair-bases together. Silica-bodies are mostly oblong, but may be oval, between cross-shaped and dumb-bellshaped, horizontally elongated and sinuous-walled, flatly rounded, deltoid, or cross-shaped. Short-cells on the intercostal zone are mostly paired but occasionally solitary. Pairs are mostly a cork-cell paired with a silica-cell but sometimes a cork-cell with a hair-base.

Solitary cells, if present, are hair-bases. Silicabodies on the intercostal zone are small and narrowly oval.

Hairs: Large prickles on the ribs and hooks in the intercostal zone are common. Angular hairs are present on the margin of the leaf. In some specimens there are no prickles or hooks in costal or intercostal zones, or occasionaly hooks are present in the intercostal zone, but large prickles are absent. In other specimens large prickles are present on the ribs and large prickles and many hooks in the intercostal zone are common. Angular hairs are present on the margin of the leaf. In some specimens there are no prickles or hooks in costal or intercostal zones, or occasionally hooks are present in the intercostal zone but large prickles are absent. In other specimens large prickles are present on the ribs and large prickles and many hooks in the intercostal zone. Stomata: Stomata are usually in one to two discontinuous rows in the intercostal zone, but they are often very rare. Sometimes stomata are completely absent on the abaxial surface, e.g. B292, B293, B284 and B289 from Iran. Long-cells: Long-cells in the intercostal zone have somewhat thin and sinuous walls. In the costal zone of small veins they are narrower than in the intercostal zone but similarly sinuous-walled. On the midrib and larger veins the long cells have sinuous but very thick and pitted walls. The above specimens from Iran have sinuous to pitted walls on ribs and non-sinuous or slightly sinuous walls in the intercostal zone. A
sand-dune specimen (B239) from Iran has highly sinuous walls in costal as well as in intercostal zones.
B. sylvaticum: Adaxial side (Plate 3.2/1B)

Short-cells: Short-cells over the ribs may be solitary or in a continuous row of up to 10 or more; groups of 3-4 are the most common. On the intercostal zone short-cells are solitary. The range of silica-cell shapes is the same as that on the abaxial epidermis, but horizontally elongated ones are much commoner. Horizontally elongated silica-cells occur either in continuous rows or interrupted by cork-cells or hair-bases. They have 3-4(5) undulations on each side.

Hairs: Large prickles are abundant on the ribs, and hooks in the intercostal zone. Long macro-hairs are common on the ribs in some cases, but only occasionally so in others. Hooks and short macro-hairs are common in the region between stomata and bulliform cells. In some specimens numerous macro-hairs of varying sizes occur in both costal and intercostal zones. The sand-dune specimen B 239 from Iran has extremely numerous macrohairs on the margins of the ribs and also on the region between stomata and bulliform cells, and hooks are present on the ribs as well as in the intercostal zone. These hooks have rather smaller bases than in other specimens of B. sylvaticum.

Stomata: Four to six rows of stomata are present in the intercostal zone, of which usually 3-4 rows are continuous. Subsidiary cells are parallel-sided.

Long-cells: Long-cells on the ribs have thick, sinuous walls and on the margins of the ribs and in the stomatal region have thin, less sinuous walls. In some cases long-cells have only slightly sinuous walls on the ribs and non-sinuous walls in the stomatal region. Longcells on the ribs are narrower than in intercostal zones.
B. glaucovirens: Abaxial side (Plate 3.2/2A) Short-cells: Short-cells on the ribs are paired or in a row of 3-4 cells or sometimes up to 6 cells together. Cork-cells are usually paired with silica-cells. When short-cells are in a group of more than two then the silica-cells, cork-cells and the hair-bases are present together. Silica-bodies are mostly oblong or flatly circular but a few other types such as those in between dumb-bell-shaped and cross-shaped, deltoid, slightly square-shaped and narrowly oval may be present. In the intercostal zone paired or solitary cells are present. Paired cells are usually cork-cells and silica-cells together and the solitary cells are hair-bases. The silica-bodies are small and either tall and narrow or narrowly oval.

Hairs: Large prickles are common on the ribs and small prickles and hooks are abundant in the intercostal zone. In some cases large prickles and a few hooks are found on the ribs. Angular hairs are present on the leaf margins.

Stomata: Usually two rows of stomata are present in the intercostal zone. Stomatal rows are mostly continuous.

Subsidiary cells are slightly dome-shaped. No specimen was found without stomata.

Long-cells: Long-cells are characterized by sinuous walls in costal as well as in intercostal zones. This character is constant but sometimes the long-cells in the intercostal zone are slightly less sinuous than those in the costal zone.
B. glaucovirens: Adaxial side (Plate 3.2/2B)

Short-cells: Short-cells are usually present in a row of many cells together on the ribs. Most of the silicabodies are horizontally elongated sinuous-walled, or are inbetween dumb-bell-shaped and cross-shaped. Rarely some other types of silica-bodies such as oblong, deltoid and cross-shaped are present. Most of the horizontally elongated silica-bodies have 3-4(5) undulations on each side.

Hairs: Large and small prickles and short macro-hairs are present on the ribs. Short macro-hairs and hooks arise from the margins of the ribs. Hooks and short macro-hairs are present between the bulliform cells and the stomatal region. In some cases many hooks but few, short macro-hairs are present on the intercostal zone.

Stomata: Five to six rows of stomata are present in the intercostal zone, of which usually four rows are continuous. The subsidiary cells are parallel-sided. Long-cells: Long-cells on the ribs are non-sinuous to slightly sinuous; they are non-sinuous in the stomatal region.

## B. pinnatum: Abaxial side (Plate $3.2 / 3 \mathrm{~A}$ )

Short-cells: In the costal zone short-cells are mostly paired, but sometimes are in a row of 4-6 (very rarely 3) cells. Those in the intercostal zone are paired or solitary. Paired cells in the costal and intercostal zones are silica-cells coupled with cork-cells. When short-cells are in a group of more than two cells then silica-cells, cork-cells and hair-bases are grouped together. Solitary cells in the intercostal zone are hair-bases. Silica-bodies in the costal zone may be oval, rounded, tall and narrow, flatly rounded or deltoid. The silica-bodies in the intercostal zone are narrowly oval, tall and narrow, or flatly rounded. Silica-cells on the large ribs are larger than cork-cells but on smaller ribs are almost equal or smaller in size.

The variants of $B$. pinnatum with $2 n=18$ and $2 \mathrm{n}=36$ did not differ from normal $B$. pinnatum in the above characters.

Hairs: Large prickles on the ribs are infrequent to common. Hooks in the intercostal zone may be absent to common. In some cases there are prickles and a few hooks in the costal zone but no hairs in the intercostal zone. Angular hairs are present on the margins of the leaves. B144 from East Germany ( $2 \mathrm{n}=18$ ) has large macro-hairs and prickles on the ribs and numerous hooks of various sizes (difference in base and length) on the margins of the ribs and also in the intercostal zone. Hooks with large bases are fewer than the hooks with small
bases. Large prickles are occasionally present in the intercostal zone in this plant. B228 and B229 from Iran have prickles on the ribs and numerous hooks in the intercostal
zone. The hooks have large bases as in normal B. pinnatum. B248 from U.S.S.R. ( $2 \mathrm{n}=36$ ) has a few small prickles and hooks on the ribs and also a few hooks in the intercostal zone. Bll5 from Italy $(2 n=36)$ has large and small prickles on the ribs and hooks are common in the intercostal zone. Hooks in B115 and B248 have large bases as in normal B. pinnatum.

Stomata: Stomata are usually present but rare in the intercostal zone, occurring in one to two or sometimes three discontinuous rows. Subsidiary cells are usually parallel-sided. The variants with $2 n=18$ from East Germany and Iran have dome-shaped subsidiary cells. The variants with $2 n=36$ from U.S.S.R. and Italy have slightly dome-shaped subsidiary cells.

Long-cells: In the costal zone long-cells usually have non-sinuous walls, butsinuous and slightly pitted walls are present in some cases. In the intercostal zone walls of the long-cells are mostly non-sinuous but sometimes slightly sinuous. Long-cells in the costal zone are thicker walled and narrower than in the intercostal zone. Bl44 from East Germany has sinuous-walled longcells in the costal as well as in the intercostal zones. B228 and B229 from Iran have highly sinuous walls on the ribs, slightly sinuous walls near the margins of the ribs, and non-sinuous walls in the intercostal zone. Bll5 has sinuous and pitted walls in the costal zone and
non-sinuous but pitted walls in the intercostal zone. B248 has sinuous walls in the costal as well as in the intercostal zones. B144 ( $2 \mathrm{n}=18$ ) and Bll5 $(2 \mathrm{n}=36)$ do not differ in the length of their long-cells from normal B. pinnatum. But B228 and B229 ( $2 \mathrm{n}=18$ ) have shorter long-cells than normal B. pinnatum and B248 ( $2 \mathrm{n}=36$ ) has long-cells almost equal to or shorter than those in normal B. pinnatum.
B. pinnatum: Adaxial side (Plate $3.2 / 3 B$ )

Short-cells: Short-cells in the costal region are paired or in groups of $3-8$ or sometimes many more cells together; rarely they may be solitary. Solitary cells are common in the intercostal zone. The combinations of types of short-cells are as on the abaxial epidermis. Silicabodies may be oval, tall and narrow with irregular shape, flatly rounded, cross-shaped, dumb-bell-shaped, or between dumb-bell-shaped and cross-shaped. The horizontally elongated sinuous-walled silica-bodies which are typical of $B$. sylvaticum are either absent or very rare in $B$. pinnatum, and when present they usually have only three undulations on each side. Variants B228, B229, and B144 ( $2 \mathrm{n}=18$ ) are not different from normal B. pinnatum. Variant Bll5 $(2 n=36)$ has horizontally elongated sinuouswalled silica-bodies with 3-4 undulations on one side. Variant B248 ( $2 \mathrm{n}=36$ ), however, does not differ from normal B. pinnatum. Bll5 has a higher frequency of oblong silica-bodies than normal B. pinnatum.

Hairs: Large prickles are present on the ribs but their frequency varies. Hooks are common in the intercostal zone. Hairs are absent from the costal and intercostal zones in some specimens. Some specimens have prickles and occasional hooks in the costal zone but neither in the intercostal zone. In some cases large and small prickles, macro-hairs of varying sizes, and a few hooks are present in the costal zone. In the intercostal zone there are hooks and short macro-hairs between the stomatal and bulliform regions. The variants of B. pinnatum with $2 \mathrm{n}=18$ and $2 \mathrm{n}=36$ do not differ from normal B. pinnatum in the above characters.

Stomata: Four to eight rows of stomata are found in the intercostal zone; usually 4 rows are continuous. The variants of $B$. pinnatum with $2 \mathrm{n}=18$ and $2 \mathrm{n}=36$ do not differ from normal B. pinnatum in the above characters except that B115 ( $2 \mathrm{n}=36$ ) has up to 10 discontinuous rows of stomata. However 4 rows are continuous as in normal B. pinnatum. Long-cells: Long-cells on the ribs have thick, nonsinuous to sinuous and rather pitted walls. Long-cells in the stomatal region also have non-sinuous to sinuous walls, but the walls are thinner and not pitted. Costal zone long-cells are narrower and rather thicker walled than those of the intercostal zone. The variants with $2 \mathrm{n}=18$ and $2 \mathrm{n}=36$ also fall in the above range of characters.

$$
\text { B. rupestre: Abaxial side (Plate } 3.2 / 4 \mathrm{~A} \text { ) }
$$

Short-cells: Short-cells in the costal zone are paired or in a row of 4 cells, and in the intercostal zone are paired. Silica-bodies in the costal zone may be deltoid,
flatly rounded or oblong. Silica-bodies in the intercostal zone may be small, narrowly oval, or tall and narrow. Hairs: In the costal zone large prickles are infrequent to common. Prickles and hooks in the intercostal zone are absent, or hooks are rarely present. Angular hairs are present on the margins of the leaves.
Stomata: Stomata are occasionally present'in l-2 rows in the intercostal zone. Subsidiary cells are parallelsided.

Long-cells: Long-cells in the costal zone have sinuous and pitted walls. Long-cells on the large ribs have sinuous and somewhat pitted walls and on the small ribs have sinuous walls. Long-cells in the intercostal zone are slightly sinuous-walled.

## B. rupestre: Adaxial side (Plate 3.2/4B)

Short-cells: Short-cells on the ribs are mostly paired or in groups of many (up to 6 or more) cells. In the intercostal zone only solitary cells are present. The combinations of cell types are as in B. pinnatum. Silica-bodies may be cross-shaped, intermediate between cross-shaped and dumb-bell-shaped, oval, squarish, flatly rounded, or tall and narrow. Horizontally elongated sinuous-walled silica-bodies are also present. The frequency of those silica-bodies inbetween cross-shaped and dumb-bell-shaped is higher than in the rest of the species. Hairs: No hairs are present.
Stomata: Stomata are present in 4-8 rows in the intercostal zone, with usually 4 rows continuous. Subsidiary cells are parallel-sided.

Long-cells: Long-cells in the costal zone have sinuous and pitted walls. In the stomatal region they have slightly sinuous walls.
B. phoenicoides: Abaxial side (Plate 3.2/5A)

Short-cells: In the costal region short-cells are usually paired or in a row of 3-4. In the intercostal zone they are paired and usually a silica-cell and a cork-cell are coupled. When short-cells are in a group of 4 they are usually a pair of couples, and groups of 3 consist of two cork-cells and a silica-cell. Silica-bodies in the costal region may be rounded to flatly rounded, tall and narrow, or oval. In the intercostal zone they are tall and narrow, or narrowly oval.

Hairs: Numerous large prickles and small prickles occur in the costal zone. In the intercostal zone small prickles and hooks are present. In some cases prickles are present only in the costal zone, with no hairs in the intercostal zone.

Stomata: One to two rows of stomata are present in the intercostal zone, where they are mostly continuous but sometimes discontinuous. Subsidiary cells are domeshaped.

Long-cells: Long-cells in the costal zone as well as in the intercostal zone are sinuous-walled. This is a constant character in B. phoenicoides. Long-cells in the costal zone are narrower than in the intercostal zone.
B. phoenicoides: Adaxial side (Plate $3.2 / 5 \mathrm{~B}$ )

Short-cells: In the costal zone short-cells are mostly paired orsolitary, but sometimes occur in a group of 4-6. Short-cells in the intercostal zone are solitary. When paired the silica-cells are coupled with corkcells. When short-cells are more than 2 together silicacells, cork-cells and hair-bases may occur in combination. Solitary cells in the intercostal zone are hair-bases. The shape of the silica-bodies may be tall and narrow, oval, or flatly rounded. Horizontally elongated silicabodies with sinuous walls are absent.

Hairs: The costal zone is densely covered with sharp and slightly curved macro-hairs of various sizes. These hairs are also present in the intercostal zone. This is a good distinguishing character in this species. Large prickles and hooks are sometimes present in the costal region. Hooks are also present in the intercostal zone. Stomata: Four to six rows of stomata are present in the intercostal zone. The stomata are not easily visible due to numerous macro-hairs spreading over the stomatal region. Subsidiary cells are parallel-sided. Long-cells: The long-cells on the costal zone as well as in the stomatal region are sinuous-walled. This is a constant character.

$$
\text { B. retusum: Abaxial side (Plate } 3.2 / 6 \mathrm{~A} \text { ) }
$$

Short-cells: Short-cells in the costal zone are paired or solitary or in a group of 3-4 cells. 'Crown-cells' are present abundantly in the costal as well as intercostal zones. Cork-cells are usually coupled with silica-cells. When short-cells are more than two then silica-cells,
cork-cells, crown-cells and hair-bases may be present together. Silica-bodies are oval to narrowly oval in both the costal and intercostal zones.

Hairs: Large prickles are present on the ribs and numerous small prickles and sometimes a few hooks are present in the intercostal zone. In some cases there are many prickles and a few hooks in the intercostal zone, with no hooks or prickles on the ribs.

Stomata: Stomata are absent.
Long-cells: Long-cells are highly sinuous-walled both in the costal and intercostal zones. This is a constant character in this species. Long-cells are shorter than in all the other species.
B. retusum: Adaxial side (Plate $3.2 / 6 B$ )

Short-cells: In the costal zone short-cells are mostly solitary or paired; rarely they are in a row of 3-4 cells. Silica-cells are usually paired with a corkcell. When short-cells are in a group of 3-4 then cork-cells, silica-cells, crown-cells and hair-bases may occur together in various combinations.

Silica-bodies are mostly tall and narrow and slightly curved, but sometimes are narrowly oval or squarish. In Bl73 from France no crown-cells were seen.

Hairs: In the costal zone large prickles are present on the ribs. Numerous slightly curved macro-hairs of various length are present on the ribs and margins of the ribs and also inbetween the stomatal and bulliform cell regions.

In some cases hooks are also present on the ribs.
Stomata: Stomata are not easily visible because of the numerous macro-hairs. Four rows of stomata are present in the intercostal zone, but the stomatal rows are discontinuous in some places.

Long-cells: Long-cells on the ribs have slightly sinuous to pitted walls and in the intercostal zone have nonsinuous or slightly sinuous walls.

## B. distachyon: Abaxial side (Plate 3.2/7A)

Short-cells: Short-cells in the costal zone are paired, solitary or in a group of up to 5 cells. Crown-cells are found on the ribs as well as in the intercostal zone. Crown-cells are common in some cases but rare in others. Silica-cells are coupled with cork-cells. Silicabodies may be dumb-bell-shaped, intermediate between dumb-bell-shaped and cross-shaped, elliptical, flatly rounded, ovoid, squarish, or horizontally elongated with sinuous walls.

Hairs: In the costal zone there are macro-hairs of varying sizes, and prickles are common. In the intercostal zone numerous hooks are present. Angular hairs are present on the margin of the leaf. In some cases a few large macro-hairs are present on the ribs and short macro-hairs and hooks are present in the intercostal zone.

Stomata: Two to three rows of stomata occur in the intercostal zone. Only 2 of these rows are continuous, which is a good character to distinguish B. distachyon from other taxa. Subsidiary cells are parallel-sided.

Long-cells: Long-cells in the costal zone as well as in the intercostal zone have non-sinuous walls, but those on the margins of the leaf have sinuous walls.
B. distachyon: Adaxial side (Plate 3.2/7B)

Short-cells: Short-cells usually occur in a row of 3 to many cells on the ribs; rarely they are paired. Silica-bodies may be horizontally elongated with sinuous walls, dumb-bell-shaped, flatly rounded, oval, oblong, or between dumb-bell-shaped and cross-shaped.

Hairs: In the costal zone there are large prickles, hooks and macro-hairs of considerable length. In the intercostal zone hooks are present between the bulliform and stomatal regions.

Stomata: Four rows of stomata are present in the intercostal zone, of which 2 rows are continuous. Subsidiary cells are parallel-sided.

Long-cells: Long-cells in the costal zone as well as in the intercostal zone have non-sinuous walls.
B. pinnatum/sylvaticum intermediates

Characters are mentioned only where they differ
in the two species B. pinnatum and B. sylvaticum.
Abaxial side (Plate 3.2/8A): Short-cells on the ribs are mostly paired or in a group of 3-4 cells together. The long-cells on the ribs are very sinuous-walled and the walls are pitted in some cases, but the walls are non-sinuous and not pitted in other cases. In the intercostal zone the long-cell walls are slightly sinuous in some cases and non-sinuous in others. The long-cells in B53 from E. Germany and B70 from Poland are longer
than in B. pinnatum and B. sylvaticum.
Adaxial side (Plate 3.2/8B): Short-cells on the ribs are usually in a group of many ( 8 or more) cells together. The frequency of horizontally elongated sinuous-walled silica-bodies varies, but is less than in B. sylvaticum and more than in B. pinnatum, except for B70 from Poland, which has almost as many as in B. sylvaticum. The frequency of silica-bodies between dumb-bell-shaped and cross-shaped in B5 from Fairlight, England, is slightly higher than in the other plants. On the ribs a few prickles and long macro-hairs are present. Long-cells in the costal zone have thin sinuous and pitted walls in some cases, while in others they have thin non-sinuous but pitted walls.
B. glaucovirens/sylvaticum intermediates

Characters are mentioned only where they differ
in the two species B. glaucovirens and B. sylvaticum. Abaxial side: Many hooks and a few prickles are present in the intercostal zone. In the case of B249 from Turkey crown-cells are present in the intercostal zone, although they are absent from both B. sylvaticum and B. glaucovirens. Long-cells in B249 from Turkey are shorter than in B. glaucovirens and B. sylvaticum, but in B230 from Greece they are not different from those of the above two species.
B. phoenicoides/pinnatum intermediate

Characters are mentioned only where they differ
in the two species B. phoenicoides and B. pinnatum.

Abaxial side (Plate $3.2 / 9 \mathrm{~A}$ ): Long-cells on the ribs have thick sinuous walls but are non-sinuous in the intercostal zone.

Adaxial side (Plate 3.2/9B): Numerous macro-hairs, which are slightly curved and of various sizes, arise from the margins of the ribs and are also present in the stomatal region. These hairs are exactly like those of B. phoenicoides.

### 3.2.2: Leaf internal anatomy

The distribution of sclerenchyma on the abaxial and adaxial sides, the number and length of bulliform cells, and the section thickness are given in Table 3.2/2.

Sclerenchyma was measured on the main ribs but excluding the midrib.

Vascular bundles in all the taxa are of two types, large vascular bundles in the large ribs and small vascular bundles in the small ribs, with no intermediates, but there are no qualitative differences between them. The midrib vascular bundle closely resembles those in the larger ribs.

Although the hairs are often a very conspicuous feature of leaf-sections; they have been described under the leaf epidermis and will not be mentioned again here.

Characters of the mesophyll and other internal parenchyma, and also of the vascular bundles, seem to be of no diagnostic value in Brachypodium, and are not described here.
B. sylvaticum (Plate 3.2/10A): The adaxial surface of the leaf is smooth, but on the abaxial side the ribs (expecially the midrib) are very slightly raised. On the adaxial surface 3-8 loosely packed bulliform cells completely fill what would otherwide be shallow intercostal grooves. Sclerenchyma distribution on both abaxial and adaxial sides is variable. In most of the ribs lateral extensions of the sclerenchyma are better developed on the abaxial side, but sometimes they are nearly equal on both sides or rarely more on the adaxial side.
B. glaucovirens: On the adaxial side leaf flatness and bulliform cell characters are as in B. sylvaticum. On the abaxial side the midrib and the adjacent row of ribs are more prominent than in B. sylvaticum. Sclerenchyma is well developed on both adaxial and abaxial sides, but it extends laterally more on the adaxial than on the abaxial side. B. pinnatum (Plate 3.2/11A): The leaf flatness and bulliform cell arrangement is similar to the case in B. sylvaticum, but the ribs are usually slightly raised on the adaxial surface, especially Bl44 ( $2 \mathrm{n}=18$ ) from E. Germany. In specimens from Fairlight, England, the bulliform cells are more compact than in other specimens, and have broader bases with pointed apices almost converging to the same point.

Sclerenchyma is well developed on both abaxial and adaxial sides. It spreads laterally more on the abaxial than on the adaxial side.
B. rupestre: B. rupestre does not differ from B. pinnatum, except that the bulliform cells are like those of the Fairlight specimens of $B$. pinnatum rather than like the majority of specimens.
B. phoenicoides (Plate 3.2/11C): On the abaxial side the rib prominence is as in B. sylvaticum and B. pinnatum. However on the adaxial side the ribs are very prominent and are flat at the tops, being separated by deep grooves with bulliform cells only at the bottom. This is a good character which separates B. phoenicoides from all the other species except B. retusum. Sclerenchyma extends laterally more on the adaxial side than on the abaxial side, except that in a good number of specimens there is a single layer of sclerenchyma cells underneath the abaxial epidermis. In the measurements of the sclerenchyma lateral extensions, this single layer, when present, has been ignored. B. retusum (Plate 3.2/10B): This species has a similar leaf-section anatomy to that of B. phoenicoides, except that the ribs on the adaxial side are rounded (not flat) at the apex, hence giving a good diagnostic character.
B. distachyon (Plate 3.2/10C): The leaf shape in section is different from that in all other species in that the regions containing the major vascular bundles bulge conspicuously as broad rounded ribs on both surfaces. The bulliform cells are less differentiated from the other epidermal cells, and are more rounded (or are rectangular),
than in the other species. The sclerenchyma is less extensive than in the other species, with rather more on the adaxial than on the abaxial side.
B. pinnatum/sylvaticum intermediates: The ribs are developed on the adaxial side as in B. pinnatum, and the bulliform cells are compact and convergent as in the specimen of B. pinnatum from Fairlight (or as in B. rupestre).
B. phoenicoides/pinnatum intermediate (Plate 3.2/11B): With regard to the ribs and grooves on the adaxial side, this plant is intermediate between $B_{\text {. }}$ phoenicoides and B. pinnatum, and the ribs are more rounded at the apex than in B. phoenicoides. The sclerenchyma is better developed on the abaxial side, as in B. pinnatum.
B. glaucovirens/sylvaticum intermediate: This plant resembles B. glaucovirens in leaf sectional anatomy.

### 3.2.3: Summary of leaf anatomy

Some leaf anatomical characters have proved to be helpful in solving the identity of certain taxa. The presence of crown-cells, leaf hairyness, distribution of stomata, type of silica-bodies (and especially the presence or absence of horizontally elongated sinuouswalled silica-bodies), sinuousness of walls of the longcells, length of long-cells on abaxial side, type of ribbing on adaxial surface, shape of bulliform cells, and distribution of sclerenchyma on abaxial and adaxial sides have been the most useful characters in the present
study.
Among the species studied, B. retusum and B. distachyon can easily be recognized because of the presence of crown-cells ('cellule en couronne' of Prat (1932)), which are absent in B. sylvaticum, B. glaucovirens, B. pinnatum, B. rupestre and B. phoenicoides. But the crown-cells have been observed in B249 from Turkey which is a B. glaucovirens/sylvaticum intermediate. In B. distachyon and B249 crown-cells are only on the abaxial side, while in B. retusum they are on both sides, except in B173 from France which has them only on the abaxial side.
B. phoenicoides and B. retusum can easily be distinguished from the rest of the species because of the presence of numerous short, slightly curved macrohairs on the margins of the ribs and in the region between the stomata and bulliform cells. B. sylvaticum, B. glaucovirens, B. pinnatum, B. rupestre and B. distachyon, in spite of some small differences, usually exhibit a broad similarity in their hairyness. Numerous hooks in the intercostal zone and prickles on the ribs were said to be present in B. rupestre by Borsos (1974), but the B. rupestre used in the present study has only occasional hooks in the intercostal zone and a few prickles on the ribs. The frequency of prickles in the costal and intercostal zones of the abaxial side of B. glaucovirens is higher than in B. sylvaticum, and in some specimens of $B$. sylvaticum prickles are absent on the abaxial side.

A sand-dune specimen of B. sylvaticum from Iran (B239) has extremely numerous macro-hairs of varying sizes on the adaxial side. Slightly curved macro-hairs, which are characteristic of B. phoenicoides, have been observed in a B. phoenicoides/pinnatum intermediate (B3I from Portugal), which indicates that it might be hybrid in origin.
B. distachyon has continuous rows of stomata on the abaxial side, while the rest of the taxa have discontinuous stomatal rows and variable distribution on the abaxial side, except for B. retusum which has no stomata on the abaxial side. In B. retusum, discontinuous rows of stomata sometimes occur even on the adaxial side, which is not the case in the rest of the species. B. phoenicoides and B. glaucovirens have a higher frequency of stomata on the abaxial side than B. sylvaticum and B. pinnatum. Metcalfe (1960) said that stomata are absent on the abaxial side of $B$. pinnatum. This is true in some cases, but some specimens have occasional stomata on the abaxial side. In B. sylvaticum the situation is the same. St. Yves (1934) said that the distribution of stomata varies on different parts of the leaves in Brachypodium species. I have observed in some cases of B. pinnatum and B. sylvaticum that stomata are absent towards the tip of the leaves on the abaxial side, yet are present from the middle towards the basal portion of the leaf-blade. Borsos (1974) described B. rupestre as without stomata on the abaxial side, but stomata have been observed on the abaxial side of $B$. rupestre in the present
study. (Actually, Borsos stated the adaxial side, but, as mentioned before, she confused the two leaf surfaces). B. sylvaticum, B. glaucovirens, and B. distachyon can be distinguished from B. pinnatum, B. rupestre, B. phoenicoides and B. retusum because of the presence of numerous horizontally elongated sinuous-walled silicabodies on the adaxial side. This type of silica-body is completely absent from B. phoenicoides and B. retusum. In B. pinnatum (including variants) such silica-bodies are occasionally present but usually absent. B. rupestre also has fewer such silica-bodies than B. sylvaticum. The specimens which are intermediate between B. pinnatum and $B$. sylvaticum usually have fewer horizontally elongated sinuous-walled silica-bodies than B. sylvaticum, but sometimes the difference is scarcely discernible. In B. rupestre the frequency of the silica-bodies which are either between cross-shaped and dumb-bell-shaped or cross-shaped is higher than in the rest of the species. But variants of B. pinnatum B248 $(2 \mathrm{n}=36)$ and B228, B229 and B283 ( $2 \mathrm{n}=18$ ) from Iran also have more such silica-bodies. Bl44 ( $2 \mathrm{n}=18$ ) from E. Germany occasionally has such silicabodies. Bll5 $(2 n=36)$ from Italy has a higher number of oblong silica-bodies than the rest of the taxa studied. The length of the long-cells in B. sylvaticum, B. glaucovirens, B. pinnatum and B. rupestre usually does not differ. B. phoenicoides has slightly shorter and $B$. distachyon slightly longer long-cells than the above mentioned species, but they also overlap with them. B. retusum usually has the shortest of all. The variants of
B. pinnatum B228, B229, and B283 ( $2 \mathrm{n}=18$ ) from Iran have long-cells almost as short as in B. retusum. The variant B248 ( $2 \mathrm{n}=36$ ) from U.S.S.R. also has long-cells shorter than in normal B. pinnatum. Some of the specimens of B. pinnatum/sylvaticum intermediates have longer long-cells than the rest of the taxa. The B. glaucovirens/sylvaticum intermediate (B249) from Turkey has shorter long-cells than either B. glaucovirens or B. sylvaticum. But in B230 from Greece the length is as in B. sylvaticum and B. glaucovirens.
B. phoenicoides and B. retusum are usually
characterized by highly sinuous-walled long-cells, B. distachyon has non-sinuous-walled long-cells. B. sylvaticum usually has long-cells with sinuous walls and B. pinnatum with non-sinuous walls, but both vary. B. glaucovirens and B. rupestre both have sinuous-walled long-cells. B. pinnatum/sylvaticum intermediates vary from sinuous-walled to non-sinuous-walled. B: glaucovirens/ sylvaticum intermediates are characterized by sinuous-walled long-cells. The B. phoenicoides/pinnatum intermediate haslong-cells with sinuous walls on the ribs and nonsinuous walls in the intercostal zone, indicating its similarity to B. pinnatum.

Three shapes of leaf-sections have been observed in the species studied. B. sylvaticum, B. glaucovirens, B. pinnatum and B. rupestre have almost smooth or slightly raised ribs on the adaxial side. B. phoenicoides and B. retusum have prominent ribs which are usually flat at
the apex in B. phoenicoides and rounded in B. retusum. The leaf-section of $B$. distachyon bulges slightly at the ribs on both surfaces.

Bulliform cells are rounded to more or less rectangular in B. distachyon, while in the rest they are broad at the base and narrow at the apex. B. glaucovirens can be distinguished from B. sylvaticum because the sclerenchyma extends laterally further on the adaxial side than on the abaxial side, while in B. sylvaticum the opposite is the case. B. pinnatum, B. rupestre and B. retusum resemble B. sylvaticum in this respect, while B. distachyon and B. phoenicoides resemble B. glaucovirens, except in some cases of B. phoenicoides where there is a single continuous layer of sclerenchyma cells underneath the abaxial epidermis. B. pinnatum/sylvaticum intermediates are more like B. pinnatum in their leaf-section anatomy. The B. phoenicoides/pinnatum intermediate is inbetween B. phoenicoides and B. pinnatum. The B. glaucovirens/ sylvaticum intermediate is more like B. glaucovirens.
3.2.4: Key to species based on leaf-blade anatomical

1. Stomata in continuous rows on abaxial side; bulliform cells rounded to slightly rectangular B. distachyon
2. Stomata not in continuous rows on abaxial side; bulliform cells broad at base and narrow at apex
3. Crown-cells present; stomata absent on abaxial side
B. retusum
4. Crown-cells absent; stomata mostly present on abaxial side 3 .
5. Ribs on adaxial side prominent; bulliform cells present only at base of intercostal grooves B. phoenicoides
6. Ribs on adaxial side not prominent; bulliform cells almost filling intercostal grooves 4 .
7. Sclerenchyma laterally more extended on adaxial side than abaxial side
B. glaucovirens
8. Sclerenchyma laterally more extended on abaxial side than adaxial side 5 .
9. Horizontally elongated silica-bodies with sinuous walls very frequent on adaxial side; long cells with sinuous walls on abaxial side
B. sylvaticum
10. Horizontally elongated silica-bodies with sinuous walls absent or rare on adaxial side; longcells usually with non-sinuous (but sometimes sinuous) walls on abaxial side
B. pinnatum


| Taxon S | Stomal length ( $\mu \mathrm{m}$ ) | Presence or absence of horizontally elongated sinuouswalled silica-bodies on adaxial side | Length of horizontally elongated sinuouswalled silica-bodies (or, if absent, length of largest silicabodies) ( $\mu \mathrm{m}$ ) |
| :---: | :---: | :---: | :---: |
| B. sylvaticum | 21.0-24.1 | Abundant | 32.5-47.5 |
| B. glaucovirens | 21.9-24.4 | Abundant | 31.6-40.6 |
| $\frac{\text { B. pinnatum }}{(2 n=28)}$ | 19.1-26.4 | Absent or rare | 25.0-30.0 |
| $\begin{aligned} & \frac{\text { B. pinnatum }}{\text { variant }} \\ & (2 n=18)(B 144) \end{aligned}$ | 20.0 | Absent |  |
| $\begin{aligned} & \text { B. pinnatum } \\ & \text { variant } \\ & (2 n=18)(\text { B228, } \\ & \text { B229) } \end{aligned}$ | 20.0-24.4 | Absent | (14.0-14.2) |
| $\begin{aligned} & \frac{\text { B. pinnatum }}{\text { Variant }} \\ & (2 n=36)(B 248) \end{aligned}$ | 23.3 | Rare | 18.8 |
| $\begin{aligned} & \frac{\text { B. pinnatum }}{\text { variant }} \\ & (2 \mathrm{n}=36)(\mathrm{Bl115)} \end{aligned}$ | 25.6 | Not common | 22.5 |
| B. rupestre | 22.5-23.3 | Not common | 15.0-32.5 |
| B. phoenicoides | s 23.3-29.4 | Absent | (10.0-12.5) |
| B. retusum | 23.1-28.8 | Absent | (12.5-15.0) |
| B. distachyon | 24.2-32.2 | Abundant | (27.5-50.0 |
| B. pinnatum/sylvaticum intermediates |  |  |  |
| B5 | 26.7 | Not common | 31.4 |
| B42 | 20.6 | Not common | 28.3 |
| B52 | 21.0 | Not common | 26.5 |
| B53 | 22.5 | Common | 37.5 |
| B. Pinnatum/sylvaticum intermediates |  |  |  |
| B70. | 21.8 | Abundant | 42.5 |
| B63 | 23.8 | Common | 29.6 |
| B78 | 25.0 | Common | 33.0 |
| B. glaucovirens/sylvaticum intermediates |  |  |  |
| $\begin{aligned} & 8249 \\ & (2 n=17) \end{aligned}$ | 23.2 | Abundant | 35.0 |
| $\begin{aligned} & \stackrel{5230}{(2 n=18)} \end{aligned}$ | 24.1 | Abundant | 36.4 |
| B. phoenicoides/pinnatum intermediate |  |  |  |
| B31 | 25.8 | Absent | 16.3 |

TABLE 3.2/2

| Taxon | $\begin{gathered} \text { Midrib } \\ \text { thickness }(\mu \mathrm{m}) \end{gathered}$ | No. rows of bulliform cells | Height of bulliform cells ( $\mu \mathrm{m}$ ) | Sclerenchyma width adjacent to epidermis ( $\mu \mathrm{m}$ ) |  |
| :---: | :---: | :---: | :---: | :---: | :---: |
|  |  |  |  | Adaxial side | Abaxial side |
| B. sylvaticum | 250-430 | 3-8 | 19.4-39.5 | 36.3-54.0 | 40.6-67.0 |
| B. glaucovirens | 360-460 | 4-9 | 25.6-39.4 | 61.8-83.1 | 53.9-75.7 |
| $\frac{\text { B. pinnatum }}{(2 n=28)}$ | 250-500 | 3-10 | 22.3-50.3 | 50.8-56.3 | 65.8-71.3 |
| $\frac{\text { B. pinnatum }}{\text { variant }} \begin{aligned} & (2 n=18)(B 144) \end{aligned}$ | 350 | 3-8 | 20-30 | 57.5 | 79.5 |
| $\begin{aligned} & \text { B. pinnatum } \\ & \frac{\text { variant }}{(2 n=18)} \\ & \text { B281, B283) } \end{aligned}$ | 250-320 | 4-9 | 21.3-42.5 | 33.3-36.3 | 46.0-66.7 |
| $\frac{\text { B. pinnatum }}{\text { variant }} \begin{aligned} & (2 n=36) \quad(B 248) \end{aligned}$ | 470 | 4-10 | 32.5-75.0 | 72.5 | 90.4 |
| $\begin{aligned} & \frac{\text { B. pinnatum }}{\text { variant }} \\ & (2 n=36) \quad(B 115) \end{aligned}$ | 380 | 5-6 | 20.0-50.0 | 50.0 | 71.3 |
|  |  |  |  |  | - |
| B. rupestre | 245-520 | 3-7 | 25.0-55.0 | 43.8-85.0 | 63.8-94.9 |
| B. phoenicoides | 350-540 | 3-7 | 18.6-44.3 | 87.5-131.0 | 68.3-95.0 |
| B. retusum | 240-280 | 4-8 | 15.0-43.8 | 38.3-62.5 | 55.0-71.5 |
| B. distachyon | 370-420 | 2-6 | 18.8-37.5 | 67.5-87.5 | 50.0-66.7 |
| B. pinnatum/sylvaticum intermediates |  |  |  |  |  |
| B5 | 330 | 5-6 | 22.5-47.5 | 46.3 | 56.9 |
| B42 | 420 | 4-7 | 20.0-42.5 | 45.6 | 50.8 |
| 851 | 300 | 3-7 | 17.5-32.5 | 35.5 | 43.5 |
| B52 | 250 | 4-8 | 22.5-62.5 | 41.9 | 54.4 |
| B63 | 400 | 4-8 | 15.0-35.0 | 35.5 | 46.0 |
| B78 | 470 | 4-10 | 25.0-47.5 | 48.8 | 58.1 |
| B. glaucovirens/sylvaticum intermediate |  |  |  |  |  |
| B249 | 230 | 3-8 | 20.0-35.0 | 47.5 | 33.6 |
| B. phoenicoides/pinnatum intermediate |  |  |  |  |  |
| B31 | 360 | 2-8(12) | 18.3-36.7 | 52.9 | 58.6 |

Plate 3.2/l: Leaf epidermis of B. sylvaticum

> A - Abaxial side

B - Adaxial side


A


B

Plate 3.2/2: Leaf epidermis of B. glaucovirens
A - Abaxial side
B - Adaxial side


A

## 1 <br> 0.2 mm



B

Plate 3.2/3: Leaf epidermis of B. pinnatum
A - Abaxial side
B - Adaxial side


A

## $\longmapsto$

0.2 mm


B

## 144

Plate 3.2/4: Leaf epidermis of B. rupestre
A - Abaxial side
B - Adaxial side


A


B

Plate 3.2/5: Leaf epidermis of B. phoenicoides
A - Abaxial side
B - Adaxial side


A


## 0.2 mm



B

Plate 3.2/6: Leaf epidermis of B. retusum
A - Abaxial side
B - Adaxial side


A

## 1

0.2 mm


B

Plate 3.2/7: Leaf epidermis of B. distachyon
A - Abaxial side
B - Adaxial side


B

Plate 3.2/8: Leaf epidermis of B. pinnatum/sylvaticum intermediate

A - Abaxial side
B - Adaxial side


B

Plate 3.2/9: Leaf epidermis of B. phoenicoides/
pinnatum intermediate
A - Abaxial side
B - Adaxial side


B

Plate 3.2/10: Leaf section of Brachypodium
A - B. sylvaticum
B - B. retusum
C - B. distachyon


Plate 3.2/11: Leaf section of Brachypodium
A - B. pinnatum
B - B. phoenicoides
$C$ - B. phoenicoides/pinnatum


B


C

## CHAPTER 3.3: ELECTROPHORESIS

The relative positions of the seed-protein bands for B. sylvaticum, B. glaucovirens, B. pinnatum (including variants), B. rupestre, B. phoenicoides, B. retusum, B. mexicanum, B. distachyon, B. pinnatum/ sylvaticum intermediates and B. glaucovirens/sylvaticum intermediates are shown in Fig. 3.3/1. The principal band positions have been designated by the numbers 1-8.* The method of pairing affinity or similarity index described by Sokal \& Sneath (1963) and Romero Lopes et al. (1979) has been used for analysing the data. The degree of pairing affinity (P.A.) between two species, based on the results of electrophoretic analysis, is calculated by the following formula:

$$
\text { P.A. }=\frac{\text { Bands common to species A \& B }}{\text { Total bands in A \& B }} \times 100
$$

The results of pairing affinity between the species are summarized in Table 3.3/lA; a dendrogram expressing the 'average linkage' (UPGMA) relationships between them is shown in Fig. 3.3/2. Plate 3.3/1 illustrates the positions and characteristics of the bands on gels from different taxa.

Among the species studied, B. sylvaticum,
B. glaucovirens, B. pinnatum, B. rupestre, B. phoenicoides and $B$. retusum showed a high affinity with each other (above 78\%). Among these, B. sylvaticum and B. glaucovirens * See note P. 163
showed the highest affinity of all (93\%). A slightly lower level of affinity was shown by B. pinnatum with B. glaucovirens and B. sylvaticum, and B. phoenicoides with B.rupestre. B. retusum linked with the above five species at a lower, though still high, level (78.1\%). B. distachyon and B. mexicanum showed a high level of affinity with each other, but linked to the other six species at the lowest level of all ( $70.5 \%$ ). B. phoenicoides showed the highest average affinity with other species.
B. pinnatum showed a high affinity with all of its variants (Table 3.3/1B). However, it showed highest affinity with B248 ( $2 \mathrm{n}=36$ ) from U.S.S.R., and comparatively less with the $2 \mathrm{n}=18$ variants B 228 , B 229 , B283 from Iran and B24I from U.S.S.R. The B. pinnatum variant Bl44 (2n=18) from E. Germany had a higher affinity with B. rupestre than with normal B. pinnatum or with the variants from Iran $(2 n=18)$ and U.S.S.R. ( $2 \mathrm{n}=18$ and 36 ).

The relationships of the intermediate plants are also shown in Plate 3.3/1B. The B. pinnatum/ sylvaticum intermediates showed higher affinity with B. pinnatum than with B. sylvaticum. The B. glaucovirens/ sylvaticum intermediate showed a slightly higher affinity with B. glaucovirens than with B. sylvaticum.

The band patterns have proved to be very useful in characterizing some of the taxa.
B. sylvaticum and B. glaucovirens have a
characteristic band pattern at position 6, where there are two dark bands with a very light band in between.

But this band pattern also differs between these two species, because in B. sylvaticum the two dark bands separate further apart from the middle light band than in B. glaucovirens. Moreover, in B. glaucovirens the bands at positions 7 and 8 are less dark than in B. sylvaticum. The glabrous members of B. sylvaticum (B239, B289, B292, B293) from Iran have been identified in this work as B. sylvaticum rather than B. pinnatum or B. glaucovirens mainly because of the characteristic band pattern at position 6.

This band pattern characteristic of B. sylvaticum and B. glaucovirens has not been found in any other species in this study. This is especially useful in distinguishing morphologically unusual plants of B. sylvaticum and B. pinnatum. The bands in B. sylvaticum are usually darker than in normal B. pinnatum and B. glaucovirens, but less dark than in B. phoenicoides and in all the variants of B. pinnatum except variant B144 ( $2 \mathrm{n}=18$ ) from E. Germany, which has bands as in normal B. pinnatum. The $2 n=18$ variants of B. pinnatum (B228, B229 and B283) from Iran and (B241, B242 and B282) from U.S.S.R., as well as the $2 \mathrm{n}=36$ (B248) variant from U.S.S.R., have a dark prominent band at position 3 . This band is either absent in normal B. pinnatum or, if present, is very light. In variant Bl44 ( $2 \mathrm{n}=18$ ) from E. Germany it is missing. B. distachyon has a prominent band at the top end of position 2, between positions 3 and 4, in the middle of position 6, and between positions 7 and 8.

The English plants which are intermediate between B. pinnatum and B. sylvaticum have the characteristic band pattern at position 6 of B. sylvaticum, even though overall (pairing affinity) they are closer to B. pinnatum. However, B63 from Czechoslovakia, which is also morphologically intermediate between B. pinnatum and B. sylvaticum, lacks the characteristic band pattern of B. sylvaticum at position 6.
*Note: Many faint bands with varying intensities were observable at position 1 on certain gels (e.g. Plate 3.3/1E). To avoid misidentification due to uncertainty, only prominent reproducible bands were considered at this position.
ueaw
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$\stackrel{i}{i}$
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| $\begin{aligned} & \boldsymbol{4} \\ & \boldsymbol{\sim} \\ & \underset{\sim}{2} \end{aligned}$ | $\overline{\text { səp!00!uә0पd }{ }^{\circ} \mathrm{g}}$ |  |  |  | 응 | N | N | ¢ |
| $$ | $\overline{\partial 17 s \partial d n \lambda ~}$ |  |  | 응 | $\stackrel{\sim}{\infty}$ | $\stackrel{\infty}{\infty}$ | ${ }_{\infty}^{\infty}$ | M |
|  | unteuuta 'g |  | ¢ | $\stackrel{\infty}{\infty}$ | $\infty$ | $\begin{aligned} & \infty \\ & \dot{1} \end{aligned}$ | 0 | ¢ |
| $\underset{+}{\underset{\sim}{E}}$ | surd!^0כne[b 'g | 응 | $\begin{aligned} & N \\ & \infty \\ & \infty \end{aligned}$ | $\underset{\sim}{n}$ | ? | $\begin{aligned} & N \\ & N \end{aligned}$ | $\cdots$ | $\begin{aligned} & \dot{0} \\ & \dot{0} \end{aligned}$ |
| $\begin{aligned} & \pi \\ & \pi \end{aligned}$ | แns!7en [1\% ${ }^{\circ} \mathrm{g}$ | ¢ | $\cdots$ | $\stackrel{N}{N}$ | N | - | $N$ 0 0 | $M$ $\infty$ 0 |



|  | B. sylvaticum | B. glaucovirans | $\begin{gathered} \text { B. } \\ \text { pinnatum } \end{gathered}$ | $\begin{gathered} \text { B. } \\ \text { rupestre } \end{gathered}$ | B. phoenicoides | $\begin{gathered} \text { B. } \\ \text { recusum } \end{gathered}$ | $\underset{\text { mexicanum }}{\text { B. }}$ | $\left.\begin{aligned} & \text { B. } \\ & \text { distachyon } \end{aligned}\right\|^{B}$ | $\begin{aligned} & \text { B.glaucoviren } \\ & \text { jsylvaticum } \\ & \text { Intermediate } \end{aligned}$ $B 230$ |  | $\begin{aligned} & \text { B. pinnatum/ } \\ & \text { sylvaticum } \\ & \text { Intermediate } \\ & \text { B63 } \end{aligned}$ | B. pinnatum <br> variant: <br> $2 n=18$ <br> B228, B229 | $\left\|\begin{array}{c} \text { B. pinnatum } \\ \text { variant: } \\ \text { 2n=18 } 8144 \end{array}\right\|$ | B. pinnatum Variant $2 n=36$ |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| 1 | EIIIX | $\square$ | वדIדI |  | VTITus |  | $\square$ | [700] | $\square$ |  | [ाएँ] |  | [ITIX |  |
| 2 |  | TIIIIA | TIITB | $\pm$ |  | - | $\square$ |  | WTIT | $\square$ | $\square$ | $\square$ | पापד | TTITM |
| 3 | Emim | $\square$ | TIIIT | [20]0 | T-ITU |  |  |  | $\square$ | PITO | [7III | - |  | -770 |
| 4 | - | $\square$ | 277\% |  |  |  | Б |  |  | [z7]0 |  | [דIד | $\pm$ | $\square$ |
| 5 | - | num |  | 27ITM | $\square$ |  |  | $\square$ | $\square$ |  |  |  |  | TIIID |
| 6 |  | - |  |  |  | 20wn |  | - |  | $\xrightarrow{\square}$ |  | $\square$ |  | - =imu |
| 7 | 唯地 |  | - | - |  | ITIIIT |  |  |  |  |  |  | - |  |
| 8 |  | (Tmmm | RTITM |  | $m$ |  |  |  |  | maz | Zous |  |  |  |

Figure 3.3/1: Relative positions of seed-protein bands on electrophoretograms obtained from taxa of Brachypodium, showing numbering system adopted


Figure 3.3/2: Dendrogram representing the 'average linkage' relationships between the species of Brachypodium
as revealed by seed-protein electrophoresis

Plate 3.3/lA: Electrophoretic seed-protein bands of Brachypodium taxa

1 - B. glaucovirens
2 - B. glaucovirens
3 - B. sylvaticum
4 - B. sylvaticum
5 - B. phoenicoides
6 - B. phoenicoides
7 - B. distachyon
8 - B. distachyon

## A



Plate 3.3/1B: Electrophoretic seed-protein bands of Brachypodium taxa

1 - B. sylvaticum
2 - B. glaucovirens
3 - B. pinnatum/sylvaticum intermediate
4 - B. pinnatum
5 - B. pinnatum
6 - B. pinnatum/sylvaticum intermediate
7 - B. pinnatum/sylvaticum intermediate
8 - B. pinnatum/sylvaticum intermediate

## B



Plate 3.3/lC: Electrophoretic seed-protein bands of Brachypodium taxa

1 - B. distachyon
2 - B. pinnatum
3 - B. rupestre
4 - B. pinnatum
5 - B. pinnatum
6 - B. pinnatum variant B144 ( $2 \mathrm{n}=18$ )
7 - B. glaucovirens
8 - B. phoenicoides
C


Plate 3.3/1D: Electrophoretic seed-protein bands of Brachypodium taxa

1 - B. sylvaticum
2 - B. pinnatum
3 - B. glaucovirens
4 - B. distachyon
5 - B. phoenicoides
6 - B. phoenicoides
7 - B. retusum
8 - B. retusum


Plate 3.3/lE: Electrophoretic seed-protein bands of Brachypodium taxa

1-B. phoenicoides
2 - B. pinnatum variant B248 ( $2 \mathrm{n}=36$ )
3 - B. pinnatum variant B229 ( $2 \mathrm{n}=18$ )
4 - B. glaucovirens
5 - B. sylvaticum
6 - B. retusum
7 - B. mexicanum
8 - B. distachyon

## $E$



### 3.4.1: Chromosome number and karyotype analysis

Chromosome number has been determined for
the following taxa of Brachypodium: B. sylvaticum, B. glaucovirens, B. pinnatum (including several variants), B. rupestre, B. phoenicoides, B. retusum, B. distachyon and certain intermediates. Chromosome counts with their locality and country of origin are given in Appendix 1. Photographs of mitotic preparations are shown in Plates $3.4 / 1$ and 3.4/2.

All 55 specimens of $B$. sylvaticum had $2 n=18$ and all four specimens of $B$. glaucovirens had $2 n=16$. The B. glaucovirens/sylvaticum intermediate from Turkey had $2 \mathrm{n}=17$, as would be expected, but that from Greece had $2 n=18$.
B. pinnatum had counts of $2 \mathrm{n}=18$ ( 5 counts), 28 ( 33 counts) and 36 ( 2 counts), of which the last is reported here for the first time.

All 6 B. pinnatum/sylvaticum intermediates had $2 n=28$. The single plant of $B$. rupestre had $2 n=36$ (a new number for this species).
B. phoenicoides had $2 \mathrm{n}=28$ (19 plants) or

36 (l plant), but two plants from Spain with $2 n=28$ also showed one B chromosome. The single B. phoenicoides/ pinnatum intermediate counted had $2 n=28$.
B. retusum had $2 \mathrm{n}=28$ (1 plant) and 32 ( 2 plants) (latter two counted by J. P. Bailey); both are new
numbers for that species.
B. distachyon was found to have $2 \mathrm{n}=10$ (2 plants) and $2 \mathrm{n}=30$ (11 plants). One of the latter from Afghanistan) had one B chromosome.
B. mexicanum was counted by J. P. Bailey as $2 n=42$, a new number for that species.

For karyotyping, total chromosome length, the length of each arm, the arm length ratio (l/s), and the centromeric position as defined by Levan et al. (1965), is given in Table 3.4/1 for most taxa.

Chromosome morphology and measurements show features characteristic for each taxon. In B. pinnatum the $2 n=28$ karytype consists of two large and two medium-sized pairs of metacentric chromosomes; two large, two medium-sized and four small pairs of submetacentric chromosomes; and two pairs of very small chromosomes. Total length of all chromosomes is $48.5 \mu \mathrm{~m}$. The $2 \mathrm{n}=18$ karyotype of B. pinnatum examined consisted of longer chromosomes than the $2 \mathrm{n}=28$ karyotype, and the longest chromosomes were submetacentries. Two or three of the nine pairs were metacentrics, while the rest were submetacentries (one pair was intermediate). Total length of all the chromosomes is $49.1 \mu \mathrm{~m}$, almost exactly as in the $2 \mathrm{n}=28$ karyotype. The $2 \mathrm{n}=36$ karyotype of B. pinnatum consisted of smaller chromosomes than in the $2 \mathrm{n}=28$ karyotype. Seven pairs were metacentrics, six were submetacentrics, and the rest were too small to measure. Total length of all the chromosomes is $44.0 / \mathrm{m}$, again close to the figure
for the $2 \mathrm{n}=18$ and 28 karyotypes.
In the B. phoenicoides $2 n=28$ karyotype the two longest pairs of chromosomes are metacentrics and there are five other pairs of metacentrics. Two pairs of chromosomes are minute and the rest are submetacentrics varying from short to long. Overall, the karyotype is rather similar to that of the B. pinnatum $2 n=28$ karyotype. Total chromosome length is $57.0 \mu \mathrm{~m}$.

The karyotype of B. sylvaticum ( $2 n=18$ ) consists of two long pairs of submetacentrics, one submetacentric and one metacentric pair of medium chromosomes, one metacentric and three submetacentric pairs of small chromosomes, and one pair of very small chromosomes. The total length of all the chromosomes is only $29.7 \mu \mathrm{~m}$.

The karyotype of B. glaucovirens $(2 n=16)$ consists of two pairs of long metacentrics and one pair of medium metacentrics, and the rest are short or medium submetacentrics. The total length of chromosome is 29.l $\mu \mathrm{m}$, which is very close to that of B. sylvaticum. The B. glaucovirens/sylvaticum intermediate had a total chromosome length of $37.5 \mu \mathrm{~m}$.

Centromeric position in B. distachyon and B. rupestre could not be worked out. In B. distachyon $(2 n=30)$ the chromosome length varies from $1.3-3.3 \mu \mathrm{~m}$, and the total length is $57.2 \mu \mathrm{~m}$. At least one pair of long and one pair of small chromosomes is metacentric.

In B. rupestre the chromosome length varies from 1.3 to $2.5 \mu \mathrm{~m}$, and the total length is $62.2 \mu \mathrm{~m}$. At least two pairs of small chromosomes are metacentrics.

TABLE 3.4/1
Chromosome measurements for various taxa of Brachypodium
B. sylvaticum ( $2 n=18$ ) (B150, B222, B239, B259, B291, B293)

| Chromosome | Total <br> length of <br> chromosome | Long arm <br> length <br> $(\mu \mathrm{m})$ | Short arm <br> length <br> $(\mu \mathrm{m})$ |
| :---: | :---: | :---: | :---: | | Arm length Centromere |
| :---: |
| ratio |


| 1 | 2.3 | 1.5 | 0.8 | 1.9 | sm |
| ---: | ---: | ---: | ---: | ---: | ---: |
| 2 | 2.1 | 1.4 | 0.7 | 2.0 | sm |
| 3 | 2.0 | 1.3 | 0.7 | 1.9 | sm |
| 4 | 2.0 | 1.3 | 0.7 | 1.9 | sm |
| 5 | 1.9 | 1.1 | 0.8 | 1.4 | m |
| 6 | 1.7 | 0.9 | 0.8 | 1.2 | m |
| 7 | 1.8 | 1.3 | 0.5 | 2.6 | sm |
| 8 | 1.7 | 1.2 | 0.5 | 2.4 | sm |
| 9 | 1.5 | 1.0 | 0.5 | 2.0 | sm |
| 10 | 1.5 | 1.0 | 0.5 | 2.0 | sm |
| 11 | 1.5 | 1.0 | 0.5 | 2.0 | sm |
| 12 | 1.5 | 1.0 | 0.5 | 2.0 | sm |
| 13 | 1.5 | 1.0 | 0.5 | 2.0 | sm |
| 14 | 1.5 | 1.0 | 0.5 | 2.0 | sm |
| 15 | 1.4 | 0.7 | 0.7 | 1.0 | m |
| 16 | 1.4 | 0.7 | 0.7 | 1.0 | m |
| 17 | 1.2 | Too | small to | measure |  |
| 18 | 1.2 | Too | small to | measure |  |

B. glaucovirens ( $2 n=16$ ) (B218, B224, B285)

| 1 | 2.7 | 1.4 | 1.3 | 1.2 | m |
| ---: | ---: | ---: | ---: | ---: | ---: |
| 2 | 2.7 | 1.4 | 1.3 | 1.2 | m |
| 3 | 2.3 | 1.3 | 1.0 | 1.3 | m |
| 4 | 2.3 | 1.3 | 1.0 | 1.3 | m |
| 5 | 1.9 | 1.3 | 0.6 | 2.2 | sm |
| 6 | 1.8 | 1.2 | 0.6 | 2.0 | sm |
| 7 | 1.7 | 1.2 | 0.5 | 2.4 | sm |
| 8 | 1.7 | 1.2 | 0.5 | 2.4 | sm |
| 9 | 1.6 | 0.9 | 0.7 | 1.3 | m |
| 10 | 1.6 | 0.9 | 0.7 | 1.3 | m |
| 11 | 1.6 | 1.0 | 0.5 | 2.2 | sm |
| 12 | 1.6 | 1.1 | 0.5 | 2.2 | sm |
| 13 | 1.5 | 1.0 | 0.5 | 2.0 | sm |
| 14 | 1.5 | 1.0 | 0.5 | 2.0 | sm |
| 15 | 1.3 | 0.9 | 0.4 | 2.3 | sm |
| 16 | 1.3 | 0.9 | 0.4 | 2.3 | sm |

## TABLE 3.4/1 CONTINUED

B. glaucovirens/sylvaticum intermediate ( $2 n=18$ ) ( $B 230$ )


| 1 | 2.9 | 1.8 | 1.1 | 1.6 | m |
| :---: | :---: | :---: | :---: | :---: | :---: |
| 2 | 2.9 | 1.8 | 1.1 | 1.6 | m |
| 3 | 2.8 | 1.8 | 1.0 | 1.8 | sm |
| 4 | 2.5 | 1.6 | 0.9 | 1.8 | sm |
| 5 | 2.5 | 1.6 | 0.9 | 1.8 | sm |
| 6 | 2.3 | 1.3 | 1.0 | 1.3 | m |
| 7 | 2.3 | 1.3 | 1.0 | 1.3 | m |
| 8 | 2.2 | 1.2 | 1.0 | 1.2 | m |
| 9 | 2.0 | Too | small to | measure |  |
| 10 | 2.0 | Too | small to | measure |  |
| 11 | 2.0 | Too | small to | measure |  |
| 12 | 2.0 | Too | small to | measure |  |
| 13 | 1.9 | Too | small to | measure |  |
| 14 | 1.8 | Too | small to | measure |  |
| 15 | 1.8 | Too | small to | measure |  |
| 16 | 1.3 | T00 | small to | measure |  |
| 17 | 1.2 | Too | small to | measure |  |
| 18 | 1.1 | Too | small to | measure |  |
| B. pinnatum ( $2 \mathrm{n}=18$ ) (B144) |  |  |  |  |  |
| 1 | 3.6 | 2.4 | 1.2 | 2.0 | sm |
| 2 | 3.0 | 2.0 | 1.0 | 2.0 | sm |
| 3 | 3.2 | 2.0 | 1.2 | 1.7 | m or sm |
| 4 | 3.0 | 1.9 | 1.1 | 1.7 | m or sm |
| 5 | 3.0 | 2.1 | 0.9 | 2.3 | sm |
| 6 | 2.9 | 1.9 | 1.0 | 1.9 | sm |
| 7 | 2.8 | 1.6 | 1.2 | 1.3 | m |
| 8 | 2.8 | . 1.6 | 1.2 | 1.3 | m |
| 9 | 2.8 | 1.9 | 0.9 | 2.1 | sm |
| 10 | 2.7 | 1.9 | 0.8 | 2.4 | sm |
| 11 | 2.7 | 1.6 | 1.1 | 1.5 | m |
| 12 | 2.7 | 1.6 | 1.1 | 1.5 | m |
| 13 | 2.5 | 1.6 | 0.9 | 1.8 | sm |
| 14 | 2.5 | 1.6 | 0.9 | 1.8 | sm |
| 15 | 2.3 | 1.5 | 0.8 | 1.9 | sm |
| 16 | 2.3 | 1.5 | 0.8 | 1.9 | sm |
| 17 | 2.2 | 1.5 | 0.7 | 2.5 | sm |
| 18 | 2.1 | 1.5 | 0.6 | 2.5 | sm |

## TABLE 3.4/1 CONTINUED

B. pinnatum $(2 n=28)(B 223, B 254, B 288)$

Chromosome Total length Long arm of length $c$
chromosome
$(\mu \mathrm{m})$ ( $\mu \mathrm{m}$ )

| 3.0 | 1.8 |
| :--- | :--- |
| 2.4 | 1.4 |
| 2.3 | 1.3 |
| 1.9 | 1.1 |
| 2.1 | 1.4 |
| 1.8 | 1.2 |
| 2.0 | 1.1 |
| 2.0 | 1.1 |
| 1.9 | 1.3 |
| 1.7 | 1.2 |
| 1.8 | 1.2 |
| 1.8 | 1.2 |
| 1.7 | 1.0 |
| 1.7 | 1.0 |
| 1.7 | 1.1 |
| 1.7 | 1.1 |
| 1.6 | 1.1 |
| 1.6 | 1.1 |
| 1.6 | 1.1 |
| 1.4 | 1.0 |
| 1.5 | 1.1 |
| 1.5 | 1.1 |
| 1.5 | 1.1 |
| 1.5 | 1.1 |
| 1.3 | Too |
| 1.3 | Too |
| 1.1 | Too |
| 1.1 | Too |

Too
Short arm length
( $\mu \mathrm{m}$ )
1.8
.4
1.3
1.1
1.2
1.1
1.1
1.2
1.2
1.0
1.1
1.1
1.1
1.0
.1
.1
Too
듵
1.2
1.0
1.
0.8
0.7
1.2
1.0
0.8
0.7
0.6
0.9
0.9
0.6
0.5
0.6
0.6
0.7
0.7
0.6
0.6
0.5
0.5
0.5
0.4
0.4
0.4
$\begin{array}{ll}0.4 & 2.8 \\ 0.4 & 2.8\end{array}$
small to measure
small to measure
small to measure

Arm length Centromere ratio position ( $1 / \mathrm{s}$ )

| 1.5 | m |
| :--- | ---: |
| 1.4 | m |
| 1.3 | m |
| 1.4 | m |
| 2.0 | sm |
| 2.0 | sm |
| 1.2 | m |
| 1.2 | m |
| 2.2 | sm |
| 2.4 | sm |
| 2.0 | sm |
| 2.0 | sm |
| 1.4 | m |
| 1.4 | m |
| 1.8 | sm |
| 1.8 | sm |
| 2.2 | sm |
| 2.2 | sm |
| 2.2 | sm |
| 2.5 | sm |
| 2.8 | sm |
| 2.8 | sm |
| 2.8 | sm |
| 2.8 | sm |

## TABLE 3.4/1 CONTINUED

B. pinnatum $(2 n=36)(B 115)$

| Chromosome | Total length |
| :---: | :---: |
| of | Long arm <br> length |
| chromosome | $(\mu \mathrm{m})$ |
| $(\mu \mathrm{m})$ |  |


| 1 | 1.7 | 1.1 |  | 0.6 |  | 1.8 | sm |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| 2 | 1.7 | 1.1 |  | 0.6 |  | 1.8 | sm |
| 3 | 1.6 | 1.1 |  | 0.5 |  | 2.2 | sm |
| 4 | 1.4 | 0.9 |  | 0.5 |  | 1.8 | sm |
| 5 | 1.5 | 0.9 |  | 0.6 |  | 1.5 | m |
| 6 | 1.5 | 0.9 |  | 0.6 |  | 1.5 | m |
| 7 | 1.4 | 0.8 |  | 0.6 |  | 1.3 | m |
| 8 | 1.4 | 0.8 |  | 0.6 |  | 1.3 | m |
| 9 | 1.3 | 0.8 |  | 0.5 |  | 1.6 | m |
| 10 | 1.3 | 0.8 |  | 0.5 |  | 1.6 | m |
| 11 | 1.3 | 0.8 |  | 0.5 |  | 1.6 | m |
| 12 | 1.3 | 0.8 |  | 0.5 |  | 1.6 | m |
| 13 | 1.3 | 0.7 |  | 0.6 |  | 1.2 | m |
| 14 | 1.3 | 0.7 |  | 0.6 |  | 1.2 | m |
| 15 | 1.2 | 0.8 |  | 0.4 |  | 2.0 | sm |
| 16 | 1.2 | 0.8 |  | 0.4 |  | 2.0 | sm |
| 17 | 1.2 | 0.8 |  | 0.4 |  | 2.0 | sm |
| 18 | 1.2 | 0.8 |  | 0.4 |  | 2.0 | sm |
| 19 | 1.2 | 0.6 |  | 0.6 |  | 1.0 | m |
| 20 | 1.2 | 0.6 |  | 0.6 |  | 1.0 | m |
| 21 | 1.1 | 0.7 |  | 0.4 |  | 1.8 | sm |
| 22 | 1.1 | 0.7 |  | 0.4 |  | 1.8 | sm |
| 23 | 1.1 | 0.6 |  | 0.5 |  | 1.2 | m |
| 24 | 1.1 | 0.6 |  | 0.5 |  | 1.2 | m |
| 25 | 1.1 | 0.7 |  | 0.4 |  | 1.8 | sm |
| 26 | 1.1 | 0.7 |  | 0.4 |  | 1.8 | sm |
| 27 | 0.9 |  | Too | small | to | measure |  |
| 28 | 0.9 |  | T00 | small | to | measure |  |
| 29 | 0.9 |  | Too | small | to | measure |  |
| 30 | 0.9 |  | Too | small | to | measure |  |
| 31 | 0.9 |  | Too | small | to | measure |  |
| 32 | 0.9 |  | T00 | small | to | measure |  |
| 33 | 0.8 |  | Too | small | to | measure |  |
| 34 | 0.8 |  | Too | small | to | measure |  |
| 35 | 0.8 |  | Too | small | to | measure |  |
| 36 | 0.8 |  | Too | small | to | measure |  |

B. phoenicoides $(2 n=28)(B 125, B 186, B 260, B 270, B 271)$

| Chromosome | Total length Long arm |  |  |
| :---: | :---: | :---: | :---: | :---: |
| of | Short arm | Arm length | Centromere |


| 1 | 3.7 | 2.3 |  | 1.4 |  | 1.6 | m |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| 2 | 3.7 | 2.3 |  | 1.4 |  | 1.6 | m |
| 3 | 2.8 | 1.6 |  | 1.2 |  | 1.3 | m |
| 4 | 2.6 | 1.5 |  | 1.1 |  | 1.4 | m |
| 5 | 2.6 | 1.7 |  | 0.9 |  | 1.9 | sm |
| 6 | 2.5 | 1.6 |  | 0.9 |  | 1.8 | sm |
| 7 | 2.4 | 1.4 |  | 1.0 |  | 1.4 | m |
| 8 | 2.3 | 1.3 |  | 1.0 |  | 1.3 | m |
| 9 | 2.3 | 1.5 |  | 0.8 |  | 1.9 | sm |
| 10 | 2.3 | 1.5 |  | 0.8 |  | 1.9 | sm |
| 11 | 2.2 | 1.3 |  | 0.9 |  | 1.4 | m |
| 12 | 2.1 | 1.2 |  | 0.9 |  | 1.3 | m |
| 13 | 2.1 | 1.3 |  | 0.8 |  | 1.6 | m |
| 14 | 2.1 | 1.3 |  | 0.8 |  | 1.6 | m |
| 15 | 1.9 | 1.1 |  | 0.8 |  | 1.4 | m |
| 16 | 1.9 | 1.1 |  | 0.8 |  | 1.4 | m |
| 17 | 1.7 | 1.2 |  | 0.5 |  | 2.4 | sm |
| 18 | 1.7 | 1.2 |  | 0.5 |  | 2.4 | sm |
| 19 | 1.7 | 0.9 |  | 0.8 |  | 1.2 | m |
| 20 | 1.7 | 0.9 |  | 0.8 |  | 1.2 | m |
| 21 | 1.6 | 1.1 |  | 0.5 |  | 2.2 | sm |
| 22 | 1.6 | 1.1 |  | 0.5 |  | 2.2 | sm |
| 23 | 1.4 | 1.0 |  | 0.4 |  | 2.5 | sm |
| 24 | 1.4 | 1.0 |  | 0.4 |  | 2.5 | sm |
| 25 | 1.3 |  | Too | small | to | measure |  |
| 26 | 1.2 |  | Too | small | to | measure |  |
| 27 | 1.1 |  | Too | small | to | measure |  |
| 28 | 1.1 |  | Too | small | to | measure |  |

## TABLE 3.4/1 CONTINUED

B. rupestre $(2 n=36)$ ( 1822 )
Chromosomes Chromosome
length ..... ( $\mu \mathrm{m}$ )

| $1-2$ | 2.7 |
| :---: | ---: |
| $3-5$ | 2.5 |
| 6 | 2.4 |
| $7-12$ | 2.2 |
| $13-14$ | 2.0 |
| $15-20$ | 1.9 |
| $21-22$ | 1.8 |
| $23-27$ | 1.7 |
| $28-30$ | 1.6 |
| $31-33$ | 1.5 |
| $34-36$ | 1.3 |

B. distachyon $(2 n=30)$ (B189, B199, B201, ..... B266)

| $1-2$ | 3.3 |
| ---: | ---: |
| 3 | 3.0 |
| $4-6$ | 2.5 |
| $7-8$ | 2.3 |
| $9-11$ | 2.0 |
| $12-14$ | 1.8 |
| $15-18$ | 1.7 |
| $19-20$ | 1.6 |
| $21-24$ | 1.5 |
| $25-27$ | 1.4 |
| $28-30$ |  |

Plate 3.4/1: Root-tip mitosis in Brachypodium taxa
A - B. sylvaticum $(2 n=18)$
$B$ - B. pinnatum $(2 n=28)$
C - B. pinnatum variant Bl44 ( $2 \mathrm{n}=18$ )
$D$ - B. phoenicoides $(2 n=28)$


Plate 3.4/2: Root-tip mitosis in Brachypodium taxa
$A$ - B. retusum $(2 n=28)$
B - B. distachyon $(2 n=30)$
C - B. distachyon $(2 n=10)$
$D$ - B. rupestre $(2 n=36)$


Meiosis has been observed in B. sylvaticum (Plate 3.4/3A), B. glaucovirens (Plate 3.4/3B), B. pinnatum $(2 n=28)$ (Plate $3.4 / 3 C)$, a variant of $B$. pinnatum (B144) with $2 n=18$, B. pinnatum/sylvaticum intermediate (Plate 3.4/3D), and B. phoenicoides.

Meiosis is regular in all the species. Spindle development was good and all the chromosomes occuped a position at the equator. During anaphase all the chromosomes moved regularly to the poles except in one specimen of B. pinnatum/sylvaticum intermediate (B63) from Czechoslovakia, where lagging chromosomes were observed (Plate $3.4 / 3 D$ ), with a mean of more than 4 univalents per pollen mother cell.

Chromosomal associations and chiasma frequency at meiosis is given in Table 3.4/2. Chiasma frequency varies from 1.03 to 1.5 per bivalent, being highest in B. sylvaticum and B. glaucovirens and lowest in B. phoenicoides. B. pinnatum ( $2 n=18$ ), with larger chromosomes than B. pinnatum $(2 n=28)$, had a higher chiasma frequency per bivalent.

Chiasma frequency per chromosome, which is calculated by dividing the total number of chiasmata per cell by the somatic chromosome number, is highest in B. glaucovirens and B. sylvaticum and the lowest in B. pinnatum/sylvaticum intermediate.
TABLE 3.4/2

| Taxon | 2 n | No. cells observed | Chromosome associations per P.M.C. |  | Chiasma frequency |  |  |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
|  |  |  | I | II | Per P.M.C. | Per chromosome | Per II |
| B. sylvaticum |  |  |  |  |  |  |  |
| b41 | 18 | 43 | 0.65 | 8.67 | 11.53 | 0.64 | 1.32 |
| B150 | 18 | 29 | 0.34 | 8.82 | 11.1 | 0.61 | 1.25 |
| B. glaucovirens |  |  |  |  |  |  |  |
| B. pinnatum |  |  |  |  |  |  |  |
| E9 | 28 | 51 | 0.12 | 13.94 | 15.3 | 0.55 | 1.1 |
| $\frac{\text { B. pinnatum }}{\text { Bi44 }}$ | 18 | 14 | 0 | 9.0 | 10.4 | 0.58 | 1.2 |
| B. pinnatum/sylvaticum intermediate |  |  |  |  |  |  |  |
| B63 | 28 | 19 | 4.42 | 11.79 | 12.73 | 0.45 | 1.08 |
| B. phoenicoides |  |  |  |  |  |  |  |
| $\overline{\mathrm{B} 5}$ | 28 | 35 | 0.63 | 13.68 | 14.20 | 0.51 | 1.03 |
| B88 | 28 | 57 | 0.25 | 13.87 | 15.20 | 0.54 | 1.1 |

Plate 3.4/3: Pollen mother cell meiosis in Brachypodium taxa

A - B. sylvaticum ( $2 \mathrm{n}=18$ )
$B-B$. glaucovirens $(2 n=16)$
C - B. pinnatum ( $2 \mathrm{n}=28$ )
D - B. pinnatum/sylvaticum intermediate ( $2 \mathrm{n}=28$ )


### 3.4.3: Pollen fertility

Pollen stainability for the taxa studied is
shown in Table 3.4/3. In all the species it was quite high (81.0-97.8\%), as expected (Plate 3.4/4A), except that it was only $52.6 \%$ in one plant of $B$. sylvaticum and only $45.8 \%$ in B. distachyon.

However, pollen stainability was very low in the two unusual cytological races of B. pinnatum ( $2 \mathrm{n}=18$ and 36 ), and very variable in the various intermediate plants. In the B. pinnatum/sylvaticum intermediates it ranged from 0.3 (Plate $3.4 / 4 \mathrm{~B}$ ) to $96.8 \%$, and in the B. glaucovirens/sylvaticum intermediate from 23.4 to 69.3\%. Both B. phoenicoides/pinnatum intermediates had very low fertility (1.0-3.8\%).

TABLE 3.4/3
Pollen stainability in the Brachypodium taxa studied

Taxon Total no. grains $\begin{gathered}\% \text { stainable } \\ \text { grains }\end{gathered}$
B. sylvaticum $(2 n=18)$

| B75 | 514 | 84.6 |
| :--- | :--- | :--- |
| B84 | 233 | 91.0 |
| B239 | 211 | 52.6 |
|  |  |  |
| B. glaucovirens $(2 n=16)$ |  | 97.3 |
| B224 | 437 | 96.1 |

B. pinnatum $(2 n=28)$

| B3 | 397 | 95.5 |
| :--- | :--- | :--- |
| B9 | 366 | 9.8 |
| B118 | 344 | 93.6 |

B. pinnatum variant ( $2 n=18$ )

B144 222
1.3
B. pinnatum variant $(2 n=36)$

B115 368
1.6
B. rupestre $(2 n=36)$

B182
234
87.7
P. phoenicoides ( $2 n=28$ )
B88 $347 \quad 91.9$

B215
494
81.0
B. distachyon ( $2 \mathrm{n}=30$ )

B266 206
45.8
B. glaucovirens/sylvaticum intermediates

| B230 <br> $(2 n=18)$ <br> B249 | 238 | 69.3 |
| :--- | :--- | :--- |
| $(2 n)$ | 526 | 23.4 |

( $2 \mathrm{n}=17$ )
B. pinnatum/sylvaticum intermediates ( $2 n=28$ )

| B5 | 322 | 0.3 |
| :--- | ---: | ---: |
| B52 | 349 | 96.8 |
| B53 | 435 | 57.2 |
| B63 | 225 | 81.8 |
| B78 | 312 | 96.5 |
| B352 | 242 | 0.4 |

B. phoenicoides/pinnatum intermediates

B31 (2n=28) 209
Herbarium specimenSpain 300

Plate 3.4/4: Stained pollen of Brachypodium taxa
A - B. glaucovirens
B - B. pinnatum/sylvaticum intermediate

$$
\begin{gathered}
\text { A } \\
1 \\
0.3 \mathrm{~mm}
\end{gathered}
$$



## CHAPTER 3.5: FLORAL BIOLOGY

### 3.5.1: Flowering behaviour

In all the species the inflorescence is a spike-like raceme with spikelets alternating up the rachis and each with at least 6 and usually over 10 florets. Hence the spikelets emerge from the uppermost leaf-sheath one at a time, and as in all grasses the top-most spikelet is the most mature. Within each spikelet, however, the lower-most floret is the most mature.

Knowledge of flowering behaviour is not only helpful in identification but is essential for breeding experiments as well. In this chapter the main aspects described for certain taxa are: start of flowering season, time taken to complete flowering, time of the day anthesis commences, response to meterological conditions, degree of chasmogamy or cleistogamy, and flowering performance of plants in green-house and field conditions.
B. pinnatum and B. phoenicoides did not flower in heated green-house conditions, since they need vernalization in winter. The same is true of most plants of $B$. sylvaticum, but in this species a few did flower in the green-house. Even in an unheated green-house, flowering took place in these three species much less readily than in the field. B. glaucovirens, B. retusum, B. distachyon and B. mexicanum flowered in
the green-house without any vernalization.
The flowering season of B. pinnatum commences earlier than in B. sylvaticum. Outdoors in Leicester it usually starts about the middle of June and ends in the middle of July. The variant of B. pinnatum Bl44 ( $2 \mathrm{n}=18$ ) from E. Germany started flowering at the end of May. Other $2 n=18$ and $2 n=36$ variants of $B$. pinnatum and plants of $B$. rupestre do not differ in their flowering season from normal B. pinnatum. B. sylvaticum starts flowering at the end of June and goes on until the middle of August. B. retusum flowers from early June to early July. B. phoenicoides flowers from the end of June to early August. Because of this difference in flowering season, I could not manage to cross B. retusum with B. phoenicoides. This problem could be solved by putting B. phoenicoides pots from the field to the warm green-house at the beginning of May. B. mexicanum starts flowering at the end of June and goes on until the middle of July. B. glaucovirens flowers from the middle of July to the end of August or even later. The flowering season of this species starts later than in all the other taxa used in this study. B. distachyon is an annual species and starts to flower within 36-45 days after sowing, the flowering period lasting for about two weeks (in heated greenhouse conditions). B. sylvaticum and B. glaucovirens have a long flowering season because some of the inflorescences complete flowering while others are
still in bud, which facilitates crossing. B. sylvaticum takes $24-36 \mathrm{hr}$ per spikelet to emerge out of the leaf-sheath, while B. glaucovirens takes $36-48 \mathrm{hr}$. Other comparative figures are: B. pinnatum 24-48 hr per spikelet; B. phoenicoides $36-50 \mathrm{hr}$ per spikelet; and B. distachyon $12-18 \mathrm{hr}$ per spikelet.

It is important to know how long it takes for an inflorescence to commence anthesis after full emergence from the leaf-sheath. In B. sylvaticum, B. glaucovirens, B. pinnatum, B. rupestre, B. phoenicoides and $B$. retusum anthesis does not commence for many days after emergence of the inflorescence. Actual figures obtained were: B. sylvaticum 13-19 days, B. pinnatum 15-22 days, B. glaucovirens 20-28 days, B. phoenicoides 19-27 days, and B. retusum 10-12 days. Even in B. distachyon and B. mexicanum there is a gap of at least four days.
B. sylvaticum takes 5-9 days to complete
anthesis from the top to the bottom spikelet, B. glaucovirens takes 7-11 days, B. pinnatum takes 4-8 days, B. phoenicoides takes 6-9 days, B. retusum takes 3-6 days, and B. mexicanum and B. distachyon take only 3-4 days. It has been observed that emasculated inflorescences complete anthesis 2-3 days earlier than unemasculated inflorescences.

Meiosis in B. sylvaticum, B. glaucovirens, B. pinnatum and B. phoenicoides takes place in the topmost spikelet when the whole inflorescence is nearly
completely emerged from the leaf-sheath, and the lower-most spikelet undergoes meiosis about 3-5 days after its emergence. Meiosis generally occurs about 2 hr after sun-rise, i.e. 6.30-7.50 hr from July to the beginning of August. All the florets within one spikelet undergo meiosis within a period of 2-5 days.

There is generally a good separation in flowering time during the day between B. sylvaticum, B. pinnatum and B. phoenicoides, although the actual time varies according to the season and the environmental conditions. In warm green-house conditions in early July B. sylvaticum exserts its anthers at about $23.00-1.30 \mathrm{hr}$ and B . pinnatum at about $1.00-2.30 \mathrm{hr}$. In field conditions on the same days the two species flower at about 3.004.00 hr and $5.00-6.00 \mathrm{hr}$ respectively. Anther dehiscence follows anther emergence by about $20-30 \mathrm{~min}$ in B. sylvaticum and $15-20 \mathrm{~min}$ in B. pinnatum in green-house conditions, and by about 35-45min and 25 - 35 min in field conditions respectively. Emergence and dehiscence of the three anthers of each floret is usually successive rather than simultaneous, two appearing together before the third. In both species a few florets may commence anthesis but the anthers become retarded and may eventually emerge and dehisce at any time during the next 12 hr .
B. phoenicoides differs markedly from the above two species in having two sorts of plants; ones which commence anthesis in the morning at about 8.00-9.00 $\mathrm{hr}_{\mathrm{r}}$ and others which commence anthesis in the early evening at
about $17.00-18.00 \mathrm{hr}$ in August in Leicester. Anther dehiscence is as in the above two species, but the flowering time does not seem to be much affected by environmental conditions.

In all three species the anthers become exserted on long, dangling filaments. In B. sylvaticum the anthers are $3.5-5.5 \mathrm{~mm}$ long, in $B$. pinnatum $4.5-6.3 \mathrm{~mm}$ and in B. phoenicoides $4.7-9.6 \mathrm{~mm}$. The anthers take only about 22-30 min for complete emergence, but the florets do not close until some hours later: 7 - 10 hr in B. sylvaticum, 6-7 in B. pinnatum, 4-6 in B. phoenicoides. The stigmas also emerge from the florets successively, the first one immediately (up to 10 min ) after the opening of the florets, i.e. before the anthers have fully emerged, the second stigma along with 3rd anther. This seems to be due simply to mechanical interference of stigma expansion by the unemerged anthers. Although these three species of Brachypodium are all perennial, B. sylvaticum is less vigorous vegetatively than the other two species and flowers at a younger stage, often in the first year after germination. In pot conditions B. sylvaticum usually produces more flowering shoots per unit area than the other two species.
B. glaucovirens commences anthesis at a time between those of $B$. sylvaticum and $B$. pinnatum, i.e. at about $4.00-5.00 \mathrm{hr}$ in late July or early August. Anthers dehisce within $30-34 \mathrm{~min}$ in field conditions.
B. retusum and B. mexicanum commence anthesis at about the same time as B. phoenicoides, i.e. 7.408.00 at the end of June.

In B. distachyon anthesis occurs from morning until afternoon at different times during the day. The florets open for about $1-2 \mathrm{hr}$ or even less during the day and then close again. It takes 24-28 min to complete the process of anthesis. Anther emergence (anthesis) takes place within $10-15$ min after the opening of the floret, but anther dehiscence occurs earlier than this, well before the florets open, when in close contact with the unemerged stigmas. Usually the stamens become re-enclosed within the florets, but sometimes one or two remain outside and eventually fall off.

Anthesis in all the species except B. phoenicoides is not affected by rainy or cloudy weather. The plants of B. phoenicoides which commence anthesis in the evening stop anthesis in rainy or densely cloudy weather. The plants of B. phoenicoides which commence anthesis in the morning do not completely stop anthesis in wet weather, but anthesis is reduced.

In emasculated plants the florets open at about the time they would normally have doneso (i.e. about 3-4 days after emasculation), or perhaps slightly earlier, except that in B. distachyon emasculated florets never re-opened.

It has been claimed for a long time (Beddows
1935) that plants of Brachypodium kept in pots produce
relatively few flowering stems. Beddows (1931b, p. 65) attributed this to poor soil conditions and unnatural conditions for the rhizomatous habit. However, in my experiments (Table 3.5/1) poor flowering was found only in plants grown in pots and kept in the green-house (unheated or heated), not in plants kept in pots and permanently plunged in the soil in open field conditions. Thus it seems more likely that poor flowering can be attributed to the absence of full vernalization.
B. mexicanum, B. retusum and B. distachyon were kept in the green-house because they do not need vernalization and are frost-sensitive.

### 3.5.2: Breeding behaviour

As mentioned in sub-chapter 3.5.1, all the species of Brachypodium are chasmogamous except B. distachyon, in which anther dehiscence occurs before the florets open.

Percentages of self seed-set for different taxa are presented in Table 3.5/2. Self-fertility is high in B. distachyon (over 80\%) and B. sylvaticum (rarely below 50\%).

In B. pinnatum (all variants), B. rupestre and B. phoenicoides self-fertility is low ( 0 - 14.9\%), except in one plant of $B$. pinnatum where a figure of $53 \%$ was obtained in emasculated and self-pollinated plants. However, the same plant gave a figure of $1.3 \%$ when unemasculated, so the high figure was probably the result
of contamination by foreign pollen. B. pinnatum/ sylvaticum and B. glaucovirens/sylvaticum intermediates also showed low self-fertility. B. glaucovirens itself showed somewhat intermediate figures (7.4-38.9\%). The two plants of B. retusum tested showed $0 \%$ and $30.6 \%$ self-fertility respectively.
A. Field conditions

| Species | No. plants <br> with flowers | No. plants <br> without flowers |
| :---: | :---: | :---: |

B. sylvaticum $\quad 57$
B. pinnatum 38
B. phoenicoides 5
B. Unheated green-house conditions
B. sylvaticum 2
B. pinnatum 3
B. phoenicoides 17

# TABLE 3.5/2 <br> Self-fertility experiments 

Accession No. florets | No. seeds \% selfed |
| :--- |
| obtained seed-set |

## B. sylvaticum

i. Florets emasculated and then pollinated with pollen from same plant

| B7 | 54 | 32 | 59.3 |
| :--- | ---: | ---: | ---: |
| B104 | 6 | 2 | 33.3 |
| B107 | 39 | 27 | 69.2 |
| B150 | 58 | 39 | 67.2 |
| B153 | 53 | 23 | 43.4 |
| B239 | 137 | 86 | 62.8 |

ii. Florets not emasculated

| B46 | 316 | 192 | 60.8 |
| :--- | ---: | ---: | ---: |
| B47 | 334 | 222 | 66.5 |
| B48 | 192 | 160 | 83.3 |
| B61 | 248 | 175 | 70.6 |
| B84 | 239 | 190 | 79.5 |
| B95 | 205 | 60 | 29.3 |
| B102 | 274 | 240 | 87.6 |
| B131 | 117 | 61 | 52.1 |
| B135 | 132 | 72 | 54.6 |
| B259 | 92 | 45 | 48.9 |

B. glaucovirens/sylvaticum intermediate (florets

| B249 | 216 | 0 | 0 |
| :--- | :--- | :--- | :--- |

B. glaucovirens (florets not emasculated)

| B218 | 122 | 9 | 7.4 |
| ---: | ---: | ---: | ---: |
| B224 | 54 | 21 | 38.9 |
| B285 | 81 | 10 | 12.3 |

B. pinnatum
i. Floret emasculated and then pollinated with pollen from same plant

| B3 | 63 | 0 | 0 |
| :--- | ---: | ---: | :---: |
| B20 | 102 | 11 | 10.8 |
| B37 | 47 | 7 | 14.9 |
| B60 | 115 | 61 | 53.0 |
| B118 | 128 | 0 | 0 |
| B220 | 74 | 0 | 0 |

## TABLE 3.5/2 CONTINUED

```
Accession No. florets No. seeds % selfed
    obtained seed-set
```

ii. Florets not emasculated

| B3 | 370 | 0 | 0 |
| :--- | :---: | :---: | :---: |
| B4 | 172 | 0 | 0 |
| B6 | 365 | 0 | 0 |
| B9 | 194 | 8 | 4.2 |
| B13 | 145 | 1 | 0.7 |
| B20 | 203 | 0 | 0 |
| B35 | 97 | 0 | 0 |
| B36 | 427 | 0 | 0 |
| B60 | 155 | 2 | 1.3 |
| B76 | 306 | 11 | 3.6 |
| B220 | 264 | 0 | 0 |

B. pinnatum variant ( $2 n=18$ ) (florets not emasculated)
B144
360
3
0.8
B. pinnatum variant ( $2 n=36$ ) (florets emasculated and pollinated with pollen from same plant)

| B115 | 82 | 0 | 0 |
| :---: | :---: | :---: | :---: |
| B248 | 76 | 5 | 6.6 |

B. pinnatum/sylvaticum intermediates
i. Florets emasculated and pollinated with pollen from same plant

| B5 | 46 | 0 | 0 |
| :--- | :--- | :--- | :--- |
| B51 | 62 | 0 | 0 |
| B52 | 97 | 0 | 0 |
| B53 | 48 | 0 | 0 |
| B78 | 85 | 1 | 1.2 |

ii. Florets not emasculated

| B5 | 405 | 36 | 8.9 |
| :--- | ---: | ---: | ---: |
| B42 | 238 | 5 | 2.1 |

B. rupestre (florets not emasculated)
B182
54
2
2

## B. phoenicoides

i. Florets emasculated and then pollinated with pollen from the same plant

| B88 | 41 | 0 | 0 |
| :--- | ---: | :--- | :---: |
| B181 | 67 | 0 | 0 |
| B210 | 121 | 1 | 0.8 |

ii. Florets not emasculated

| B2 | 342 | 0 | 0 |
| :--- | ---: | :---: | :---: |
| B18 | 490 | 0 | 0 |
| B39 | 225 | 7 | 3.1 |
| B55 | 206 | 0 | 0 |
| B71 | 338 | 0 | 0 |
| B88 | 1112 | 25 | 2.4 |
| B91 | 205 | 9 | 4.4 |
| B125 | 312 | 0 | 0 |
| B181 | 107 | 0 | 0 |
| B186 | 46 | 6 | 13.0 |
| B216 | 113 | 15 | 13.3 |

B. retusum (florets emasculated and pollinated with pollen from the same plant)

| B173 | 36 | 11 | 30.6 |
| :---: | :---: | :---: | :---: |
| B227 | 30 | 0 | 0 |

B. distachyon (florets not emasculated)

| B199 | 70 | 59 | 84.3 |
| :--- | :--- | :--- | :--- |
| B266 | 98 | 75 | 76.5 |

## CHAPTER 3.6: ARTIFICIAL HYBRIDIZATION

3.6.1: Percentage seed-set after hybridization Hybridization experiments were carried out from the end of June to the middle of September in 1980 and 1981. The seeds were harvested in October 1980 and 1981 respectively. Average percentage seedset obtained from different combinations of Brachypodium taxa and hybrids is given in Tables $3.6 / 1,2,3$. Intraspecific crosses in B. sylvaticum, B. glaucovirens, B. pinnatum and B. phoenicoides produced 20.3 to $65.0 \%$ seed-set. For the most part, interspecific crosses among these same four species produced similar results ( 20.1 to $39.1 \%$ ), i.e. interspecific crosses were only slightly less successful than intraspecific crosses. With B. phoenicoides as female parent, however, lower seed-set was achieved ( $9.3 \%$ and $15.8 \%$ ). B. retusum gave reasonably high seed-set as a female parent to B. sylvaticum and B. pinnatum, but B. mexicanum and B. distachyon gave relatively low figures in crosses with B. sylvaticum, B. pinnatum and B. phoenicoides. B. distachyon also produced rather low figures when used as a female parent with the same three species, and no seed at all when used as a male parent. In the last case the very low pollen output of $B$. distachyon was probably responsible.

Reciprocal differences were notable only in the case of B. phoenicoides, which produced much less
seed when used as a female parent crossed with B. sylvaticum and B. pinnatum than when used as the male parent.

Backcrosses of B. glaucovirens x B. sylvaticum, B. pinnatum $x$ B. phoenicoides and B. phoenicoides $x$ B. pinnatum produced variable results. In general, the hybrids were better as male parents ( 31 to $52.5 \%$ ) than as female ( 4.3 to $32.8 \%$ ), which was unexpected.

### 3.6.2: Embryo culture and hybrid plants

Caryopses produced in hybridization experiments had little endosperm in many cases, so the embryo culture technique was found to be essential to obtain good germination results. Embryo culture results for 42 combinations are presented in Table 3.6/4. The total number of caryopses used in embryo culture was 18,255.

All the caryopses of intraspecific crosses at one chromosomal level had sufficient endosperm and large embryos, as in the parents. Interchromosomal level crosses within $B$. pinnatum ( $2 \mathrm{n}=18,28,36$ ) mostly had imperfect caryopses, as in many of the interspecific hybrids.

The structure of the caryopses from different interspecific combinations can be divided into four categories:

1. B. pinnatum $(2 n=28) \times$ B. phoenicoides and B. pinnatum/sylvaticum intermediate $x$ B. pinnatum/ sylvaticum intermediate. Caryopses with sufficient endosperm and a large embryo almost as in the parents.
2. B. sylvaticum $\times$ B. glaucovirens; B. pinnatum $(2 n=28) \times$ B. pinnatum $(2 n=18) ;$ B. glaucovirens/ sylvaticum intermediate x B. sylvaticum, B. glaucovirens and B. phoenicoides; and B. pinnatum/sylvaticum intermediate $x$ B. sylvaticum, B. pinnatum ( $2 \mathrm{n}=28$ ) and B. phoenicoides. Caryopses normally with large to medium embryos but much reduced endosperm.
3. B. pinnatum $(2 n=28) \times$ B. sylvaticum. Caryopses with variable morphology, i.e. some with sufficient endosperm and medium to large embryos, but most with small embryo and reduced endosperm.
4. B. phoenicoides $x$ B. sylvaticum, B.
retusum $x$ B. sylvaticum, B. retusum $x$ B. pinnatum ( $2 \mathrm{n}=28$ ), B. mexicanum $x$ B. sylvaticum, B. mexicanum $x$ B. phoenicoides, B. distachyon $\times$ B. sylvaticum, B. distachyon $x$ B. pinnatum $(2 n=28)$, and B. distachyon $x$ B. phoenicoides. Caryopses mostly with small and shrunken endosperm and small to medium embryos.
B. phoenicoides $\times$ B. pinnatum $(2 n=28)$ and B. glaucovirens $\times$ B. sylvaticum hybrids, when backcrossed to either parent, produced caryopses with sufficient endosperm and a large embryo almost like that of the parents.

Embryos on the agar germinated with 5-9 days, mostly within 6 days. Usually the seedling was ready to pot out when it had $2-3$ leaves and several roots. About 80 vials had fungal infection but most of the seedlings were saved. Some of the embryos did not germinate at all. Some embryos germinated but, because
of lack of chlorophyll, grew only about $2-4 \mathrm{~mm}$. Some had leaves but no roots, and others had roots but the leaves did not grow.

About 282 mature hybrid plants were obtained from embryo culture experiments.

In terms of success of embryo culture, ten crosses produced no hybrid plants at all. These involved various combinations between and within chromosome levels, with little taxonomic pattern apparent, except that B. mexicanum produced no hybrids, and B. distachyon produced hybrids only with B. pinnatum ( $2 \mathrm{n}=18,28$ and 36).

The successful crosses can be divided into two categories:

1. When the chromosome numbers of the plants were identical or similar (within two, i.e. $16 / 18$ or $28 / 30$ ), over $50 \%$ of the embryos placed in culture grew to mature plants. Exceptions were B. sylvaticum $x$ B. pinnatum $(2 n=18)(30 \%)$, B. phoenicoides $x$ B. phoenicoides (37\%) and B. pinnatum/ sylvaticum intermediates x B. phoenicoides (26.3\%).
2. Where the chromosome number of the plants was less similar (i.e. $17 / 28,18 / 28,18 / 30$, $28 / 32,28 / 36,30 / 36$ ) a lower proportion (10-32.1\%) of the embryos placed in culture grew to mature plants. Exceptions were: B. glaucovirens/sylvaticum intermediate x B. phoenicoides ( $63.6 \%$ ), B. pinnatum/sylvaticum intermediate $x$ B. sylvaticum ( $66.7 \%$ ), B. pinnatum ( $2 \mathrm{n}=28$ )
$x$ B. pinnatum $(2 n=18)(70 \%)$, B. retusum $(2 n=32) x$ B. pinnatum $(2 n=28)(61.9 \%)$ and B. distachyon $x$ B. pinnatum ( $2 \mathrm{n}=36$ ) (57.1\%). Therefore no taxonomic pattern, other than the chromosome number referred to above, is apparent.
3.6.3: Behaviour and success of hybrid plants

Hybrid plants of different combinations obtained by embryo culture grew well in the warm greenhouse ( $19^{\circ} \mathrm{C}$ ). After about 2 to 3 months, the plants were removed to the cold green-house for vernalization, and their growth continued to appear satisfactory. Finally the hybrids were moved to the field where most of them survived without problem.

Hybrids involving B. sylvaticum, B. glaucovirens, B. pinnatum and B. phoenicoides were mostly quite vigorous, even in field conditions, and flowered well in the field or cold green-house (after vernalization) within twelve months of the hybridization experiment. Of these, only B. glaucovirens $x$ B. sylvaticum and its reciprocal flowered in the warm green-house without vernalization

Plants of $B$. retusum $\times B$. pinnatum were not put into the field, but they grew successfully and flowered in the cold green-house twelve months after hybridization.
B. distachyon $\times$ B. pinnatum plants were grown in the warm green-house, where they flowered within about 5 months of hybridization.


|  |  |  |  | TABLE |  |  |  |  |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
|  | res a | of | inte <br> For | in intra ssion cros dual resu | intersp <br> obtained <br> see App | $\begin{aligned} & \text { fic } \mathrm{cl} \\ & \text { or th } \\ & i \times 2 \end{aligned}$ | omi | tion |
| Female parent |  | $n$ <br> 0 <br> 0 <br>  <br> 0 <br> 0 <br> 0 <br> 0 <br> 0 <br> 0 <br> 0 <br> $\infty$ |  |  | (1) | 笠\| |  |  |
| B. sylvaticum | 47.6 | 23.9 | 25.4 | 75.2 | 18.5 | 30.0 | - | - |
| B. glaucovirens | 39.1 | 65.0 | 20.1 | - | 5.0 | 30.3 | - | - |
| B. pinnatum ( $2 \mathrm{n}=28$ ) | 27.6 | - | 33.2 | 26.2 | 33.7 | 29.5 | - |  |
| $\frac{\text { B. pinnatum variant }}{(2 n=18)(B 144)}$ | 30.0 | - | 40.6 | - | 82.1 | - | - | - |
| $\frac{\text { B. pinnatum variant }}{(2 n=36)(B 115, ~ B 248)}$ | 0 | - | 72.2 | - | - | - | - | - |
| B. phoenicoides | 9.3 | - | 15.8 | - | - | 20.3 | - |  |
| B. retusum | 68.1 | - | 32.1 | - | - | - | - |  |
| B. mexicanum | 3.4 | - | 0 | - | - | 5.9 | - | - |
| B. distachyon | 7.7 | - | 21.6 | 35.0 | 46.5 | 7.4 | - |  |

TABLE $3.6 / 2$
Percentage seed-set in crosses between taxonomically intermediate plants and typical species

mineuu!d ${ }^{\circ} g \quad, \quad \infty_{\infty}^{\infty}$

$N$
$N$
20.4
$\begin{array}{lll}\sim & \sim \\ \sim & \infty & \sim\end{array}$
For individual results,

$\stackrel{0}{\infty}$

.
i
11 the
 $\qquad$
Figures are means of all

$$
\overline{\mathrm{un} \partial!7 \mathrm{en}\lfloor K s}
$$

6.0
19.9
quased alew
Female parent
B. sylvaticum
$\frac{\text { B. glaucovirens/ }}{\frac{\text { Sylvaticum }}{\text { intermediates }}}$
B. glaucovirens B. pinnatum/
$\frac{\text { sylvaticum }}{\text { intermediates }}$ B. pinnatum
B. phoenicoides
B. distachyon


quased əLeW
Female parent
B. sylvaticum
B. glaucovirens $x$
B. sylvaticum $(2 n=17)$
B. glaucovirens
B. pinnatum ( $2 n=28$ )
B. pinnatum $x$
$\frac{\text { B. phoenicoides }}{(2 n=28)}$
B. phoenicoides
B. phoenicoides $x$
B. pinnatum $(2 n=28)$
TABLE 3.6/4

| Embryo culture results <br> Numbers in the first three columns refer to totals of all inter-plant crosses for that For individual results, see Appendix 3 |  |  |  |  |  |
| :---: | :---: | :---: | :---: | :---: | :---: |
| Cross | No. embryos in embryo culture (No. caryopses with no embryo) | No. seedlings obtained | No. mature plants obtained | \% Success using embryo culture (column 3 over column $1 \times 100$ ) | chr. no. <br> (2n) of hybrids |
| B. sylvaticum $(2 n=18) x$ <br> B. Sylvaticum ( $2 n=18$ ) | 45 | 29 | 24 | 53.3 | 18 |
| B. sylvaticum ( $2 n=18$ ) $x$ <br> B. glaucovirens ( $2 n=16$ ) | 18 | 11 | 9 | 50.0 | 17 |
| B. glaucovirens ( $2 n=16$ ) $x$ <br> B. sylvaticum ( $2 \mathrm{n}=18$ ) | 27(3) | 18 | 16 | 53.3 | 17 |
| B. glaucovirens/sylvaticum intermediate $(2 n=17) \times$ B. sylvaticum $(2 n=18)$ | 4 | 3 | 2 | 50.0 | 17 |
| B. glaucovirens/sylvaticum intermediate ( $2 n=17$ ) $\times$ B. glaucovirens ( $2 n=16$ ) | 2 | 2 | 1 | 50.0 | 16 |
| B. glaucovirens/sylvaticum intermediate $(2 n=17) \times$ B. phoenicoides ( $2 n=28$ ) | 11 | 10 | 7 | 63.6 | 22 |chr. no.

$(2 n)$ of
hybrids
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$\stackrel{\infty}{\sim}$ $\stackrel{\infty}{\sim}$ $\stackrel{\infty}{\sim} \quad \underset{\sim}{0}$ ~~~ $\stackrel{\sim}{\sim}$ $\underset{\sim}{\sim}$
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 No. embryos
in embryo
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(No. caryopses
with no embryo) 134(14) 23(8) ๓ ㄴ ก
요
$\bullet$ ~ $0 \quad 0+\frac{\text { ® }}{\circ}$ TABLE 3.6/4 CONTINUED
Cross


$\frac{\text { B. pinnatum }}{(2 n=28) \times \frac{\text { sylvaticum }}{\text { sylvaticum }}(2 n=18)}$ $\frac{\text { B. pinnatum/sylvaticum }}{(2 n=28) \times \text { B }}$ intermediate

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CONTINUED
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$04 \%$
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\% Success using
embryo culture
(column 3 over
column $1 \times 100$ )


|  |  |
| :---: | :---: |
| $\stackrel{m}{\sim}$ | ल |





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Cross
B. pinnatum $(2 n=28) x$
B. phoenicoides $(2 n=28)$ B. sylvaticum $(2 n=18) x$ B. phoenicoides $(2 n=28)$
 B. phoenicoides $(2 n=28) x$
B. sylvaticum $(2 n=18)$
B. phoenicoides $(2 n=28) x$
B. pinnatum $(2 n=28)$
B. phoenicoides $(2 n=28) x$ $\frac{\text { B. pinnatum }}{(2 n=28)}$ sylvaticum intermediate B. pinnatum/syl vaticum intermediate $(2 n=28) \times$ B. phoenicoides $(2 n=28)$
No. mature \% Successs using chr. no. غi4.我觡


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| No. embryos <br> in embryo <br> cul ture <br> (No. caryopses <br> with no embryo) | No. <br> seedlings <br> obtained |
| :---: | :---: |
| $21(7)$ | 17 |
| $2(1)$ | 0 |
| 1 | 0 |
| $8(1)$ | 3 |
| $5(2)$ | 5 |
| $6(1)$ | 3 |
| $7(9)$ | 7 |
| 8 | 3 |

$\dot{\circ} \underset{\sim}{\circ} \underset{\sim}{\circ} \frac{5}{3}$



ก
Cross
$\frac{\text { B. retusum }}{(2 n=28)}(2 n=32) \times$ B. pinnatum
$\frac{\text { B. mexicanum }}{(2 n=18)}(2 n=42) \times$ B. sylvaticum
$\frac{\text { B. mexicanum }}{\text { B. phoenicoides }}(2 n=42) \times$
$\frac{\text { B. distachyon }}{}(2 n=38)$
$\frac{(2 n=18)}{\text { B. }} 2 n=$ B. sylvaticum
$\frac{\text { B. distachyon }}{(2 n=28)}(2 n=30) \times$ B. pinnatum
$\frac{\text { B. distachyon }}{\text { variant }(2 n=18)}(2 n=30) \times$ B. pinnatum
$\frac{\text { B. distachyon }}{\text { variant }(2 n=36)}(2 n=30) \times$ B. pinnatum
$\frac{\text { B. sylvaticum }}{\times \text { B. sylvaticum })}(2 n=18) \times(2 n=17)$
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plants
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$11(1)$

TABLE 3．6／4
Cross

| B．pinnatum $(2 n=28) \times$（B．pinnatum <br> B．phoenicoides） $2 n=28$ |  |
| :---: | :---: |
|  |  |



## CHAPTER 3.7: HYBRID CHARACTERISTICS

### 3.7.1: Vegetative morphology of hybrids

Vegetative characters of the hybrids are summarized in Table $3.7 / 1$, and Plates $3.7 / 1$ - $3.7 / 5$ illustrate some hybrid plants. In the whole of this chapter female parents are given first.
B. sylvaticum $x$ B. glaucovirens resembles its male parent in its glabrous culms, leaf-blades and leaf-sheaths, and in its patent leaf-blades, but is like its female parent in its ciliated leaf-sheath and abaxial tuft of hairs at the junction of leaf-blade and leaf-sheath. The reciprocal cross also resembles its male parent in the pubescence of the culms and leaves, broadness of the leaf-blades, ciliated leaf-sheath margins and abaxial tuft of hairs at the junction of the leaf-blade and leaf-sheath. The backcross of this pubescent hybrid (female) to B. glaucovirens produced a glabrous plant (i.e. again close to the male parent), except for the ciliated leaf-sheaths and the abaxial tuft of hairs at the junction of leaf-blade and leafsheath. The B. glaucovirens/sylvaticum intermediate $x$ B. sylvaticum also resembles the male parent in its vegetative pubescence. The same is true of $B$. glaucovirens/sylvaticum intermediate x B. glaucovirens except that in this case the culm varies from glabrous to shortly pubescent.

The plants of B. pinnatum x B. sylvaticum resemble their male parent in their number of internodes (4-5), densely pubescent nodes and leaf-sheaths, and broadness of leaf-blades. The reciprocal crosses (Plate $3.7 / 1 a$ ) also resemble their male parent in the number of internodes (3-4), shortly pubescent nodes and leaf-sheaths, and narrow leaf-blades. However, culm pubescence varies from pubescent to glabrous in hybrids arising from crosses made in both directions.

## B. sylvaticum $\times$ B. pinnatum/sylvaticum

intermediate (Plate3.7/lb) differs from both parents in its densely pubescent leaves.

## B. pinnatum/sylvaticum intermediate $x$

B. sylvaticum differs from both parents in its glabrous leaf-sheaths, but resembles its male parent in the sparsely pubescent leaves and its female parent in the glabrous culms.

The B. sylvaticum $\times$ B. phoenicoides hybrid (Plate $3.7 / 3 a$ ) resembles its male parent in the glabrous culms, pubescent margins of the ribs, glabrous leafsheaths, and long narrow leaf-blades with prominent ribs. It resembles its female parent in the occasional presence of long spreading hairs on the face of the leaf-blade ribs. Because of this character it can be recognised as a hybrid and can be distinguished from B. phoenicoides. The reciprocal crosses (Plate $3.7 / 3 b$ ) also resemble their male parent in their pubescent culms and leaf-sheaths, ciliated leaf-sheath margins, short, broad leaf-blades, and abaxial tuft of hairs at the junction of leaf-blade and leaf-sheath. None
of the female parent characters is obvious. This cross, in fact, cannot be distinguished as a hybrid on the basis of vegetative morphology.
B. glaucovirens/sylvaticum intermediate $x$. B. phoenicoides (Plate $3.7 / 5 \mathrm{~B}$ ) resembles its male parent in its glabrous culms, pubescent margins of the ribs, long leaf-blades and prominent leaf-blade ribs. It resembles the female parent in its ciliated leaf-sheath and occasional pubescence of long spreading hairs on the face of the leaf-blade ribs. Superficially the plant looks like its male parent.
B. glaucovirens $\times$ B. pinnatum (Plate $3.7 / 2 \mathrm{~A}$ )
resembles its male parent in its pubescent leaf-blades and leaf-sheaths and ciliated leaf-sheath margins. None of the female parent characters is obvious. The pubescent culm of the hybrid differs from that of both parents.
B. glaucovirens $x$ B. phoenicoides (Plate 3.7/4B) resembles its male parent in its prominent leaf-blade ribs with pubescent margins. The ciliated margins of the leaf-sheath differ from those of both parents.
B. pinnatum $\times$ B. phoenicoides (Plate $3.7 / 4 \mathrm{~A}$ ) resembles its male parent in the prominent leaf-blade ribs with pubescent margins, but resembles its female parent in its ciliated margins of the leaf-sheath and in the presence of long spreading hairs on the face of the ribs. The pubescence of the leaf-sheath
either resembles the female parent or differs from both of the parents. The reciprocal cross resembles its female parent in the prominent leaf-blade ribs with pubescent margins, but resembles its male parent in that the faces of the ribs have long spreading hairs and the margins of the leaf-sheath are ciliated.
B. phoenicoides $\times$ B. pinnatum/sylvaticum intermediate also resembles its female parent in the prominent leaf-blade ribs with pubescent margins, but resembles its male parent in the pubescent leaf-sheath and the ciliated margins of the leaf-sheath.
B. pinnatum/sylvaticum intermediate $x$ B. phoenicoides resembles its male parent in the prominent leaf-blade ribs with pubescent margins, but resembles the female parent in the pubescence of the leaf-sheath and its ciliated margins.

$$
\text { B. pinnatum } \times \text { (B. pinnatum } \times \text { B. phoenicoides) }
$$

backcross still resembles the male parent (B. pinnatum x B. phoenicoides) in the prominent leaf-blade ribs with pubescent margins and in the face of the ribs with spreading hairs, but differs from both parents in the glabrous leaf-sheaths.

The (B. pinnatum $\times$ B. phoenicoides) $x$
B. phoenicoides backcross once more resembles its female parent (B. pinnatum $\times$ B. phoenicoides) in the leaf-blade rib pubescence and in the pubescent leafsheaths with ciliated margins. The main diagnostic character of the male parent, i.e. prominent leaf~ blade ribs with pubescent margins, is also present
in the female parent.
B. pinnatum variant $(2 n=18) \times$ B. pinnatum
( $2 \mathrm{n}=28$ ) (Plate $3.7 / 2 \mathrm{~B}$ ) does not differ from either of the parents, which are very similar morphologically.
B. retusum $x$ B. pinnatum is intermediate between its parents in the number of internodes and the length and width of the leaf-blades. It resembles its female parent in the prominent leaf-blade ribs with pubescent margins, but resembles its male parent in the pubescent leaf-sheath with ciliated margins.
B. distachyon $x$ B. pinnatum (Plate $3.7 / 5 \mathrm{~A}$ ) resembles its male parent in the height of the plant, and its female parent in the ciliated margins of the leaf-sheath. It is intermediate in the length and width of leaf-blades and has more internodes than either of the parents. It is longer-lived than B. distachyon, but appears to be only a weak perennial, with few innovations.

### 3.7.2: Floral morphology of hybrids

Floral morphology of the hybrids is summarized in Table 3.7/2.
B. sylvaticum $x$ B. glaucovirens differs from both parents in having fewer spikelets per raceme, fewer florets per spikelet, and longer awns. It resembles its female parent in its sparsely pubescent spikelets, length of pedicel and length of glumes, but its male parent in its patent spikelets. The
reciprocal cross resembles the male parent in the pubescent spikelets and the longer awns, but resembles the female parent in its patent spikelet and longer glumes. It differs from both parents in the slightly longer pedicel.
B. glaucovirens/sylvaticum intermediate $x$ B. sylvaticum resembles the male parent in its overlapping and pubescent spikelets and longer awns.
B. glaucovirens/sylvaticum intermediate $x$
B. glaucovirens resembles the male parent in its patent spikelets. Other characters do not differ from those of both parents.

The backcross (B. glaucovirens $x$ B. sylvaticum)
x B. glaucovirens differs from both parents in its shorter pedicels.
B. sylvaticum $\times$ B. pinnatum is intermediate
in pedicel length, glume length and awn length. It differs from both parents in its shorter anthers. The same is true of the reciprocal cross.
B. sylvaticum $\times$ B. pinnatum/sylvaticum
intermediate is intermediate in the lengths of pedicel, glumes and awn, but the anthers are shorter than in either parent.
B. pinnatum/sylvaticum intermediate x
B. sylvaticum is intermediate in the length of pedicel, glumes and awns, but the anthers are shorter than in either of the parents.
B. sylvaticum $x$ B. phoenicoides is intermediate in the length of pedicel, glumes and awns, but differs
from both parents in the shorter anthers. The reciprocal cross resembles the male parent in the shortly pubescent spikelets, and in the length of glumes and awns. It differs from both parents in the longer pedicel and the shorter anthers.
B. glaucovirens/sylvaticum intermediate $x$ B. phoenicoides resembles the male parent in the length of pedicel, but the female parent in the length of glumes. It is intermediate in the length of anthers and awns, but awn length is much closer to that of the female parent than to that of the male. It differs from both parents in the longer spikelets. B. glaucovirens $\times$ B. pinnatum (Plate $3.7 / 6 \mathrm{~A}$ ) resembles the male parent in the length of awn, but the female parent in the patent spikelets. It is intermediate in the length of pedicel.
B. glaucovirens x B. phoenicoides (Plate $3.7 / 6 B$ ) resembles the male parent in the length of pedicel, but the female parent in its patent spikelets. It is intermediate in awn length and differs from both parents in the shorter glumes.
B. pinnatum $\times$ B. phoenicoides resembles the male parent in the length of awn. Pedicel length in the hybrids is either more than in both parents or more closely resembles that of the female parent. The reciprocal crosses also resemble the male parent in the length of awn, but the female parent in the length of pedicel.
B. phoenicoides $\times$ B. pinnatum/sylvaticum intermediate resembles the male parent in the length of awn but is intermediate in the length of pedicel.
B. pinnatum/sylvaticum intermediate $x$
B. phoenicoides is intermediate in the length of pedicel and awn.

The B. pinnatum $\times$ (B. pinnatum $\times$ B. phoenicoides) backcross differs from both parents in its shorter pedicel and longer awn.

The (B. pinnatum $x$ B. phoenicoides) $x$ B. phoenicoides backcross resembles the male parent in its length of awn, but differs from both parents in its shorter pedicel.
B. pinnatum variant $(2 n=18) \times$ B. sylvaticum resembles the male parent in the length of awn and is intermediate in the length of pedicel.
B. pinnatum variant $(2 n=18) \times$ B. pinnatum $(2 n=28)$ is intermediate in the length of pedicel.
B. retusum $\times$ B. pinnatum resembles the male
parent in the pubescent spikelets and the length of awn, and is intermediate in the length of pedicel.
B. distachyon $x$ B. pinnatum resembles the female parent in the length of pedicel and awn.
3.7.3: Leaf epidermal anatomy of hybrids

Table $3.7 / 3$ summarises the stomatal length, presence/absence and length of horizontally elongated sinuous-walled silica-bodies, and length of abaxial
long-cells in the artifical hybrids. Plates 3.7/73.7/8 illustrate epidermal characters of the artificial hybrids.
B. sylvaticum $\times$ B. pinnatum (Plate 3.7/7A)
resembles the male parent in having rows of $3-4$ shortcells on the abaxial epidermis, in having long-cells with non-sinuous walls over costal and intercostal zones of both epidermises, and in lacking hairs on both epidermises. It resembles the female parent in the presence of abundant large prickles on the abaxial surface. It is intermediate in the frequency of horizontally elongated sinuous-walled silicabodies on the adaxial epidermis. Reciprocal crosses resemble the male parent in the presence of hooks on both surfaces, in having sinuous-walled long-cells on both costal and intercostal zones of the adaxial surface. They are intermediate in the frequency of horizontally elongated sinuous-walled silica-bodies.
B. glaucovirens $\times$ B. sylvaticum resembles the male parent in the occasional occurrence of stomata on the abaxial epidermis.
B. phoenicoides $\times$ B. sylvaticum (Plate $3.7 / 8 \mathrm{~A}$ )
resembles the male parent in having horizontally elongated sinuous-walled silica-bodies but in lacking macro-hairs on the adaxial surface, and in the occasional presence of stomata on the abaxial surface. The reciprocal cross (Plate $3.7 / 8 \mathrm{~B}$ ) resembles the male parent in the absence of horizontally elongated sinuous-
walled silica-bodies and in the presence of slightly curved macro-hairs on the adaxial surface.
B. pinnatum $x$ B. phoenicoides (Plate 3.7/7B) resembles the male parent in the presence of abundant large prickles on the abaxial surface and of slightly curved macro-hairs on the adaxial surface, and in the highly sinuous-walled long-cells on both epidermises. It resembles the female parent in having rows of up to 7 short-cells. The reciprocal cross resembles the male parent in the absence of hairs on the intercostal zone on the abaxial surface, but resembles the female parent in having rows of $2-3$ short-cells on the abaxial surface, and in having slightly curved macro-hairs on the adaxial surface. It is intermediate in the undulation of the walls of the long-cells.
B. glaucovirens $\times$ B. phoenicoides resembles the male parent in the presence of slightly curved macro-hairs on the adaxial epidermis.

### 3.7.4: Leaf internal anatomy of hybrids

Table 3.7/4 summarises the width of subepidermal sclerenchyma strands and the number and length of bulliform cells. Plates $3.7 / 9-3.7 / 11$ illustrate leaf section anatomy of the artificial hybrids.

$$
\text { B. sylvaticum } x \text { B. pinnatum (Plate } 3.7 / 9 \mathrm{~A} \text { ) }
$$

resembles the two parents, which do not differ in internal anatomy.

$$
\text { B. glaucovirens } \times \text { B. sylvaticum resembles the }
$$

female parent in the slightly more extended distribution of sclerenchyma on the adaxial than on the abaxial side.
B. glaucovirens $\times$ B. pinnatum resembles the male parent in the slightly more extended distribution of sclerenchyma on the abaxial than on the adaxial side, but resembles the female parent in having shallow intercostal grooves.
B. pinnatum $\times \underline{\text { B. phoenicoides (Plate } 3.7 / 10 A \text { ) }}$
and the reciprocal cross (Plate $3.7 / 10 B$ ) resemble B. phoenicoides in the deep intercostal grooves. Both crosses resemble $B$. pinnatum in having more extensive sclerenchyma on the abaxial side than on the adaxial side.
B. glaucovirens $\times$ B. phoenicoides resembles the male parent in the deep intercostal grooves and prominent ribs, but differs from both the parents in having more laterally extended sclerenchyma on the abaxial side than on the adaxial side.
B. phoenicoides $\times$ B. sylvaticum (Plate $3.7 / 11 \mathrm{~A}$ )
resembles the male parent in having more extensive sclerenchyma on the abaxial side than on the adaxial side, and in the shallow intercostal adaxial grooves. In reciprocal crosses (Plate $3.7 / 11 B$ ) sclerenchyma distribution is variable; in two plants it resembles the female parent but in one plant it resembles the male parent. The reciprocal hybrid also resembles the male parent in the deep intercostal grooves.

$$
\text { B. distachyon } \times \text { B. pinnatum }(2 n=28,2 n=18
$$


#### Abstract

and $2 n=36$ variants)(Plate $3.7 / 9 B$ ) resembles the female parent in the sclerenchyma being more extensive on the adaxial than on the abaxial side. All the three crosses also resemble the female parent in the shallow intercostal grooves and the rounded to oval bulliform cells.


### 3.7.5: Chromosome numbers and meiotic behaviour of hybrids

Chromsome numbers have been determined from root-tip preparations of a number of hybrids (Table 3.7/5, Plate 3.7/12); further chromosome numbers have been obtained from the meiotic analyses.

Chromosomal associations and chiasma frequency at meiosis are given in Table 3.7/6, and some meiotic preparations are shown in Plates 3.7/13$3.7 / 15$.

In Table 3.7/6, the relative number of chain and ring bivalentscan be ascertained from the last column, the number of chiasmata per bivalent, since figures significantly above 1.0 are derived from plants showing a good number of ring bivalents. The overall pairing affinity in hybrids can be seen in the penultimate column, which is calculated by dividing the total number of chiasmata per pollen mother cell by the somatic chromsome number.
Trivalents are rare in all cases (up to
0.41 per P.M.C.), and all those observed closely were Y-shaped.

Hybrids can be divided into three groups according to their meiotic behaviour.

In the first group meiosis is not markedly irregular or is almost regular. This group includes B. glaucovirens $\times$ B. sylvaticum (Plate 3.7/13A), B. pinnatum $\times$ B. phoenicoides and reciprocal (Plate $3.7 / 14 \mathrm{~B}$ ), B. pinnatum/sylvaticum intermediate ( $2 \mathrm{n}=28$ ) $\times$ B. phoenicoides, B. glaucovirens/sylvaticum intermediate ( $2 \mathrm{n}=17$ ) x B. sylvaticum, B. glaucovirens/sylvaticum intermediate ( $2 \mathrm{n}=17$ ) x B. sylvaticum, B. glaucovirens/ sylvaticum intermediate ( $2 \mathrm{n}=17$ ) $\times$ B. glaucovirens and (B. glaucovirens $\times$ B. sylvaticum) $(2 n=17) \times$ B. glaucovirens backcross (Plate 3.7/15B). These crosses are between parents whose chromosome numbers differ by no more than two, and there are fewer than two univalents found in each P.M.C., except for one plant of B. phoenicoides $\times$ B. pinnatum, where 3.05 were found. The number of chiasmata divided by the somatic chromosome number is usually above 0.5 ( $0.48-0.64$ ). The two cases which have figures below 0.5 ( 0.48 and 0.49 ) are the two crosses of the parentage B. phoenicoides $x$ B. pinnatum; the reciprocal of this had 0.58. The highest figure (0.64) was shown by B. glaucovirens $x$ B. sylvaticum and by the backcross of this to B. glaucovirens. Lagging chromsomes were observed at anaphase in all of these crosses except B. pinnatum $x$ B. phoenicoides and its reciprocal (despite the relatively low chiasma frequency of the latter), where meiosis appeared more or less regular.

Meiosis in the second group is less regular. This group includes B. sylvaticum $\times$ B. pinnatum and reciprocal (Plate 3.7/13B), B. sylvaticum $x$ B. phoenicoides and reciprocal (Plate $3.7 / 14 \mathrm{~A}$ ), B. sylvaticum $x$ B. pinnatum/sylvaticum $(2 n=28)$ and B. glaucovirens/ sylvaticum intermediate $(2 n=17) \times$ B. phoenicoides. These crosses are between parents with chromosome numbers differing by ten or eleven, and there are 3.25 to 6.42 univalents in each P.M.C. The number of chiasmata divided by the somatic chromosome number is usually much less than 0.5 ( $0.37-0.43$ ), except in the cross B. glaucovirens/sylvaticum intermediate x B. phoenicoides, where the figure was 0.49. Lagging chromosomes are visible in all meioses in this group, and these lead to the appearance of micronuclei at later stages.

The third group consists of the combinations involving B. distachyon (female) $x$ B. pinnatum ( $2 \mathrm{n}=28$ and 36 races) (Plate $3.7 / 15 \mathrm{~A}$ ). There meiosis is very irregular, with 8.24-14.13 univalents and mean chiasma frequencies per chromosome of 0.26-0.41. Later stages of meiosis were very disturbed, and sometimes even spindle development was poor.

### 3.7.6: Pollen stainability of hybrids

For practical purposes pollen fertility was measured by pollen stainability in acetocarmine. Results are shown in Table 3.7/7.

In crosses between parents with chromosome numbers differing by more than two, pollen fertility
was very low ( $0-6.0 \%$ ), except in B. pinnatum/sylvaticum intermediate x B. sylvaticum where it was $11.9 \%$.

Crosses involving B. distachyon all had 0\% stainable pollen (Plate 3.7/16B).

Crosses between parents both with $2 \mathrm{n}=28$ (B.pinnatum, B. phoenicoides, B. pinnatum/sylvaticum intermediate) showed the highest pollen fertility(68.9-93.5\%) (Plate $3.7 / 16 \mathrm{~A}$ ) . Next highest were parents differing in chromosome number by one or two chromosomes, i.e. (B. glaucovirens $\times$ B. sylvaticum) $\times$ B. glaucovirens backcross (54.0\%) and B. glaucovirens $x$ B. sylvaticum (37.2\%). However, two crosses similar to these two showed much lower pollen fertilities, i.e. B. glaucovirens /sylvaticum intermediate $x$ B. glaucovirens (9.9\%) and B. glaucovirens $\times$ B. sylvaticum ( $0 \%$ ).

The most remarkable of these results concerns the two plants B. glaucovirens x B. sylvaticum, where the hairy variant showed $0 \%$ and the glabrous variant 37.2\%. Since in both cases the pollen used was a mixture of that from four male parents, the explanation is probably to be found in different parentage.

### 3.7.7: Seed-set of hybrids

In the case of seed-set following artificial
interspecific hybridization, it was found that usually all 'good' (full, hard, embryo-containing) caryopses were capable of germination. Therefore, seed-set in $F_{1}$ hybrids was taken as a rough guide to seed-fertility
of these plants. Results are presented in Table 3.7/8.

Once again, there is a correlation between seed-set and similarity of parental chromosome number. Where the chromosome number of the parents differed by more than one, seed-set was generally $0 \%$, but sometimes up to $1.8 \%$. Where the difference was one chromosome, seed-set was $4.5 \%$ in B. glaucovirens/ sylvaticum intermediate $x$ B. sylvaticum, $0 \%$ in B. glaucovirens/sylvaticum intermediate x B. glaucovirens, and $18.4 \%$ in the backcross (B. glaucovirens $\times$ B. sylvaticum) x B. glaucovirens.

Where the chromosome number of the two parents was the same, seed-set was $2.8 \%$ in B. pinnatum variant $(2 n=18) \times$ B. sylvaticum, and $15.6-47 \%$ in hybrids between parents both with $2 \mathrm{n}=28$. Highest seed-set was in B. pinnatum $x$ B. phoenicoides and the reciprocal (25.1-47\%).

As in the case of pollen-fertility, seedset was different in the hairy and non-hairy variants of B. glaucovirens x B. sylvaticum ( $0 \%$ and $1.39 \%$ respectively).

The hybrid involving B. distachyon set no seed.

### 3.7.8: Floral biology of hybrids

Flowering behaviour in hybrids was mostly observed in July and early August. All the hybrids were chasmogamous.

Details of the length of time taken for inflorescence and spikelet to emerge from the sheath were observed only in B. distachyon $x$ B. pinnatum. In this hybrid each spikelet took $20-25 \mathrm{hr}$ to emerge from the leaf-sheath, and the inflorescence underwent anthesis 10-12 days after complete emergence from the leaf-sheath. Florets opened in the morning at 8.309.00 in June. Anthers dehisced after $30-35 \mathrm{~min}$.
B. pinnatum $\times$ B. sylvaticum and the reciprocal crosses mostly commenced anthesis at a time intermediate for both parents, but some hybrid plants underwent anthesis at the same time as one or other of the parents. Florets opened at 3.00-4.00(5.00) in July. Anthers dehisced after $40-45 \mathrm{~min}$.
B. glaucovirens $\times$ B. sylvaticum commenced anthesis at 4.30-5.00 in July, thus resembling the female parent (slightly later than the male parent). Anthers dehisced after about 45 min .
B. glaucovirens $\times$ B. pinnatum commenced anthesis at 3.00-4.00 in late July. Anthers dehisced after about 45 min . Anthesis time resembled that of the female parent.
B. glaucovirens $\times$ B. phoenicoides underwent anthesis in the morning as well as in the evening. Florets opened in the morning at 4.30-5.00 and in the evening at about 18.45-19.00. Dehiscence took place after $40-45 \mathrm{~min}$. The morning anthesis time resembled that of the female parent. The evening time anthesis differed from that of both parents, although some other
variants of B. phoenicoides undergo evening anthesis.
B. sylvaticum $x$ B. phoenicoides also commenced anthesis in the morning as well as in the evening. The florets opened at 5.00-6.00 (slightly later than female parent) and 18.00-1900 (different from both parents) in July and early August. Anthers dehisced after 1 hr or even longer. The reciprocal cross commenced anthesis only in the morning. Florets opened at 5.00-5.30 in July and early August (very slightly later than the female parent). Anthers dehisced after about 1 hr . B. pinnatum variant $(2 n=18) \times$ B. pinnatum ( $2 \mathrm{n}=28$ ) commenced anthesis at $5.00-5.30$ in late July. Dehiscence took place after 40-45 min. Anthesis time did not differ from that of both parents.

The intraspecific hybrid between the two variants of B. phoenicoides (i.e. those commencing anthesis in the morning and those in the evening) underwent anthesis at 17.30-18.00 in July. Anthers dehisced after 23-25 min. Anthesis time therefore resembled that of the female parent.
B. pinnatum $\times$ B. phoenicoides and the reciprocal commenced anthesis in the morning as well as in the evening. Florets opened at 8.30-9.30 and 17.30-18.30 at the end of June and in July. Anthers dehisced after 23-25 min. The morning anthesis time was the same as that of the male parent. One of the hybrids (B55 x B35) commenced anthesis only in the morning at 8.45 in early July.

[^1]B. phoenicoides commenced anthesis in the morning as well as in the evening. The florets opened at 5.006.00 and 18.00-19.00 in July to early August. Dehiscence took place after about 1 hr or even later. Morning anthesis time was between that of both parents, but evening anthesis time resembled that of the male parent.
B. pinnatum $\times$ ( $B$. pinnatum $\times$ B. phoenicoides) backcross commenced anthesis 17.30-18.30 at the end of July. Anthers dehisced after $25-30 \mathrm{~min}$. Anthesis in the backcross resembled that in B. phoenicoides.
B. pinnatum/sylvaticum intermediate $x$ B. phoenicoides commenced anthesis at 9.00-9.30 at the end of June and in July. Anthers dehisced after 25-30 min. Anthesis time is somewhat later than that of both parents.
B. retusum $\times$ B. pinnatum commenced anthesis at 7.30-8.00 at the end of June and in early July. Anthers dehisced after about 30 min. Anthesis time resembled that of the female parent.


| Hyorid | Plant height (cm) | Culm pubescence | No. internodes | Internode length (cm) | Node pubescence | Leaf length $x$ width (cm x mm) | Pubescence of leaves on upper side | Pubescence of leaf-sheath | Margin of leafsheath | Ligule length (mm) |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| B. phoenicoides $\times$ B. sylvaticum |  |  |  |  |  |  |  |  |  |  |
| B55 $\times$ B84 | 74.5 | Pubescent | 3-4 | 15.9 | Densely pubescent | $14.6 \times 8.3$ | Face of ribs slightly pubescent | Densely pubescent | Ciliated | 1.8 |
| B. glaucovirens/sylvaticum intermediate $\times$ B. phoenicoides |  |  |  |  |  |  |  |  |  |  |
| B249 x B39 | 145.5 | Glabrous | 4-5 | 23.2 | Shortly pubescent | $41.3 \times 5.3$ | Margin and face of ribs pubescent | Glabrous | Ciliated | 2.8 |
| B249 x B181 | 67.0 | Glabrous | 5 | 12.7 | Shortly pubescent | $25.5 \times 4.5$ | Margin and face of ribs pubescent | Glabrous | Ciliated | 1.7 |
| Hybrid | Plant height (cm) | Culm pubescence | No. internodes | Internode length (cm) | Node pubescence | Leaf length x width (cm x mm) | Pubescence of leaves on upper side | $\begin{aligned} & \text { Pubes cence } \\ & \text { of } \end{aligned}$ leaf-sheath | Margin of leafsheath | Ligule length (mm) |
| B. glaucovirens $\times$ B. pinnatum |  |  |  |  |  |  |  |  |  |  |
| B224 x 88 | 45.3 | Glabrous | 3-4 | 10.5 | Shortly pubescent | $13.1 \times 5.9$ | Slightly pubescent | Glabrous | Ciliated | 2.5 |
| $\text { B226 } \times \text { B220 }$ |  | Slightly pubescent | 4-7 | 10.4 | Shortly pubescent | $18.8 \times 4.0$ | Pubescent | Pubescent | Ciliated | 2.1 |
| B. glaucovirens $\times$ B. phoenicoides |  |  |  |  |  |  |  |  |  |  |
| B224 $\times$ B55 | 83.0 | Glabrous | 4 | 18.1 | Shortly pubescent | $33.6 \times 4.5$ | Margin of ribs pubescent | Glabrous | Slightly <br> ciliated | 3.0 |
| B. pinnatum $\times$ B. sylvaticum |  |  |  |  |  |  |  |  |  |  |
| B4 $\times$ B48 | 55.0 | Glabrous | 4 | 10.5 | Densely pubescent | $27.0 \times 6.5$ | Pubescent | Glabrous | Ciliated | 2.8 |
| B10 $\times 829$ | 96.0 | Densely pubescent | 5 | 18.6 | Densely pubescent | $27.5 \times 7.5$ | Densely pubescent | Densely Pubescent | Ciliated | 2.8 |
| B17 $\times 894$ | 60.8 | Glabrous | 4 | 12.7 | Densely pubescent | $15.2 \times 5.0$ | Pubescent | Densely pubescent | Ciliated | 1.6 |
| Hybrid | Plant height (cm) | Culm pubescence | No internodes | Internode length (cm) | Node pubescence | Leaf length $x$ width ( $\mathrm{cm} \times \mathrm{mm}$ ) | Pubescence of leaves on upper side | Pubescence of leaf-sheath | Margin of leafsheath | Ligule length (mm) |
| B. pinnatum $\times$ B. phoenicoides |  |  |  |  |  |  |  |  |  |  |
| в36 $\times$ B88 | 73.3 | Glabrous | 3-4 | 15.1 | Pubescent | $17.5 \times 5.0$ | Margin and face of ribs pubescent | Pubescent | Ciliated | 1.9 |
| B220 $\times 181$ | 52.5 | Glabrous | 4 | 11.5 | Shortly pubescent | $15.3 \times 5.5$ | Margin and face of ribs pubescent | Glabrous | Ciliated | 1.6 |
| B. pinnatum variant ( $2 \mathrm{n}=18$ ) $\times$ B. sylvaticum |  |  |  |  |  |  |  |  |  |  |
| B144 $\times$ B257 | 67.0 | Glabrous | 3-4 | 16.5 | Shortly pubescent | $13.6 \times 8.4$ | Glabrous <br> to sparsely pubescent | Glabrous | Ciliated | 1.9 |
| B. pinnatum variant ( $2 \mathrm{n}=18$ ) $\times$ B. pinnatum $(2 n=28)$ |  |  |  |  |  |  |  |  |  |  |
| B144 $\times 88$ | 58.5 | Glabrous | 4 | 11.4 | Shortly pubescent | $13.6 \times 8.4$ | Glabrous to sparsely pubescent | Glabrous | Ciliated | 1.9 |



Hybrid Raceme Distance from Distance Spikelet Pedice uppermost
leaf-sheath to
between len length $\substack{\text { leaf-sheath to } \\ \text { inflorescence } \\(\mathrm{cm})}$
$(\mathrm{cm})$
B. sylvaticum $\times$ B. glaucovirens

| B364 $\times$ B226 | 9.5 | 10.3 | 1.3 | 4.3 | 0.9 |
| :---: | :---: | :---: | :---: | :---: | :---: |
| B. glaucovirens $\times$ B. sylvaticum |  |  |  |  |  |
|  | 2.1 | 20.7 | 2.1 | 5.6 | 1.4 |

B. glaucovirens/sylvaticum intermediate $\times$ B. sylvaticum $\begin{array}{l:lccc}\text { B249 } \times \text { B253 } & 10.4 & 20.4 & 1.8 & 3.6 \\ \text { B. } \text { glaucovirens } / \text { sylvaticum } & 0.8 \\ \text { intermediate }\end{array} \times \begin{aligned} & \text { B. glaucovirens }\end{aligned}$ $\begin{array}{llllll}\text { B249 } \times \text { B226 } & 12.5 & 18.3 & 1.6 & 4.1 & 0.7\end{array}$ (B. glaucovirens $\times$ B. sylvaticum) $\times$ B. glaucovirens backcross
 B. sylvaticum $\times$ B. pinnatum

| -829 $\times 810$ | 15.5 | 27.6 | 2.4 | 4.7 | 1.2 |
| :---: | :---: | :---: | :---: | :---: | :---: |
| B95 $\times$ 89 | 10.2 | 16.0 | 3.0 | 3.1 | 1.5 |
| B102 $\times$ B36 | 12.6 | 17.1 | 1.5 | 3.3 | 1 |
| B258 $\times$ B76 | 16.6 | 18.5 | 2.1 | 3.2 | 1.3 |
| B. sylvaticum $\times$ B. pinnatum/sylvaticum intermediate |  |  |  |  |  |
| B61 $\times 85$ | 9.9 | 12.4 | 1.2 | 2.6 | 1.5 |
| B. pinnatum/sylvaticum intermediate $\times$ B. sylvaticum |  |  |  |  |  |
| B42 $\times 846$ | 7.9 | 26.7 | 1.6 | 3.5 | 1.3 |
| B. sylvaticum $\times$ B. phoenicoides |  |  |  |  |  |
| B28 $\times$ B2 | 11.0 | 5.0 | 1.9 |  |  |
| $841 \times 839$ | 14.8 | 22.0 | 1.8 | 5.4 | 1.2 |
| 884 $\times 855$ | 9.7 | 14.8 | 1.6 | 3.2 | 1.0 |



| $3-4$ | $9-12$ | 8.4 | 10.4 | 5 | 6 | 21.1 | 10.7 | 5 |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| $5-10$ | $12-26$ | 7.8 | 10.2 | $4-6$ | $6-7$ | 20.5 | 9.7 | $5-8$ |

Sparsely pubescent

| Glabrous to pubescent | 9.4 | 1.9 | 3.8 |
| :--- | :--- | :--- | :--- |


| Slightly pubescent | 8.6 | 1.9 | 3.4 | - |
| :--- | :--- | :--- | :--- | :--- |


| Glabrous | 8.6 | 1.7 | 3.2 |
| :--- | :---: | :---: | :---: |
| Glabrous to shortly pubescent | 8.1 | 1.6 | 3.8 |

Glabrous
Densely pube
Glabrous
Shortly pubescent

Densely pubescent
$\begin{array}{lll}6.6 & 1.9 & 2.9\end{array}$

Densely pubescent

Minutely pubescent Glabrous
$\begin{array}{ll}9.0 & 1.9 \\ 9.4 & 2.1 \\ 8.0\end{array}$
$\underset{\substack{\text { Pa lea } \\ \text { length } \\(\mathrm{mm})}}{\substack{\text { Lemma } \\ \text { width } \\(\mathrm{mm})}} \begin{gathered}\text { Anther } \\ \text { length } \\ (\mathrm{mm})\end{gathered} \begin{gathered}\text { Grain } \\ \text { lingth } \times \\ \text { width } \\ (\mathrm{rm} \times \mathrm{mm})\end{gathered}$ $\underset{(\mathrm{mm}}{\substack{\text { width } \\ \text { mim }}}$
-

| $7-8$ | $9-12$ | 9.8 | 10.5 | 6 | $8-9$ | 20.4 | 9.3 | 6 |
| :--- | :--- | :--- | :--- | :--- | :--- | :--- | :--- | :--- |


| $4-7$ | $14-19$ | 5.6 | 8.3 | $4-7$ | $5-7$ | 13.5 | 4.7 | 5 |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| $4-6$ | $8-19$ | 7.5 | 9.5 | $4-7$ | $6-7$ | 14.2 | 5.3 | 5 |
| $4-8$ | $10-21$ | 8.1 | 10.1 | 4 | 5 | 13.8 | 5.5 | 6 |
| $3-8$ | $8-12$ | 6.3 | 8.3 | $3-5$ | $6-7$ | 15.7 | 7.0 | 5 |
|  |  |  |  |  |  |  |  |  |
| $5-10$ | $8-10$ | 5.1 | 6.6 | 4 | 5 | 11.7 | 4.5 | 4 |
|  |  |  |  |  |  |  |  |  |
| $5-9$ | $10-14$ | 7.2 | 8.6 | 4 | 5 | 14.6 | 5.1 | 5 |
|  |  |  |  |  |  |  |  |  |
|  |  |  |  |  |  |  |  |  |
| $5-6$ | $6-7$ | 8.7 | 10.4 | 5 | 5 | 15.5 | 5.2 | 5 |
| $4-9$ | $15-23$ | 7.9 | 10.0 | $5-6$ | $6-7$ | 16.1 | 4.7 | $3-5$ |
| 5 | 9 | 8.7 | 10.5 | 5 | 0 | 17.3 | 7.8 | 6 |

$\begin{array}{cc}\begin{array}{c}3.4 \\ 3.4 \\ 4.4\end{array} & \vdots\end{array}$

| Hybrid | Raceme <br> length <br> (cm) | Distance from uppermost leaf-sheath t inflorescence (cm) | Distance between spikelets (cm) | Spikelet <br> length (cm) | Pedicel length (mm) | $\begin{aligned} & \text { No. } \\ & \text { spikelets } \end{aligned}$ | No. florets | Lower glume $\underset{\substack{\text { (mm) } \\ \text { (mgth }}}{ }$ | $\begin{aligned} & \text { Upper } \\ & \text { glume } \\ & \text { length } \\ & \text { (mm) } \end{aligned}$ | $\begin{aligned} & \text { No. } \\ & \text { lower } \\ & \text { glume } \\ & \text { veins } \end{aligned}$ | No. glume veins | Lemma (incl. awn) (mm) | $\begin{gathered} \text { Aun } \\ \substack{\text { length } \\ (\mathrm{mm})} \end{gathered}$ | $\begin{aligned} & \text { No. } \\ & \text { lemma } \\ & \text { veins } \end{aligned}$ | Lemma pubescence | $\begin{aligned} & \text { Palea } \\ & \text { length } \\ & (\mathrm{mm}) \end{aligned}$ | $\begin{gathered} \text { Lemma } \\ \text { width } \\ (\mathrm{mm}) \end{gathered}$ | Anther length (mm) | Grain length $x$ width ( $m \mathrm{~m} \times \mathrm{mm}$ ) |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| B. phoenicoides $\times$ B. sylvaticum |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| B55 $\times 884$ | 14.1 | 23.2 | 1.8 | 4.5 | 1.6 | 8-9 | 7-17 | 7.3 | 10.0 | 4 | 6-7 | 18.6 | 8.0 | 5 | Shortly pubescent | 8.9 | 1.8 | 3.6 | - |
| B. glaucovirens/sylvaticum intermediate $\times$ B. phoenicoides |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| $\begin{aligned} & B 249 \times B 39 \\ & B 249 \times 8181 \end{aligned}$ | $\begin{aligned} & \begin{array}{c} 23.6 \\ 11.4 \end{array} \end{aligned}$ | $\begin{aligned} & 31.6 .6 \\ & 18.9 \end{aligned}$ | $\begin{aligned} & 2.6 \\ & 1.0 \end{aligned}$ | $\begin{aligned} & 7.4 \\ & \hline .7 \end{aligned}$ | $\begin{aligned} & 2.4 \\ & 0.9 \end{aligned}$ | $7-9$ | 25-27 18 | 7.6 5.1 | 9.6 6.0 | ${ }_{4}^{6}$ | $7-8$ 5 | 16.1 9.1 | ${ }_{1}^{5.6}$ | $5-8$ 5 | Glabrous Glabrous | 9.3 | 2.2 | 3.7 3.0 | - |
| B. glaucovirens $\times$ B. pinnatum |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| $\begin{aligned} & 8224 \times \text { B8 } \\ & \text { B226 } \times \text { B220 } \\ & \text { B. glaucov } \end{aligned}$ | $\begin{array}{r} 10.7 \\ 11.2 \\ \text { rens } \times 3 . \end{array}$ | $\begin{gathered} 17.4 \\ 13.5 \\ \text { phoenicoides } \end{gathered}$ | 1.4 | 3.8 5.6 | 1.5 | $7-8$ 7 | $18-19$ $11-24$ | $\begin{aligned} & 7.2 \\ & 6.4 \end{aligned}$ | 8.6 8.4 | 4-5 | $\begin{aligned} & 6-8 \\ & 5-8 \end{aligned}$ | 12.3 14.2 | 3.7 5.0 | 5 5 | $\underset{\text { Glabrous }}{\text { Glabrous }}$ | $\begin{aligned} & 7.8 \\ & 8.7 \end{aligned}$ | 1.7 | 4.4 | - |
| $\mathrm{B} 224 \times 855$ <br> B. pinnat | $\begin{array}{r}8.0 \\ \times \text { B. } \text { sy } \\ \hline 9 .\end{array}$ | ${ }^{22.7}$ | 2.3 | 3.2 | 1.4 | 4-6 | 13-14 | 4.2 | 5.9 | 4-5 | 5-7 | 11.7 | 2.9 | 6-8 | Glabrous | 8.4 | 2.1 | 4.0 | - |
| $\begin{aligned} & 84 \times B 48 \\ & B 10 \times 829 \\ & B 11 \times 894 \end{aligned}$ | $\begin{array}{r} 9.7 \\ 10.2 \\ 11.7 \end{array}$ | $\begin{array}{r} 11.2 \\ 26.2 \\ 5.8 \end{array}$ | 1.4 1.8 1.3 | 4.5 3.6 4.1 | 0.9 1.3 1.0 | $6-7$ $3-6$ $5-7$ | $18-20$ $13-21$ $15-16$ | 6.7 6.8 7.0 | 7.9 8.9 9.5 | 6 5 5 | 8 6 7 | 15.1 14.4 16.5 | 5.5 5.7 6.8 | 5 7 | Glabrous to minutely pubescent | 8.7 8.4 8.4 | 1.9 1.8 2.0 | 3.6 3.3 3.8 | - |
| B. pinnatum $\times$ B. phoenicoides |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| $\begin{aligned} & 836 \times \mathrm{B88} \\ & \mathrm{B220} \times \mathrm{B} 181 \end{aligned}$ | $\begin{array}{r} 14.5 \\ 9.4 \end{array}$ | $\begin{array}{r} 7.9 \\ 13.6 \end{array}$ | 1.7 1.2 | 4.5 4.0 | 4.5 | $\begin{aligned} & 6-9 \\ & 6-8 \end{aligned}$ | $\begin{aligned} & 21-22 \\ & 18-26 \end{aligned}$ | 6.5 4.7 | 8.0 | $3-4$ 3 | 6 5 | 12.9 9.7 | 2.5 | 3 5 | Glabrous Glabrous | 9.5 6.9 | 2.4 2.0 | 4.3 | $7.3 \times 1.9$ |
| B. pinnatum variant ( $2 \mathrm{n}=18$ ) $\times$ B. sylvaticum |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| B144 $\times 8257$ B. pinnatu | 14.4 variant | 23.2 $(2 n=18) \times$ B. pi | 2.0 ${ }_{\text {nnatum }}(2 n=$ | 28) 5.0 | 1.3 | 3-9 | 12-15 | 7.4 | 9.4 | 4-5 | 5-8 | 17.1 | 7.5 | 5-6 | Shortly pubescent | 8.6 | 1.8 | 3.9 | $5.2 \times 1.0$ |
| $8144 \times 88$ <br> B. phoenic | 10.6 ides $\times$ B | 15.9 pinnatum | 1.1 | 3.6 | 1.2 | 9-10 | 18-21 | 6.0 | 7.1 | 3-5 | 6-7 | 11.5 | 2.7 | 5-6 | Glabrous | 7.6 | 1.8 | 3.3 | - |
| $\begin{aligned} & 888 \times 836 \\ & 855 \times 835 \end{aligned}$ | $\begin{array}{r} 13.9 \\ 9.6 \end{array}$ | $\begin{aligned} & 14.9 \\ & 19.7 \end{aligned}$ | $\begin{aligned} & 1.9 \\ & \hline 2 \end{aligned}$ | $\begin{aligned} & 3.2 \\ & 3.0 \end{aligned}$ | 3.2 | $\begin{gathered} 6-8 \\ 5-7 \end{gathered}$ | $9-11$ $14-24$ | 7.0 5.3 | 8.3 6.5 | $\frac{4}{3-5}$ | ${ }_{5-6}^{6}$ | $\begin{aligned} & 12.3 \\ & 11.2 \end{aligned}$ | $\begin{aligned} & 3.4 \\ & 1.7 \end{aligned}$ | $\begin{aligned} & 6 \\ & 5 \end{aligned}$ | Glabrous Glabrous | $\begin{aligned} & 8.6 \\ & 8.6 \end{aligned}$ | 1.7 2.0 | 4.2 | $8.0 \times 2.0$ |
| B. phoenicoides $\times$ B. pinnatum/sylvaticum intermediate |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| B97 $\times$ B5 | 13.4 | 22.8 | 1.9 | 5.4 | 1.1 | 5 | 21-24 | 5.6 | 7.6 | 3 | 5 | 11.6 | 2.4 | 5 | Shortly pubescent | 8.8 | 2.0 | 5.4 | - |


| Hybrid | Raceme <br> length (cm) | Distance from uppermost leaf-sheath inflorescence (cm) | Distance between spikelets (cm) | Spikelet length (cm) | Pedicel (mm) | $\begin{aligned} & \text { No. } \\ & \text { spikelets } \end{aligned}$ | No. florets | Lower glume (mm) | $\begin{aligned} & \text { Upper } \\ & \text { glume } \\ & \text { length } \\ & \text { (mm) } \end{aligned}$ | No. glume veins | No. glume veins | Lemma (incl. awn) (mm) | $\begin{gathered} \text { Awn } \\ \text { length } \\ (\mathrm{mm}) \end{gathered}$ | $\begin{gathered} \text { No. } \\ \text { lemma } \\ \text { veins } \end{gathered}$ | Lemma pubescence | Palea <br> length (mm) | $\begin{aligned} & \text { Lemma } \\ & \text { width } \\ & \text { (mm) } \end{aligned}$ | Anther length (mm) | Grain length x (mm x mm) |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| B. pinnatum/sylvaticum intermediate $\times$ B. phoenicoides |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| B5 $\times$ B55 | 14.3 | 19.9 | 1.4 | 3.1 | 1.4 | 8-9 | 12-15 | 5.7 | 6.7 | 5 | 6 | 9.7 | 2.2 | 5 | Glabrous | 7.9 | 1.8 | 5.3 | - |
| B. pinnatu | $\times$ (B. | natum $\times$ B. ph | enicoides) | backcross |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| ${ }_{888)}^{B 14} \times(B 36 \times$ | 8.3 | 27.1 | 2.3 | 3.5 | 1.1 | 3-5 | 13-15 | 6.1 | 7.6 | 3-5 | 5-6 | 13.3 | 4.8 | 5 | Sparsely pubescent | 8.1 | 1.9 | - | $6.3 \times 1.7$ |
| (B. pinnatum $\times$ B. phoenicoides) $\times$ B. phoenicoides backcross |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  | 7.6 | 1.7 | - | $6.7 \times 1.6$ |
| $\begin{aligned} & (\mathrm{B} 36 \times 888) \\ & \times 888 \end{aligned}$ | 9.1 | 10.3 | 1.5 | 1.8 | 2.3 | 6-7 | 7-9 | 5.3 | 6.7 | 4-5 | 6-7 | 8.9 | 0.9 | 5 | Glabrous |  |  |  |  |
| B. retusum $\times$ B. pinnatum |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| B173 $\times$ B60 | 9.0 | 20.7 | 1.2 | 3.0 | 1.5 | 5-8 | 10-13 | 5.3 | 6.8 | 3-4 | 5 | 12.3 | 4.0 | 5 | Pubescent | 7.0 | 1.9 | 3.7 | - |
| B. distachyon $\times$ B. pinnatum |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| B199 x B8 | 6.1 | 20.9 | 1.4 | 3.5 | 0.6 | 1-7 | 11-20 | 4.8 | 6.6 | 5 | 6 | 18.7 | 10.1 | 5 | Densely pubescent | 8.3 | 1.7 | - | - |

Hybrid \begin{tabular}{c}
Mean stomatal length <br>
Abaxial <br>
$(\mu \mathrm{m})$

 side 

Adaxial <br>
$(\mu \mathrm{m})$
\end{tabular} side

B. sylvaticum $\times$ B. pinnatum.
$3258 \times$ B76 $26.8 \quad 26.8$
io
24.0
22.1
22.9
21.3
24.2
$\stackrel{\sim}{\sim}$
ベ
웅 TABLE $3.7 / 3$
Leaf epidermal anatomy of hybrids TABLE $3.7 / 3$
Leaf epidermal anatomy of hybrids Mean length of
long-cells on
abaxial-side $(\mu \mathrm{m})$
114.4
Ono
in
ion
111.6
$\stackrel{-}{\sim}$
끌
126.6
$\stackrel{ \pm}{-}$
일

Present
Present
Present
Absent
Absent
Absent
Present Present
Present
28.1
32.9
14.0
13.8
$\stackrel{\infty}{\dot{\sim}} \quad \stackrel{\infty}{\dot{m}}$
io
$n$
$\cdots$
4.0
No. rows of




> TABLE $3.7 / 5$
> Chromosome numbers of artificial hybrids obtained from mitotic preparations

Hybrid $2 n$
B. sylvaticum $(2 n=18) \times$ B. pinnatum $(2 n=28)$

B29 $\times$ B10
23
B95 x B9
23
B102 x B36
23
B258 x B76
23
B261 × B4, B9
23
B. pinnatum $(2 n=28) \times$ B. sylvaticum $(2 n=18)$

B10 x B29 23
B35 $\times$ B131 23
B. pinnatum/sylvaticum intermediate $(2 n=28) \times \frac{\text { B. sylvaticum }}{(2 n=18)}$
$B 42 \times B 46$
23
B. glaucovirens $(2 n=16) \times$ B. sylvaticum $(2 n=18)$

B224 x B48, B61, B134, 17
B261
B. glaucovirens $(2 n=16) \times$ B. phoenicoides $(2 n=28)$

B224 $\times$ B55
22
B. sylvaticum $(2 n=18) \times$ B. phoenicoides $(2 n=28)$

B41 $\times 839$
B. phoenicoides $(2 n=28) \times$ B. sylvaticum $(2 n=18)$

B55 $\times$ E84 23
TABLE 3.7/6
Mean and range of chromosome associations and mean number of chiasmata at meiosis in synthetic hybrids

III II I

$\stackrel{M}{+}$
$\bigcirc$
Per bivalent (excl.
trivalents) $0.43-1.0$
.
$0.40 \quad 1.01$
어우
No
No்
0.41
$\square$
0
0
TABLE 3.7/6 CONTINUED
Hybrid $2 n$ (calculated No. cells
Mean no. chiasmata

$$
10.43
$$

9.13
10.29
16.13
13.73
12.83

TABLE 3.7/6 CONTINUED
Hybrid $2 n$ (calculated No. cells
from meiosis)

$$
\text { Mean no. chiasmata }
$$

| Per P.M.C. |
| :--- |
| (bivalents |
| trivalents) |

0.49
5
0.41
0.37

| 6 |
| :---: |
|  |
| 0 |

1.02
1.04
1.07
Per bivalent
(excl.
trivalents)
1.16
1.05
$\begin{array}{ll}\sigma & 0 \\ - & -\end{array}$

$$
\begin{gathered}
\text { TABLE } 3.7 / 7 \\
\text { Pollen stainability of artificial hybrids }
\end{gathered}
$$

| Hybrid | Total no. pollen-grains scored | pollen-grains stained |
| :---: | :---: | :---: |
| B. sylvaticum $(2 n=18) \times$ B. pinnatum $(2 n=28)$ |  |  |
| B95 x B9 | 375 | 0 |
| B29 $\times$ B10 | 301 | 2.3 |
| B258 $\times$ B76 | 398 | 5.8 |
| B. pinnatum $(2 n=28) \times$ B. sylvaticum $(2 n=18)$ |  |  |
| $B 10 \times B 29$ $B 4 \times B 48$ | 256 | 0 |
| B4 $\times$ B48 | 326 | 0 |
| $\text { B. sylvaticum }(2 n=18) \times \text { B. pinnatum/sylvaticum } \frac{\text { intermediate }(2 n=28) ~}{\text { B }}$ |  |  |
| B61 $\times$ B5 | 243 | 6.6 |
| B. pinnatum/sylvaticum intermediate ( $2 n=28$ ) $x$ B. sylvaticum ( $2 n=18$ ) |  |  |
| B42 $\times 846$ | 252 | 11.9 |
| B. glaucovirens $(2 n=16) \times$ B. sylvaticum $(2 n=18)$ |  |  |
| $\begin{aligned} & \text { B224 } \times \text { B48, B61, } \\ & \text { B134, B261 } \\ & \text { (Hairy plants) } \end{aligned}$ | 510 | 0 |
| $\begin{aligned} & \mathrm{B} 224 \times \mathrm{B} 48, \mathrm{~B} 61, \\ & \text { B134, B261 } \\ & \text { (Non-hairy plants } \end{aligned}$ | 454 | 37.2 |
| B. glaucovirens/sylvaticum intermediate ( $2 n=17$ ) $x$ B. glaucovirens $(2 n=16)$ |  |  |
| B249 $\times$ B226 | 355 | 9.9 |
| (B. glaucovirens $\times \underline{\text { B. sylvaticum) }}(2 n=17) \times \underset{(2 \mathrm{~B}=16) \text { blaucovirens }}{(2 \mathrm{Backcross}}$ |  |  |
| $\begin{aligned} & (B 224 \times B 48, B 61, \\ & \text { B134, B261) } \times \\ & \text { B224 } \end{aligned}$ | 374 | 54.0 |

## TABLE 3.7/7 CONTINUED

| Hybrid | Total no. pollen-grains scored | pollen-grains stained |
| :---: | :---: | :---: |
| B. glaucovirens $(2 n=16) \times$ B. phoenicoides $(2 n=28)$ |  |  |
| B224 x B55 | 408 | 0 |
| B. pinnatum $(2 n=28) \times$ B. phoenicoides $(2 n=28)$ |  |  |
| B36 $\times 888$ | 322 | 68.9 |
| B. phoenicoides $(2 n=28) \times$ B. pinnatum $(2 n=28)$ |  |  |
| $\begin{aligned} & B 88 \times B 36 \\ & B 55 \times B 35 \end{aligned}$ | $\begin{aligned} & 262 \\ & 307 \end{aligned}$ | $\begin{aligned} & 84.7 \\ & 93.5 \end{aligned}$ |
| $\text { B. phoenicoides }(2 n=28) \times \frac{\text { B. pinnatum } / \text { sylvaticum }}{\text { intermediate }}(2 n=28)$ |  |  |
| B91 $\times$ B5 | 398 | 71.1 |
| B. phoenicoides $(2 n=28) \times$ B. sylvaticum $(2 n=18)$ |  |  |
| B55 $\times$ B84 | 219 | 4.6 |
| B. sylvaticum $(2 n=18) \times$ B. phoenicoides $(2 n=28)$ |  |  |
| B28 $\times$ B2 | 511 | 0 |
| B. glaucovirens/sylvaticum intermediate ( $2 n=17$ ) $\times$ B. phoenicoides ( $2 n=28$ ) |  |  |
| B249 x B39 | 506 | 1.6 |
| B. distachyon $(2 n=30) \times$ B. pinnatum $(2 n=28)$ |  |  |
| B199 x B8 | 518 | 0 |
| B. distachyon $(2 n=30) \times$ B. pinnatum variant $(2 n=36)$ |  |  |
| B199 $\times$ B115 | 513 | 0 |

TABLE 3.7/8
Percentage seed-set of artificial hybrids

| Hybrid | No. florets dissected | No. full caryopses obtained | \% caryopsis fertility |
| :---: | :---: | :---: | :---: |
| B. sylvaticum ( $2 n=18$ ) $\times$ B. glaucovirens $(2 n=16)$ |  |  |  |
| B364 $\times$ B226 | 131 | 0 | 0 |
| B. glaucovirens $(2 n=16) \times$ B. sylvaticum $(2 n=18)$ |  |  |  |
| $\begin{aligned} & \text { B224 x B48, B61, } \\ & \text { B134, B261 } \\ & \text { (Hairy plants) } \end{aligned}$ |  | 0 | 0 |
| $\begin{aligned} & \text { B224 x B48, B61, } \\ & \text { B134, B261 } \\ & \text { (Non-hairy plants } \end{aligned}$ | $2021$ | $28$ | $1.39$ |
| B. glaucovirens/s | icum interme | $(2 n=17)$ | $\frac{\text { sylvaticum }}{(2 n=18)}$ |
| B249 $\times$ B153 | 242 | 11 | 4.5 |
| B. glaucovirens/sylvaticum intermediate $(2 n=17) \times$ B. glaucovirens ( $2 n=16$ ) |  |  |  |
| B249 $\times$ B226 | 442 | 0 | 0 |
| (B. glaucovirens $\times$ B. sylvaticum) $(2 n=17) \times \frac{\text { B. glaucovirens }}{(2 n=16) \text { backcross }}$ |  |  |  |
| $\begin{aligned} & \text { (B224 } \times \text { B48, B61, } \\ & \text { B134, B261) } \\ & \text { B224 } \end{aligned}$ |  | 72 | 18.4 |
| B. sylvaticum $(2 n=18) \times$ B. pinnatum $(2 n=28)$ |  |  |  |
| B29 x B10 | 468 | 0 | 0 |
| B95 x B9 | 198 | 0 | 0 |
| B102 x B36 | 236 | 0 | 0 |
| B258 x ${ }^{\text {P76 }}$ | 50 | 0 | 0 |
| B. pinnatum $(2 n=28) \times$ B. sylvaticum $(2 n=18)$ |  |  |  |
| B42 $\times$ B46 | 502 | 3 | 0.6 |
| B10 $\times$ B29 | 713 | 0 | 0 |
| B4 $\times$ B48 | 112 | 2 | 1.8 |


| Hybrid | No. florets <br> dissected |
| :---: | :---: | | No. full |
| :--- |
| caryopses |
| obtained |$\quad \%$ caryopsis


B. glaucovirens/sylvaticum intermediate $(2 n=17) \times \frac{\text { B. phoenicoides }}{(2 n=28)}$

| B249 $\times$ B39 | 129 | 0 | 0 |
| :--- | :--- | :--- | :---: |
| B249 $\times$ B181 | 224 | 2 | 0.9 |

B. pinnatum $(2 n=28) \times$ (B. pinnatum $\times$ B. phoenicoides) $(2 n=28)$ backcross
$B 118 \times(B 36 \times B 88) \quad 162 \quad 46 \quad 28.4$

$\begin{array}{llll}(B 36 \times B 88)\end{array} \times 888 \quad 32 \quad 15.6$
B. distachyon $(2 n=30) \times$ B. pinnatum $(2 n=28)$
$\begin{array}{llll}\mathrm{B} 199 \times \mathrm{B8} & 522 & 0\end{array}$
B. distachyon $(2 n=30) \times$ B. pinnatum variant $(2 n=36)$
$81.99 \times 8115$

# Plate 3.7/l: Living plants of synthetic Brachypodium hybrids (female parent first) <br> A - B. sylvaticum $\times$ B. pinnatum <br> B - B. sylvaticum x B. pinnatum/ <br> sylvaticum intermediate 



Plate 3.7/2: Living plants of synthetic Brachypodium hybrids (female parent first)

A - B. glaucovirens $\times$ B. pinnatum B $-\frac{\text { B. pinnatum }}{}(2 n=18) \times \frac{\text { B. pinnatum }}{(2 n=28)}$


Plate 3.7/3: Living plants of synthetic Brachypodium hybrids (female parent first)

A - B. sylvaticum $\times$ B. phoenicoides
B - B. phoenicoides $\times$ B. sylvaticum


Plate 3.7/4: Living plants of synthetic Brachypodium hybrids (female parent first)

A - B. pinnatum $\times$ B. phoenicoides
B - B. glaucovirens $\times$ B. phoenicoides



```
Plate 3.7/5: Living plants of synthetic Brachypodium
    hybrids (female parent first)
    A - B. distachyon x B. pinnatum
    B - B. glaucovirens/sylvaticum intermediate
        x B. phoenicoides
```



Plate 3.7/6: Inflorescences of synthetic Brachypodium hybrids (female parent first)

A - B. glaucovirens $\times$ B. pinnatum
B - B. glaucovirens $\times$ B. phoenicoides

$\infty$


Plate 3.7/7: Leaf epidermis of synthetic Brachypodium hybrids (female parent first)

A - B. sylvaticum $x$ B. pinnatum
B - B. pinnatum $\times$ B. phoenicoides

A. Abaxial

A. Adaxial

B. Abaxial

B. Adaxial

Plate 3.7/8: Leaf epidermis of synthetic Brachypodium hybrids (female parent first)

A - B. phoenicoides $x$ B. sylvaticum
B - B. sylvaticum $\times$ B. phoenicoides

A. Abaxial

A. Adaxial

B. Abaxial
B. Aclaxial
Plate 3.7/9: Leaf sections of synthetic Brachypodium hybrids (female parent first)
A - B. sylvaticum $x$ B. pinnatum
B - B. distachyon $\times$ B. pinnatum


[^2]

B

Plate $3.7 / 11:$ Leaf sections of synthetic Brachypodium hybrids (female parent first)

A - B. phoenicoides $x$ B. sylvaticum
B - B. sylvaticum $x$ B. phoenicoides


Plate 3.7/12: Root-tip mitosis in synthetic Brachypodium hybrids (female parent first)

A - B. pinnatum $x$ B. sylvaticum
B - B. sylvaticum $\times$ B. phoenicoides


A

$$
10 \mu \mathrm{~m}
$$


$\begin{aligned} \text { Plate } 3.7 / 13: & \text { Pollen mother cell meiosis in synthetic } \\ & \text { Brachypodium hybrids (female parent first) }\end{aligned}$
A - B. glaucovirens $x$ B. sylvaticum
B - B. pinnatum $\times$ B. sylvaticum


Plate 3.7/14: Pollen mother cell meiosis in synthetic Brachypodium hybrids (female parent first)

A - B. phoenicoides $\times$ B. sylvaticum
B - B. phoenicoides $\times$ B. pinnatum


## A



Plate 3.7/15: Pollen mother cell meiosis in synthetic Brachypodium hybrids (female parent first)

A - B. distachyon $x$ B. pinnatum
$B-\frac{\text { B. glaucovirens }}{\mathrm{x} \text { B. glaucovirens }}$ B. sylvaticum)


A


B

Plate 3.7/16: Stained pollen of synthetic Brachypodium hybrids (female parent first)

A - B. distachyon $\times$ B. pinnatum
B - B. pinnatum $\times$ B. phoenicoides


B

## SECTION4

DISCUSSION

### 4.1.1: Relationship of the species as shown by hybridisation

Hybridization has formed the most important part of this research as it has been carried out here for the first time in this genus, and it was expected to reveal many facts about the genetic relationships of the taxa studied. Figs $4.1 / 1$ and $4.1 / 2$ show the relationships between the taxa.

It is clear from Fig. 4.1/1 that most of the crosses were successful, but that some were not, e.g. B. mexicanum as a female parent and B. distachyon as a male parent. B. mexicanum is genetically isolated from B. pinnatum due to gametophytic isolation and from B. phoenicoides and B. sylvaticum due to seed incompatibility.

The degree of success between the taxa is very often proportional to the closeness of their chromosome numbers. For example B. pinnatum ( $2 \mathrm{n}=18$ ) x B. sylvaticum $(2 n=18)$, B. pinnatum $(2 n=28) \times$ B. phoenicoides $(2 n=28)$, and B. glaucovirens $(2 n=16) \times$ B. sylvaticum $(2 n=18)$ were successful crosses. But an exception to this rule is provided by B. distachyon $(2 n=30) \times$ B. phoenicoides ( $2 \mathrm{n}=28$ ) .

Taxonomic (morphological) closeness is equally
important for successful crossing, because B. pinnatum with $2 n=18,28$ and 36 when crossed with each other give good seed-set, while B. pinnatum $(2 n=36) \times$ B. sylvaticum (2n=18) gives a lower seed-set.

|  | $\begin{aligned} & \text { Z } \\ & \text { O} \\ & \text { U } \\ & \text { n } \\ & 0 \\ & 0 \end{aligned}$ | $\begin{gathered} M \\ \dot{\sim} \\ 1 \\ \dot{\sim} \\ \dot{M} \end{gathered}$ | $\begin{aligned} & 0 \\ & \dot{N} \\ & 1 \\ & \infty \\ & \end{aligned}$ | $\begin{gathered} M \\ \dot{M} \\ M \\ \dot{M} \\ \dot{N} \\ \underset{N}{\prime} \end{gathered}$ | 10 $\dot{0}$ + 1 $\dot{\sim}$ $M$ | $\bar{\sim}$ <br> $\infty$ <br> 1 <br> 0 <br> 0 |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| $\begin{gathered} \bar{u} \\ w \\ \sim \\ \text { か○ } \end{gathered}$ | 1 1 $\downarrow$ 1 | $\psi$ |  | . | - |  |



Figure 4.1/1: Chart showing the percentage seed-set in artificial
interspecific crosses of Brachypodium taxa

| Pollen fertility of Fi |  |
| :---: | :---: |
| $\leftarrow-$ | cross failed $0 \%$ seed set |
| $\checkmark$ | Seeds produced but no $F_{1}$ plants |
|  | Good Fi plants with up $12 \%$ pollen fertility |
|  | Good $F_{1}$ plants with variable ( $0 \%-37 \%$ ) pertility |
|  | Good F1 plants with $68 \%$ or more pollen fertility |
|  |  |


Figure 4.1/2: Chart showing pollen fertility of artificial $\mathrm{F}_{1}$

Success measured by percentage seed-set is not necessarily the same as that measured by $F_{1}$ hybrid development. For example in the two crosses B. retusum $(2 n=32) \times$ B. sylvaticum $(2 n=18)$ and B. retusum $(2 n=32) x$ B. pinnatum $(2 n=28)$ the former gave good seed-set but no $F_{1}$ seedlings were obtained, while the latter gave less than half the seed-set but a high proportion of $\mathrm{F}_{1}$ plants were obtained. Similarly to the former, good seed-set but no hybrids were obtained in the crosses B. pinnatum $(2 n=36) \times$ B. pinnatum $(2 n=28)$ and B. pinnatum $(2 \mathrm{n}=18) \times$ B. pinnatum $(2 \mathrm{n}=36)$.

Nettancourt (1977) pointed out that in general self-fertile species are more successful as female parents in crosses between self-fertile and self-sterile plants. This phenomenon (unilateral interspecific incompatibility) is found in some of the taxa of Brachypodium, e.g. selfsterile B. phoenicoides $(2 n=28)$ or B. pinnatum $(2 n=36)$ $x$ self-fertile B. sylvaticum $(2 n=18)$ and the reciprocal crosses. On the other hand the two self-compatible species B. distachyon and B. sylvaticum also showed unilateral interspecific incompatibility. However, the failure of $B$. distachyon to cross as a male parent might have been due at least to some extent to its low level of pollen production. But similarly, B. sylvaticum and B. pinnatum were almost equally successful as female parents; in fact, in terms of $F_{1}$ hybrid fertility, B. pinnatum (self-sterile) was more successful as the female. These results suggest that taxa of Brachypodium
do not always obey the rule that self-compatible species are more sucessful than self-incompatible species as female parents.

Meiotic pairing was more regular in the hybrids between species having similar numbers of chromosomes, e.g. B. pinnatum $(2 n=28) \times$ B. phoenicoides $(2 n=28)$ and B. glaucovirens $(2 n=16) \times$ B. sylvaticum $(2 n=18)$. The species with more regular pairing had good stainable pollen and the ones with irregular pairing had nonstainable pollen. Since only very few meiotic studies were made within each hybrid combination, variation in the chromosome number of hybrids from parents with irregular meiosis was not detected. However, it surely exists. For exampleB. glaucovirens/sylvaticum intermediate ( $2 n=17$ ) $x$ B. phoenicoides $(2 n=28)$ had $2 n=22$, but B: glaucovirens/ sylvaticum intermediate ( $2 \mathrm{n}=17$ ) $\times$ B. glaucovirens $(2 n=16)$ had $2 \mathrm{n}=17$. In these two crosses the same female parent contributed 8 and 9 chromosomes respectively.

There is no indication from my results that polyploid taxa are more successful than diploid species in hybridization, although this point is often made in other plants (Stace 1975).

Clearly several factors contribute to the success of hybridization in Brachypodium, but the most important appear to be morphological similarity and closeness of chromosome number.
4.1.2: Taxonomic status of the main taxa of Brachypodium Brachypodium pinnatum, which is the lectotype of the genus (Niles \& Chase 1925), closely resembles B. sylvaticum. St. Yves (1934) considered B. sylvaticum as a variety of B. pinnatum, but Smith (1980) and most other more recent authors have considered B. pinnatum and B. sylvaticum as separate species. The nodding inflorescence, long awns, and long, dense pubescence of $B$. sylvaticum differentiate it from B. pinnatum. Anatomically B. sylvaticum differs from B. pinnatum in the more abundant horizontally elongated sinuous-walled silica-bodies. Although seedprotein bands in B. sylvaticum have a characteristic pattern distinguishing that species from B. pinnatum, the two species have a high affinity (Fig. 3.3/2). These two species can be crossed but produce highly sterile hybrids with irregular meiosis.
B. glaucovirens has a disputed taxonomic position. Murbeck (1892) and Smith (1980) treated it as a subspecies of B. sylvaticum. St. Yves (1934) considered B. glaucovirens as another variety of B. pinnatum. Kozuharov (1974) believed that B. glaucovirens is similar to B. tenerum Velenovsky on morphological grounds, and to B. ponticum Velenovsky on overall characteristics. He suggested that B. glaucovirens and B. ponticum should be assigned to B. sylvaticum, as the awn in all three is twice as long as in B. pinnatum and the glumes are longer than in B. pinnatum. However, he concluded that $B$. glaucovirens seems to have an intermediate position between B. pinnatum and B. sylvaticum and he
thought that the latter two might have hybridized in the past at the diploid level ( $2 \mathrm{n}=14$ and $2 \mathrm{n}=18$ ) to form it. However, in my opinion B. glaucovirens is a good species and can ine easily distinguished from the closely related B. sylvaticum due to its patent leaves and spikelets and different chromosome number. Although anatomically it is close to B. sylvaticum it differs from that species in its sclerenchyma distribution. The characteristic seedprotein band pattern of B. sylvaticum is also different from that of $B$. glaucovirens. Its anthesis time is intermediate between those of B. sylvaticum and B. pinnatum, and its flowering season is later than in either of the latter species. Meiosis in the hybrids between B. glaucovirens and B. sylvaticum is not markedly irregular, but their seed-set and pollen-stainability are less than in the B. pinnatum $\times$ B. phoenicoides hybrids. All this information supports the idea that B. glaucovirens is a separate species from B. sylvaticum.
B. rupestre was recognized as a subvariety of B. pinnatum var. glabrum by St Yves (1934). Nevski (1934), in Flora U.S.S.R., treated B. rupestre as a separate species, as did Borsos (1974) and Sustar (1976) on the basis of its leaf anatomy. Smith (1980) in Flora Europaea treated it as a subspecies of B. pinnatum.

However, material of $B$. rupestre was limited in the present study because most of the material sent from different countries as that species was actually some other taxon of Brachypodium.

According to Smith (1980) awn length in
B. rupestre is not more than 3 mm , but in the present study it was found to be $4.3-4.5 \mathrm{~mm}$. The glaucous-green leaves are the only morphological character that I have found to distinguish B. rupestre from B. pinnatum. Borsos (1974) mentioned that stomata were only on the adaxial leaf surface, but stomata were found on both abaxial and adaxial sides in my studies.

Anatomically B. rupestre does not differ from B. pinnatum, but the bulliform cells are like those of Fairlight specimens of $B$. pinnatum rather than like those of most specimens of B. pinnatum. My observations on B. rupestre do not agree with those of Borsos (1974), because the anatomical characters she mentioned for B. ruprestre overlap with those $I$ found in B. pinnatum. The chromosome number characteristic of B. rupestre ( $2 \mathrm{n}=36$ ) is also found in B. pinnatum. According to seed-protein band analysis, B. rupestre is not far from B. pinnatum. Plants of B. pinnatum var. rupestre with $2 \mathrm{n}=14$, such as those studied by Kozuharov (1974), were not available in the present study. In the light of present information $I$ believe $B$. rupestre is best treated as a variety of B. pinnatum.
B. phoenicoides is a good species and has the most close resemblance to $B$. pinnatum. The evidence of their close relationship is confirmed by hybridization, as has been discussed in sub-chapter 4.1.1.

St Yves (1934) distinguished B. phoenicoides from B. pinnatum on the basis of leaf epidermal anatomy, and the present study supports this. Robertson(1980) said that in the Mediterranean these two species are sometimes difficult to distinguish on the basis of gross morphology, but, according to my observations in the Botanic Garden field study, they can be easily distinguished by their dark green leaves, prominent ribs, and characteristic pubescence on the margins on the ribs. Darker and characteristic seed-protein bands in B. phoenicoides and different flowering behaviour from B. pinnatum also support the idea that these are separate species.
B. retusum is a species very distinct from all the above, and can be easily identified vegetatively by the presence of numerous internodes, culms branched and woody at the base, and narrow leaves. Among the other species studied, this is closest to B. phoenicoides, due to its leaf anatomy. This species was crossed with B. pinnatum and B. sylvaticum but hybrid seeds from only the former cross germinated and produced hybrid plants, as mentioned in sub-chapter 4.1.1. Smith (1980) treated B. retusum as a distinct species and the present study agrees with that. Various relatives of B. retusum, e.g. B. boissieri, which is treated as a distinct species by Tateoka (1968), and B. arbuscula from the Canary Islands, need further investigation.
B. distachyon is very different from the rost
of the species in many characters. Mainly it is

## TABLE 4.1/1

Classification of representatives of the genus Brachypodium used in this study

Brachypodium Beauv.
Section Brachypodium

1. B. retusum (Pers.) Beauv.
2. B. phoenicoides (L.) Roemer \& Schultes
3. B. pinnatum (L.) Beauv.
a. var. pinnatum
b. var. rupestre (Host) Reichenb.
4. B. sylvaticum (Hudson) Beauv.
5. B. glaucovirens (Murbeck) Fritsch
6. B. mexicanum (Roemer \& Schultes) Link

Section Trachynia (Link) Nyman
7. B. distachyon (L.) Beauv.
distinguished from the rest by its annual habit, laterally compressed spikelets and very small anthers of distinct shape. It also has a different leaf anatomy. Its chromosomes are small as in the rest of the species, but its basic chromosome number ( $x=5$ ) is unique in the genus. Seed-protein band analysis showed that it is closer to B. mexicanum than to the rest of the species examined. B. distachyon was crossed with B. pinnatum and B. sylvaticum, but produced hybrid plants only with B. pinnatum and these were very sterile.

Link (1827) described a new genus, Trachynia, with two species, T. distachyon and T. rigida. Maire \& Weiller (1955) and Smith (1980) included Trachynia in Brachypodium as a section and I agree with this. It is the most distinct species of the genus, and its separation at the generic level does have some justification.

The classification I adopt for representatives of the genus is given in Table 4.1/l.

### 4.1.3: Problems in identification

The rather complex pattern of morphological variation in the genus Brachypodium is genetically rather than environmentally based, because the variations concerned have all been found to breed true from seed (e.g. spikelet and leaf pubescence, awn length, pedicel length, flowering behaviour). But, in B. pinnatum variants with $2 \mathrm{n}=18$ and $2 \mathrm{n}=36$, it was found that there is a strong correlation between morphological characters and geographical origin.

Some B. sylvaticum seed-samples from Iran (especially B239 from sand dunes) were sent as B. pinnatum, probably because of the glabrous nature of the plants, but when these plants were examined fully they were recognized as $B$. sylvaticum. These plants were particularly so identified due to the characteristic seed-protein band pattern and the long lemma-awns. Apart from such glabrous plants, specimens of $B$. sylvaticum were identified easily, as shown in the keys previously presented.

Morphologically the variants of B. pinnatum with $2 \mathrm{n}=18$ and 36 , in spite of some variations, were within the range of normal B. pinnatum ( $2 \mathrm{n}=28$ ). These chromosomal variants could be considered as varieties of $B$. pinnatum, but they are not always absolutely distinguishable from normal plants with $2 \mathrm{n}=28$.

The main problems of identification concern the interspecific intermediate plants, i.e. B. pinnatum/ sylvaticum intermediates, B. pinnatum/phoenicoides intermediates, and B. glaucovirens/sylvaticum intermediates.
B. pinnatum/sylvaticum intermediates have morphological similarities to synthetic hybrids of B. pinnatum $x$ B. sylvaticum, as shown in Table 4.1/2. If we take notice of the pedicel length, glume length and awn length especially, it seems most reasonable that these intermediate plants may be hybrid in origin. In addition, the very characteristic seed-protein band pattern at position 6, which always distinguishes B. sylvaticum from B. pinnatum, resembles that of

## TABLE 4.1/2

Diagnostic characters of wild intermediates between, and synthetic hybrids of, B. pinnatum and B. sylvaticum

|  |  |  | - |  |  |
| :---: | :---: | :---: | :---: | :---: | :---: |
| Culm | Glabrous | Glabrous to pubescent | Glabrous to densely <br> pubescent | Glabrous to pubescent | Glabrous to pubescent |
| Leaf-sheath | Glabrous to pubescent | Glabrous to pubescent | Glabrous to densely pubescent | Glabrous to densely pubescent | Densely pubescent to glabrous |
| Margins of leaf-sheath | Non- <br> ciliated (to <br> ciliated) | Ciliated (to nonciliated) | Ciliated | Ciliated (to <br> slightly <br> ciliated) | Ciliated (to nonciliated) |
| $\begin{aligned} & \text { Pedicel } \\ & \text { length ( } \mathrm{mm} \text { ) } \end{aligned}$ | 1.4-2.7 | 1.3-2.4 | 0.9-1.3 | 1.2-1.5 | 0.4-1.7 |
| $\begin{aligned} & \text { Lower glume } \\ & \text { length }(\mathrm{mm}) \end{aligned}$ | 4.7-8.0 | 4.7-8.8 | 5.6-8.1 | 6.7-7.0 | 7.8-9.7 |
| $\begin{aligned} & \text { Upper glume } \\ & \text { length (mm) } \end{aligned}$ | 6.5-9.6 | 7.2-10.4 | 8.3-10.1 | 7.9-9.5 | 9.3-11.3 |
| $\begin{aligned} & \text { Lemma-awn } \\ & \text { length (mm) } \end{aligned}$ | 2.6-4.0 | 1.6-5.2 | 5.5-6.8 | 4.7-7.0 | 7.2-13.9 |

B. sylvaticum in the case of the intermediates B5 and B5l, but that of $B$. pinnatum in the case of B63. Examples of intermediates have been seen from Fairlight (B5), Milton Keynes (B51, B52) and Cambridgeshire (B351, B352) in England; from France (B42); from East Germany (B53); from Poland (B7O); from Czechoslovakia (B63); and from Hungary (B78). Natural hybrids of B. pinnatum x B. sylvaticum had been reported by Camus (1958) from France, and Stace (1975) stated that such hybrids had been recorded from Denmark, Czechoslovakia, France and Ireland. However chromosome number in the artificial $F_{1}$ hybrids of B. pinnatum $(2 n=28) \times$ B. sylvaticum ( $2 n=18$ ) was always $2 n=23$, whereas all the B. pinnatum/sylvaticum intermediates counted had $2 \mathrm{n}=28$, as in B. pinnatum. This leads to the possibility that the B. pinnatum/sylvaticum intermediates $(2 n=28)$ were the results of backcrosses with B. pinnatum ( $2 \mathrm{n}=28$ ). An alternative explanation for the wild intermediates is that they are in fact B. pinnatum showing an unusual extreme of variation. However, the very characteristic seed-protein band pattern at position 6 in two of the wild intermediates, and their very variable (and often low) pollen stainability, is good evidence that they are in fact hybrid in origin. Despite the constant chromosome number of $2 n=23$, plants of B. pinnatum $\times$ B. sylvaticum did set some good seed. The morphology and chromosome number of such $F_{2}$ (or backcross) plants remains to be investigated.

The B. glaucovirens/sylvaticum intermediate
( $2 \mathrm{n}=17$ ) which was grown from wild collected seed (Turkey, B249) indicates the occurrence of cross-pollination between the species of Brachypodium in nature. From the situation discussed above it seems most probable that the B. glaucovirens/sylvaticum intermediate ( $2 \mathrm{n}=18$ ) from Greece (B230) may have arisen as a result of introgression, since this plant has more stainable pollen than the B. glaucovirens/sylvaticum intermediate with $2 \mathrm{n}=17$. Despite its chromosome number it is more similar to B. glaucovirens than to B. sylvaticum. The B. phoenicoides/pinnatum intermediates resemble the synthetic hybrids of $B$. phoenicoides $x$ B. pinnatum in the margins of the ribs being shortly pubescent and the face of the ribs having spreading hairs, in the deep intercostal grooves, and in the face of the ribs being rounded rather than flat as in B. phoenicoides. Pollen stainability in the B. phoenicoides/pinnatum intermediates is very much lower than in the synthetic $F_{1}$ hybrids of B. phoenicoides and B. pinnatum. This may be an effect of the different strains of the parents involved. The evidence is very strong that these wild intermediates are hybrids. They come from Portugal (B31) as wild collected seeds, and from Spain and Greece as herbarium specimens.

So hybridization seems to be one of the major factors which cause variation and problems of identification in the genus.

An interesting point noticed in the synthetic hybrids is that most of their characters resemble the male parents, as shown by reciprocal crosses. This phenomenon was found to be widespread and often very marked, especially in crosses between B. sylvaticum and B. phoenicoides. Such male dominance is scarcely mentioned in the literature. The phenomenon of xenia discussed by Focke (1881) and Crane \& Lawrence (1952) (i.e. the effect of foreign pollen on the maternal tissue of the female parent) is possibly close to this situation. Nixon (1928-36), working with Phoenix dactylifera and Phoenix canariensis, found that seedsize and time of ripening vary according to the pollen used in fertilization. Harrison (1931) found that, in certain varieties of cotton, length of lint differs in different pollinations.

Crane \& Lawrence (1952) state that the effect of xenia is not in the resulting zygote or its offspring, as in heterosis. But according to my study the effects of the male parent are seen in the resulting offspring. However, the genetical mechanism of this effect is not known and needs further investigation.

### 4.1.4: Evolution in Brachypodium

More than half of the species of the genus Brachypodium are distributed in the Mediterranean and neighbouring regions, but others are scattered as far as eastern Asia, northern Africa, and southern and central America. This wide-spread distribution in different
parts of the world indicates that Brachypodium is an ancient genus and that its present day distribution is largely relict.

This possibility can be investigated by a consideration of the primitiveness/advancedness of the characters of Brachypodium.

Chromosome size in the genus is overall very small, which is a primitive feature in grasses. Of the 5 or 6 subfamilies, only Pooideae has large chromosomes. Therefore small chromosomes in Brachypodium suggest it is very primitive within the Pooideae.

The racemose inflorescence of Brachypodium is also considered to be a primitive feature within the Pooideae.

Perennial/annual habit; woody and branched/ herbaceous and unbranched stems; long, well-developed/ short, poorly developed rhizomes; many-flowered/fewflowered spikelets; and short or no awns/long awns are generally considered primitive/advanced characters in grasses according to Stebbins (1956).

Three conditions of anthesis exist in Brachypodium: morning anthesis, evening anthesis, and no fixed anthesis time. The absence of a fixed anthesis time is found only in the annual species B. distachyon, in which it may be derived from the other two character states. It is difficult to decide whether the evening anthesis is derived from the morning anthesis or vice versa. However, there is some indication from crossing experiments
that evening anthesis could be an advanced character. Reciprocal crosses of B. pinnatum and B. phoenicoides, both showing only morning anthesis, commenced anthesis both in the morning and in the evening, and the B. pinnatum $x$ (B. pinnatum $x$ B. phoenicoides) backcross commenced anthesis only in the evening.

Crown-cells, which may be repressed hairs, are found in B. distachyon, B. glaucovirens/sylvaticum intermediate (B249), and B. retusum. This may be an advanced character.

Chasmogamy and self-sterility are primitive characters since they are characteristic of perennial species.

The species may be categorized according to their primitiveness/advancedness into five groups:

1. B. retusum group

These are chasmogamous and probably selfsterile species with one fixed anthesis time, a short lemma-awn, well developed rhizomes, spikelets with many florets, and perennial, branched aerial stems. These are all primitive characters. In addition, B. arbuscula, a close relative of $B$. retusum, has markedly woody stems. However, these species have crown-cells, which are probably an advanced character.
2. B. pinnatum, B. rupestre and B. phoenicoides

These differ from the first group in their herbaceous, unbranched stems. B. pinnatum has one and B. phoenicoides two fixed anthesis times. There are no crown-cells.
3. B. glaucovirens and B. sylvaticum

These are perennials but rather short-lived. They have short rhizomes and a long lemma-awn, and are usually over $50 \%$ self-fertile and largely inbreeding. However, out-breeding may also take place to some extent since anther dehiscence takes place after the florets are fully opened.
4. B. mexicanum

This is a much shorter-lived perennial with poor rhizomes and spikelets with few florets. The plants are fully self-fertile and inbreeding; the short anthers are only slightly exserted at anthesis.
5. B. distachyon
B. distachyon is a fully self-fertile annual
with only partially chasmogamous florets, long lemmaawns and crown-cells. It is probably the most advanced species in the genus.

Different basic chromosome numbers, i.e. 9,
8,7 and 5 , show a trend of evolution in different directions in the genus.

According to the present study B. pinnatum exists at three chromosome levels ( $2 \mathrm{n}=18,28,36$ ), with two different basic chromosome numbers (7 and 9). Kožuharov (1974) also found $2 \mathrm{n}=14$. B. pinnatum ( $2 \mathrm{n}=28$ ) is the most common cytotype, and since almost only bivalents were observed at meiosis it seems to be an allotetraploid. The existence of $2 \mathrm{n}=18$ in B. pinnatum would suggest that the $2 n=36$ race could be a polyploid, and since it is completely sterile it might well be an autotetraploid.

However, B. pinnatum with $2 \mathrm{n}=28$ has a total chromosome length ( $48.5 \mu \mathrm{~m}$ ) almost the same as that ( $49.1 \mu \mathrm{~m}$ ) in B. pinnatum with $2 n=36$ from Italy. B. pinnatum with $2 n=18$ from E. Germany has longer chromosomes than B. pinnatum with $2 \mathrm{n}=28$ or 36 , but in B. pinnatum with $2 n=18$ from Iran and U.S.S.R. the chromosomes appeared to be small but could not be measured. The existence of small chromosomes in B. pinnatum with $2 n=18$ supports the idea that $2 \mathrm{n}=36$ is a polyploid. But the existence of large chromosomes in the $B$. pinnatum $2 n=18$ from E. Germany supports the possibility that $2 n=18$ could have given rise to $2 \mathrm{n}=36$ by splitting. However, this is very unlikely because the chromosomes in the $2 n=36$ cytotype are metacentrics.
B. sylvaticum showed no intraspecific chromosome variation in the present study. However, Kozuharov (1974) found $2 n=28,42+2 B$ and 56 , which are based on $x=7$, not $\mathrm{x}=9$ as is the usual $2 \mathrm{n}=18$.
B. glaucovirens is a diploid with $2 \mathrm{n}=16$ ( $\mathrm{x}=8$ ), and might have arisen as the result of hybridization between B. pinnatum with $2 \mathrm{n}=14$ and B. sylvaticum with $2 \mathrm{n}=18$, as suggested by Kožuharov (1974). An alternative explanation could be aneuploidy from a parent with $2 \mathrm{n}=18$.
B. phoenicoides is a tetraploid with $2 \mathrm{n}=28$ and $2 n=36$ with different basic chromosome numbers ( $x=7$ and 9 ). B. phoenicoides with $2 n=28$ had almost only bivalents at meiosis so it would be an allotetraploid. B. phoenicoidos with $2 n=36$ is fully fertile, unlike the B. pinnatum with $2 \mathrm{n}=36$, and could be derived from an unknown parent with $2 n=18$.
B. retusum, with $2 n=28$ * found in the present study, and $2 \mathrm{n}=32$ and 42 found by J. P. Bailey (pers. comm.), seems to have undergone a lot of chromosome evolution. The reports by Robertson (1981) of $2 n=36$ are errors as the plants concerned have been recounted by J. P. Bailey as above. The counts suggest base numbers of 7 and 8, although no diploids are known in B. retusum based on these numbers. The only relevant diploid is the closely related B. arbuscula with $2 n=18(x=9)$ (Larsen 1963, Robertson 1981, recently confirmed by J. P. Bailey). The count of $2 \mathrm{n}=27$ for $B$. retusum Natarjan (1978) can also be explained by the presence of a base number of $x=9$. B. arbuscula therefore appears to be a patroendemic of the Canary Islands. B. boisseri, another very close relative of B. retusum, has $2 \mathrm{n}=42$ (J. P. Bailey, pers. comm.) and was suggested to be a paleoendemic by Tateoka (1968). However, its chromosome number suggests it is much more likely to be a neoendemic.
B. mexicanum, with $2 \mathrm{n}=38$ according to Tateoka (1962) and $2 \mathrm{n}=42$ according to J. P. Bailey (pers. comm.), needs more extensive studies before any detailed conclusions can be drawn, but the two numbers known are clearly advanced rather than primitive.

* $2 \mathrm{n}=28$ has also recently been reported by Romero Zarco \& Devesa (Lagascalia, 12: 124, 1983) in material from Córdoba, Spain.
B. distachyon has been found with $2 n=10,2 n=20$ and $2 \mathrm{n}=30$, of which $2 \mathrm{n}=30$ is the most common cytotype. This suggests increasing polyploidy to tetraploid and hexaploid levels from the $2 \mathrm{n}=10$ diploid. The reports of $2 \mathrm{n}=28$ by Mimeur (1950), Gould (1972) and Kliphuis \& Wieffering (1972), if correct, seem to be derived by decreasing aneuploidy. The basic chromosome number $x=5$ in B. distachyon seems to be derived by decreasing base number within the genus.

Within the grasses the basic chromosome number $x=7$ is common only in the Pooideae, an advanced subfamily. More primitive subfamilies have mostly higher basic chromosome numbers (mostly 12, 10, 9). Therefore in Brachypodium the direction of chromosome evolution is probably from higher to lower basic chromosome numbers, as follows: 9 to 8 to 7 to 5. Thus, again, Brachypodium seems to be a primitive genus in the Pooideae, and B. distachyon the most advanced species in the genus.

## APPENDIX 1

List of Brachypodium accessions mentioned in the text, together with their chromosome numbers where known, and their taxonomic identity

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DIS = B. distachyon
GLA = B. glaucovirens
GS = B. glaucovirens/sylvaticum intermediate
MEX = B. mexicanum
PHO = B. phoenicoides
PIN = B. pinnatum
PP = B. phoenicoides/pinnatum intermediate
PS = B. pinnatum/sylvaticum intermediate
RET = B. retusum
RUP = B. rupestre
SYL = B. sylvaticum
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Bl Miller's Dale, Derbyshire, England 2n=18 SYL
B2 Escalet, Ramatuelle, Var, France $2 n=28$ PHO
B3 Le Canadel, Cavalaire, Var, France $2 n=28$ PIN
B4 Ambleteuse dunes, Pas de Calais, France $2 \mathrm{n}=28$ PIN
B5 Fairlight, East Sussex, England $2 n=28$ PS
B6 Fairlight, East Sussex, England 2n=28 PIN
B7 Fairlight, East Sussex, England 2n=18 SYL
B8 Fairlight, East Sussex, England 2n=28 PIN
B9 Fairlight, East Sussex, England $2 n=28$ PIN
Blo Fairlight, East Sussex, England $2 n=28$ PIN
Bll Fairlight, East Sussex, England $2 n=28$ PIN
Bl3 St Seine L'Abbaye, Cote D'Or, France $2 n=28$ PIN
Bl4 Priestcliff Lees, Miller's Dale, Derbyshire, England $2 \mathrm{n}=28$ PIN

Bl8 Between Comps and Montferrat, Var, France $2 n=28$ PIN


B70 Mitosna, Poland PS
B71 Mornas, Near Orange, Vaucluse, France $2 n=28$ PIN B72 Slovakia, Czechoslovakia, ex Košice $2 n=28$ PIN

B73 Slovakia, Czechoslovakia, ex Košice $2 n=18$ SYL
B74 Stara Boleslav, Drisy, C. Bohemia, Czechoslovakia $2 n=18 \quad S Y L$

375 Eichof Zeihen, Switzerland 2n=18 SYL
B77 Astrup, Sjaelland, Denmark 2n=18 SYL
B78 Hungary, ex Vácrátót $2 \mathrm{n}=28 \mathrm{PS}$
B81 700 m, Savoie, France $2 \mathrm{n}=18$ SYL
B82 Floby, Mőnarp, Sweden $2 \mathrm{n}=28$ PIN
B83 Harz, Rübeland, E. Germany $2 n=28$ PIN
B84 Jena, E. Germany $2 \mathrm{n}=18$ SYL
B88 Portugal, ex Lisbon $2 \mathrm{n}=28$ PHO
B90 Poroa das Pegas, Portugal 2n=18 SYL
B91 Oeiras, Portugal $2 \mathrm{n}=28$ PHO
B93 Bos Dahg, Greece $2 \mathrm{n}=18$ SYL
B94 Bos Dahg, Greece $2 n=18$ SYL
B95 S. Jugoslavia $2 \mathrm{n}=18$ SYL
Bl02 2 km N.W. of Gouvia, Central Corfu, Greece $2 \mathrm{n}=18$ SYL
BlO4 Near Hurley, Berkshire, England $2 n=18$ SYL
Blo7 Hamoir, Liège, Belgium 2n=18 SYL
Bl08 Machico, Madère, Belgium 2n=18 SYL
Bll5 Tornimarte, L'Aquila, Italy $2 n=36$ PIN
Bl17 Lot, France $2 n=18$ SYL
Bll8 Lot, France $2 \mathrm{n}=28$ PIN
B120 Gran Sasso D'Italia, C. Italy $2 \mathrm{n}=28$ PIN
Bl23 England, ex Kew 2n=18 SYL
Bl25 Portugal, ex Coimbra 2n=28 pHO
B131 Hungary, ex Vácrátót $2 \mathrm{n}=18$ SYL
Bl33 Villers-sur-Nancy, Lorraine, France 2n=18 SYL
Bl34 Moncel-sur-Vair, Vosges, France $2 n=18$ SYL
Bl39 St Georges-sur-Fontaine, France 2n=18 SYL
Bl44 E. Germany, Ex Leipzig 2n=18 PIN
Bl46 Bad Kosen, Halle, E. Germany $2 \mathrm{n}=18$ SYL
B150 Catania, Sicily $2 \mathrm{n}=18$ SYL
Bl52 Sicily, ex Catania GLA
B153 Colombé, Trana, 470m, Italy $2 n=18$ SYL
B161 Asnaes, Denmark $2 n=18$ SYL
B167 Méounes, Var, France RET
Bl73 Méounes, Var, France 2n=32* RET
B175 Clipsham, Rutland, England 2n=28 PIN
Bl77 Clipsham, Rutland, England 2n=28 PIN
Bl78 Clipsham, Rutland, England 2n=28 PIN
B181 Bollène, Vaucluse, France $2 n=28$ PHO
Bl82 Istria, Jugoslavia 2n=36 RUP
Bl86 N. Portugal, ex Coimbra 2n=28 PHO
Bl89 Near Darling, Cape Province, S. Africa 2n=30 DIS
B194 Jabal-uS-Siraj, 4800ft, Kabul, Afghanistan$2 \mathrm{n}=30+1 \mathrm{~B}$ DIS
B195 15 miles S. of Shushtar, Iran $2 \mathrm{n}=30$ DIS
B199 Kalafabad, Iran $2 \mathrm{n}=30$ DIS
B200 Kaman, Kirsehir, Turkey $2 \mathrm{n}=10$ DIS
B2O1 6 miles S. of Pabbi, Pakistan $2 n=30$ DIS
B202 17 miles N.W. of Ongda, Morocco $2 \mathrm{n}=30$ DIS
B207 Uruguay, ex U.S. Dept Agriculture $2 n=30$ DIS
B209 France, ex U.S. Dept Agriculture 2n=28 pHO
B210 20 km S.W. of Teruel, Spain $2 \mathrm{n}=28$ pHO
B215 Zaragoza, Spain 2n=28 PHO
B216 Sos del Rey Catolico, Zaragoza, Spain $2 n=28$ PHO
B218 Iraq, ex U.S. Dept Agriculture $2 n=16$ GLA
B220 Near Malatya, Turkey $2 n=28$ PIN
B221 Greece, ex U.S. Dept Agriculture $2 n=30$ DIS
B222 Greece, ex U.S. Dept Agriculture 2n=18 SYL
B223 Samsum, Turkey $2 \mathrm{n}=28$ PIN
B224 Between Akhisan and Soma, Marisa, Turkey 2n=16 GLA
B226 Near Karacali, Mugla, Turkey $2 \mathrm{n}=16$ GLA
B227 Iran, ex U.S. Dept Agriculture 2n=32* RET
B228 35 miles $S$. of Kalom, 6000-6500ft Caspian slopeof Alborz, Iran $2 n=18$ PIN
B229 35 miles $S$. of Kalom, 7000 ft Caspain slopeof Alborz, Iran $2 n=18$ PIN
B230 Yannina, l800ft, Greece $2 \mathrm{n}=18$ GS
B239 Costal sand dunes 70 km E. of Ramsar, Iran $2 \mathrm{n}=18$ SYL
B241 19 km S. of Stavropol on Hillside, U.S.S.R. PIN
B242 Cossach village of Isprovnaya, U.S.S.R. PIN
B243 Klukhov River, 1556 m, Stavropol region, U.S.S.R.$2 \mathrm{n}=18$ PIN
B248 U.S.S.R., ex U.S. Dept Agriculture $2 \mathrm{n}=36$ PIN
B249 Soguk Su, Trabzon, Turkey $2 n=17$ GS
B250 Congara, Gerze, Sinop, Turkey $2 \mathrm{n}=18$ SYL
B254 Greece, ex U.S. Dept Agriculture 2n=28 PIN
3257 Spain, ex U.S. Dept Agriculture 2n=18 SYL
B258 6 miles N. of Veles, Macedonia, Jugoslavia an=18 SYL
B259 172 km E. of Gorgan, Iran $2 \mathrm{n}=18 \mathrm{SYL}$
B260 Toledo, Spain $2 \mathrm{n}=28$ PHO
B261 Candeleda, Avila, Spain $2 \mathrm{n}=18$ SYL
B264 Norway, ex U.S. Dept Agriculture $2 n=18$ SYL
B265 Ardebil, on E. side of grade to Astara, 1500m,
Iran $2 n=18$ SYL
B266 Kaschmar, Iran $2 \mathrm{n}=30$ DIS
B269 30km S. of Arbet, Iraq $2 \mathrm{n}=30$ DIS
B270 Zaragoza, Spain $2 \mathrm{n}=28$ PHO
B271 Jaen, Spain $2 \mathrm{n}=36$ РНО
B277 Albacete, Spain $2 n=28+1 B$ PHO
B279 Teruel, Spain $2 \mathrm{n}=28$ PHO
B28O Jaen, Spain $2 \mathrm{n}=28$ PHO
B281 3km S.E. of Kislovodsk, on N. slope, 732m, Stavropol
region, U.S.S.R. $2 \mathrm{n}=18$ PIN
B282 Stavropol region, above forest at 915 m on Mt Mashuk,
U.S.S.R. PIN
B283 8 miles S. of Kalom, l000ft, Caspian side of Alborz,
Iran $2 n=18$ PIN
B284 54 miles E. of Gorgan, Iran $2 n=18$ SYL
B285 Golbasi, on road to Mara, Turkey $2 n=16$ GLA
B286 50km E. of Artrivin, Turkey $2 n=18$ SYL
B288 Ankara, Turkey $2 \mathrm{n}=28$ PIN
B289 Dashte Nazgame reserve, $32 \mathrm{~km} N$. of Sari, Iran
$2 \mathrm{n}=18 \quad \mathrm{SYL}$
B290 S. of Split, Dalmatia, Jugoslavia 2n=28 RET
B291 Dashte Naz game reserve, $32 \mathrm{~km} N$. of Sari, Iran
$2 n=18 \quad$ SYL
B292 Dashte Naz game reserve, 32 km N. of Sari, Iran
2n=18 SYL
B293 Raza Shah wild-life park, 12 km inside W. boundary,
Iran $2 \mathrm{n}=18 \mathrm{SYL}$
B295 19 km E. of Texoco on highway to Apizaco, 2850m,
Edo Mexico, Mexico MEX
B306 Octon, Hérault, France $2 \mathrm{n}=10$ DIS
B316 France, Ex Bordeaux $2 \mathrm{n}=28$ PIN
B347 Sierra de Pachuca, Hidalgo, Mexico 2n=42* MEX
B351 Cambridgeshire, England PS
B352 Cambridgeshire, England PS
B366 Aitana, Alicante, Spain $2 \mathrm{n}=18$ SYL

* Counted by Mr. J. P. Bailey


## APPENDIX

Hybridization experiments: seed-set in individual crosses

Female parent Male parent \begin{tabular}{l}
No. florets <br>
pollinated

 

No. caryopses <br>
formed
\end{tabular} \% seed-set

B. sylvaticum $(2 n=18) \times$ B. sylvaticum $(2 n=18)$

| B1 | B61 | 126 | 67 | 53.2 |
| :--- | :--- | ---: | ---: | ---: |
| B7 | B47 | 64 | 32 | 50.0 |
| B70 | B7, B104 | 23 | 11 | 47.8 |
| B81 | B258 | 66 | 35 | 53.0 |
| B102 | B7, B107, B153 | 62 | 32 | 51.6 |
| B153 | B250 | 32 | 14 | 43.8 |
| B239 | B250 | 41 | 38 | 92.7 |
| B250 | B153 | 30 | 8 | 26.7 |
| B258 | B84, B108 | 12 | 5 | 41.7 |
| B284 | B104 | 38 | 13 | 34.2 |
| B284 | B46 | 38 | 11 | 29.0 |

B. sylvaticum $(2 n=18) \times$ B. glaucovirens $(2 n=16)$

| B70 | B218 | 23 | 1 | 4.4 |
| :--- | ---: | ---: | ---: | ---: |
| B84 | B226 | 20 | 2 | 10.0 |
| B90 | B226 | 66 | 25 | 37.9 |
| B364 | B226 | 37 | .16 | 43.2 |

B. sylvaticum $(2 n=18) \times$ B. pinnatum $(2 n=28)$

| B7 | B102 | 48 | 0 | 0 |
| :--- | :--- | ---: | ---: | ---: |
| B29 | B10 | 146 | 19 | 13.0 |
| B48 | B4 | 177 | 17 | 9.6 |
| B95 | B9 | 118 | 20 | 17.0 |
| B102 | B36 | 62 | 9 | 14.5 |
| B104 | B4, B5 | 55 | 22 | 40.0 |
| B108 | B4, B5 | 37 | 14 | 27.8 |
| B131 | B4, B5 | 48 | 10 | 20.8 |
| B131 | B6 | 05 | 0 |  |
| B239 | B60, B220 | - | 23 | 15 |
| B239 | B14 | 37 | 26 | 65.2 |
| B257 | B35 | 102 | 10 | 70.3 |
| B258 | B4 | 24 | 2.8 | 8.8 |
| B258 | B76 | 254 | 16 | 6.3 |
| B261 | B4, B9 | 35 | 23 | 65.7 |
| B284 | B4, B5 | 139 | 43 | 30.9 |
| B289 | B4 | 56 | 18 | 32.0 |

B. sylvaticum $(2 n=18) \times$ B. pinnatum variant $(2 n=36)$

| B41 | B115 | 30 | 8 | 26.7 |
| :--- | :--- | :--- | :--- | :--- |
| B257 | B115 | 49 | 5 | 10.2 |

## APPENDIX 2 CONTINUED



## APPENDIX 2 CONTINUED

| B. glaucovirens ( $2 n=16) \times$ B. phoenicoides $(2 n=28)$ |  |  |  |  |
| :---: | :---: | :---: | :---: | :---: |
| B224 | B39 | 46 | 27 | 58.7 |
| B224 | B55 | 50 | 18 | 36.0 |
| B226 | B18, B39, B181 | 59 | 3 | 5.1 |
| B226 | B88, 1186 | 28 | 6 | 21.4 |
| B. pinnatum ( $2 n=28$ ) $\times$ B. pinnatum ( $2 n=28$ ) |  |  |  |  |
| B8 | B20 | 201 | 102 | 50.8 |
| B8 | B36 | 63 | 18 | 28.6 |
| B20 | B36 | 45 | 9 | 20.0 |
| B120 | B14 | 36 | 12 | 33.3 |
| B. pinnatum ( $2 \mathrm{n}=28$ ) $\times$ B. pinnatum variant ( $2 \mathrm{n}=18$ ) |  |  |  |  |
| B20 | B144 | 130 | 34 | 26.2 |
| B. pinnatum ( $2 \mathrm{n}=28$ ) $\times$ B. pinnatum variant ( $2 \mathrm{n}=36$ ) |  |  |  |  |
| B20 | B115 | 55 | 15 | 27.3 |
| B36 | B115 | 268 | 157 | $\stackrel{58.6}{ }$ |
| B220 | B248 | 46 |  | 15.2 |
| B. pinnatum $(2 n=28) \times$ B. sylvaticum $(2 n=18)$ |  |  |  |  |
| B4 | B48 | 132 | 15 | 11.4 |
| B6 | B131 | 113 | 11 | 9.7 |
| B8 | 861 | 189 | 34 | 17.2 |
| B9 | B134 | 182 | 7 | 3.9 |
| B9 | B107 | 63 | 33 | 52.4 |
| B10 | B29 | 70 | 28 | 40.0 |
| 811 | B94 | 290 | 51 | 17.6 |
| B13 | B47 | 205 | 57 | 27.8 |
| B14 | B150 | 31 | 10 | 32.3 |
| B35 | B131 | 52 | 15 | 28.8 |
| B72 | B29 | 120 | 56 | 46.7 |
| B120 | B150 | 60 | 33 | 55.0 |
| B177 | B61 | 115 | 20 | 17.4 |
| B. pinnatum ( $2 n=28$ ) $\times$ B. phoenicoides $(2 n=28)$ |  |  |  |  |
| B3 | B55, B88 | 70 | 21 | 30.0 |
| B34 | B88, B91, B186 | 104 | 34 | 32.7 |
| B36 | B88 | 353 | 52 | 14.7 |
| B220 | B181, B279 | 37 | 15 | 40.5 |
| B. pinnatum variant ( $2 n=18$ ) $\times$ B. sylvaticum ( $2 n=18$ ) |  |  |  |  |
| B144 | B257 | 40 | 12 | 30.0 |

## APPENDIX 2 CONTINUED

B. pinnatum variant $(2 n=18) \times$ B. pinnatum $(2 n=28)$

| B144 | B8 | 32 | 13 | 40.6 |
| :---: | :---: | :---: | :---: | :---: |
| B. pinnatum variant ( $2 n=18$ ) $\times$ B. pinnatum variant $(2 n=36)$ |  |  |  |  |
| B144 | B115 | 28 | 23 | 82.1 |
| B. pinnatum variant ( $2 n=36$ ) $\times$ B. sylvaticum $(2 n=18)$ |  |  |  |  |
| B115 | B29 | 35 | 0 | 0 |
| B. pinnatum variant $(2 n=36) \times$ B. pinnatum $(2 n=28)$ |  |  |  |  |
| B115 | B20 | 115 | 83 | 72.2 |

B. phoenicoides $(2 n=28) \times$ B. phoenicoides $(2 n=28)$

| B18 | B125 | 55 | 4 | 7.3 |
| :--- | :--- | ---: | ---: | ---: |
| B55 | B71 | 35 | 8 | 22.9 |
| B71 | B55 | 158 | 17 | 15.7 |
| B91 | B279 | 13 | 4 | 30.8 |
| B125 | B181 | 59 | 22 | 37.3 |
| B181 | B18 | 42 | 9 | 21.4 |
| B210 | B91 | 38 | 5.3 |  |
| B216 | B55 | 85 | 1 | 1.2 |
| B260 | B39, B181 | 78 | 15 | 19.2 |
| B279 | B55 | 72 | 13 | 18.0 |
| B280 | B270 | 48 | 21 | 43.8 |

B. phoenicoides $(2 n=28) \times$ B. sylvaticum $(2 n=18)$

| B2 | B75, B264 | 264 | 41 | 15.5 |
| :--- | :--- | ---: | ---: | :---: |
| B18 | B108 | 73 | 0 | 0 |
| B55 | B84 | 270 | 124 | 45.9 |
| B88 | B7 | 48 | 0 | 0 |
| B91 | B135 | 132 | 0 | 0 |
| B125 | B150 | 209 | 11 | 5.3 |
| B181 | B7 | 72 | 3 | 4.2 |
| B270 | B123 | 46 | 6 | 13.0 |
| B279 | B7, B153 | 35 | 0 | 0 |

B. phoenicoides $(2 n=28) \times$ B. pinnatum $(2 n=28)$

| B55 | B35 | 54 | 2 | 3.7 |
| :--- | :--- | ---: | ---: | ---: |
| B71 | B35 | 237 | 43 | 18.1 |
| B88 | B36 | 240 | 4 | 1.7 |
| B88 | B9 | 38 | 23.7 |  |
| B260 | B4, B9 | 19 | 6 | 31.6 |


| APPENDIX 2 CONTINUED |  |  |  |  |
| :---: | :---: | :---: | :---: | :---: |
| B. phoenicoides $(2 n=28) \times$ B. distachyon $(2 n=30)$ |  |  |  |  |
| B91 | B266 | 25 | 0 | 0 |
| B. retusum $(2 n=32) \times$ B. sylvaticum $(2 n=18)$ |  |  |  |  |
| B173 | B250 | 22 | 15 | 68.1 |
| B. retusum $(2 n=32) \times$ B. pinnatum $(2 n=28)$ |  |  |  |  |
| $\begin{aligned} & \text { B173 } \\ & \text { B173 } \end{aligned}$ | $\begin{aligned} & \text { B60 } \\ & \text { B118 } \end{aligned}$ | $\begin{aligned} & 39 \\ & 37 \end{aligned}$ | 25 | 64.1 0 |
| B. mexicanum $(2 n=42) \times$ B. sylvaticum ( $2 n=18$ ) |  |  |  |  |
| $\begin{aligned} & \text { B347 } \\ & \text { B347 } \end{aligned}$ | $\begin{aligned} & \text { B150 } \\ & \text { B250 } \end{aligned}$ | $\begin{aligned} & 44 \\ & 28 \end{aligned}$ | 3 0 | 6.8 0 |
| B. mexicanum $(2 n=42) \times$ B. pinnatum $(2 n=28)$ |  |  |  |  |
| $\begin{aligned} & \text { B347 } \\ & \text { R347 } \end{aligned}$ | $\begin{aligned} & \mathrm{B} 60 \\ & \mathrm{~B} 220 \end{aligned}$ | $\begin{array}{r} 17 \\ 6 \end{array}$ | 0 0 | 0 0 |
| B. mexicanum $(2 n=42) \times$ B. phoenicoides $(2 n=28)$ |  |  |  |  |
| B347 | B186 | 17 | 1 | 5.9 |
| B. distachyon $(2 n=30) \times$ B. sylvaticum $(2 n=18)$ |  |  |  |  |
| B199 | B47 | 15 | 0 | 0 |
| B199 | B84 | 8 | 0 | 0 |
| B201 | B253 | 14 | 1 | 7.1 |
| B201 | B104 | 30 | 6 | 20.0 |
| B201 | B153 | 26 | 3 | 11.5 |
| B. distachyon ( $2 n=30$ ) $\times$ B. pinnatum $(2 n=28)$ |  |  |  |  |
| B199 | B8 | 15 | 3 | 20.0 |
| B199 | B36 | 34 | 7 | 20.6 |
| B201 | B118 | 32 | 4 | 12.5 |
| B266 | B14, B118 | 37 | 11 | 32.4 |
| B. distachyon ( $2 n=30$ ) $\times$ B. pinnatum variant ( $2 n=18$ ) |  |  |  |  |
| B266 | V133 | 20 | 7 | 35.0 |
| B. distachyon $(2 n=30) \times$ B. pinnatum variant $(2 n=36)$ |  |  |  |  |
| $\begin{aligned} & \text { B199 } \\ & \text { B201 } \end{aligned}$ | $\begin{aligned} & \text { B115 } \\ & \text { B115 } \end{aligned}$ | $\begin{aligned} & 11 \\ & 40 \end{aligned}$ | 5 19 | 45.5 47.5 |

## APPENDIX 2 CONTINUED

B. distachyon $(2 n=30) \times$ B. phoenicoides $(2 n=28)$

| B199 | B88, B186 | 18 | 1 | 5.6 |
| :---: | :---: | :---: | :---: | :---: |
| B201 | B88 | 12 | , | 8.3 |
| B266 | B186 | 12 | 1 | 8.3 |
| B. sylvaticum ( $2 n=18$ ) $\times$ B. pinnatum/sylvaticum intermediate $(2 n=28)$ |  |  |  |  |
| B46 | B42 | 70 | 7 | 10.0 |
| 861 | B5 | 172 | 49 | 28.5 |
| B250 | B52 | 120 | 33 | 27.5 |
| B. pinnatum $(2 n=28) \times$ B. pinnatum/sylvaticum intermediate $(2 n=28)$ |  |  |  |  |
| B10 | B42 | 110 | 30 | 27.3 |
| B14 | B78 | 102 | 61 | 59.8 |
| B60 | B63 | 84 | 34 | 40.5 |
| B118 | B51 | 52 | 22 | 42.3 |
| E. phoenicoides $(2 n=28) \times$ B. pinnatum/sylvaticum intermediate ( $2 n=28$ ) |  |  |  |  |
| B91 | B5 | 33 | 6 | 18.2 |
| B. distachyon ( $2 n=30$ ) $\times$ B. pinnatum/sylvaticum intermediate $(2 n=28)$ |  |  |  |  |
| B266 | B5 | 106 | 2 | 1.9 |
| B266 | B63 | 35 | 1 | 2.9 |

B. pinnatum/sylvaticum intermediate $(2 n=28) \times \frac{\text { B. pinnatum } / \text { sylvaticum }}{\text { intermediate }} \frac{(2 n=28)}{(28)}$

| B63 | 113 | 23 | 20.4 |
| :--- | :--- | :--- | :--- | :--- |

B. pinnatum/sylvaticum intermediate $(2 n=28) \times$ B. sylvaticum $(2 n=18)$

| B5 | B103, B150 | 40 | 13 | 32.5 |
| :--- | :--- | ---: | ---: | ---: |
| B42 | B46 | 138 | 18 | 13.0 |
| B51 | B250 | 49 | 22 | 44.9 |
| B51 | B104 | 35 | 3 | 8.6 |
| B53 | B250 | 51 | 1 | 2.0 |
| B53 | B108 | 56 | 0 | 0 |
| B53 | B104 | 35 | 3 | 8.6 |
| B63 | B150, B250 | 307 | 151 | 49.2 |

B. pinnatum/sylvaticum intermediate $(2 n=28) \times$ B. pinnatum $(2 n=28)$

| B5 | B13 | 169 | 20 | 11.8 |
| :--- | :--- | :---: | ---: | ---: |
| B5 | B220 | 161 | 3 | 1.9 |
| B51 | B118 | 52 | 19 | 36.5 |
| B53 | B4 | 32 | 0 | 0 |
| B53 | B118 | 50 | 18 | 36.0 |
| B53 | B220 | 42 | 2.8 |  |
| B63 | B14 | 66 | 11 | 16.7 |
| B63 | B118 | 100 | 37 | 37.0 |
| B78 | B118 | 45 | 19 | 42.2 |

## APPENDIX 2 CONTINUED

| B5 | B88, B186 | 112 | 57 | 50.9 |
| :---: | :---: | :---: | :---: | :---: |
| B5 | B55 | 81 | 31 | 38.3 |
| B53 | B181, B186 | 27 | 0 | 0 |

B. glaucovirens/sylvaticum intermediate $(2 n=17) \times$ B. sylvaticum $(2 n=18)$

| B249 | V7 | 52 | 2 | 33.8 |
| :---: | :---: | :---: | :---: | :---: |
| B249 | B253 | 37 | 3 | 8.1 |
| B. glaucovirens/sylvaticum intermediate $\times$ B. glaucovirens ( $2 n=16$ ) |  |  |  |  |
| B249 | B226 | 48 | 2 | 4.2 |
| (2n=17) B230 | B226 | 39 | 6 | 15.4 |
| (2n=18) |  |  |  |  |
| B230 | B218 | 16 | 1 | 6.3 |

B. glaucovirens/sylvaticum intermediate $(2 n=17) \times$ B. pinnatum $(2 n=28)$

| B249 | B35 | 34 | 3 | 8.8 |
| :--- | :--- | :--- | :--- | :--- |


| B249 | B39, B71 | 72 | 6 | 8.3 |
| :---: | :---: | :---: | :---: | :---: |
| ( $2 \mathrm{n}=17$ ) |  |  |  |  |
| B249 | B181 | 65 | 5 | 7.7 |
| $(2 n=17)$ B230 | B186 | 43 | 1 | 2.3 |
| ( $2 \mathrm{n}=18$ ) |  |  |  | 2.3 |

(B. pinnatum $\times$ B. phoenicoides) $(2 n=28) \times \frac{\text { B. phoenicoides }}{\text { backcross }}(2 n=28)$

| B36 $\times$ B88 | 64 | 21 | 32.8 |
| :--- | :--- | :--- | :--- | :--- |

(B. pinnatum $\times$ B. phoenicoides) $(2 n=28) \times$ B. pinnatum $(2 n=28)$ backcross

| $\mathrm{B} 36 \times \mathrm{B} 88$ | $\mathrm{B9}, \mathrm{~B} 60$ | 96 | 8 | 8.3 |
| :--- | :--- | :--- | :--- | :--- |

(B. phoenicoides $\times$ B. pinnatum) $(2 n=28) \times$ B. phoenicoides $(2 n=28)$ backcross

| B88 $\times$ B36 | 7288 | 16 | 22.2 |
| :--- | :--- | :--- | :--- | :--- |

B. pinnatum $(2 n=28) \times$ (B. pinnatum $\times$ B. phoenicoides) $(2 n=28)$ backcross

| B14 | B36 $\times$ B88 | 16 | 10 | 62.5 |
| :--- | :--- | :--- | :--- | :--- |
| B118 | B36 $\times$ B88 | 59 | 25 | 42.4 |



## APPENDIX

Hybridization experiments: growth of embryos in individual crosses

Size of embryo
Type of
Endosperm
B. sylvaticum $(2 n=18) \times$ B. sylvaticum $(2 n=18)$

| B1 $\times$ B61 | 6 | 3 | 2 | 18 | Large | Hard |
| :--- | ---: | :--- | :--- | :--- | :--- | :--- |
| B7 $\times$ B47 | 4 | 4 | 3 | 18 | Large | Hard |
| B81 $\times$ B258 | 6 | 3 | 2 | 18 | Large | Hard |
| B102 $\times$ B7 | 4 | 3 | 3 | 18 | Large | Hard |
| B153 $\times$ B250 | 6 | 5 | 5 | 18 | Large | Hard |
| B239 $\times$ B250 | 10 | 7 | 5 | 18 | Medium | Hard |
| B258 $\times$ B84 | 3 | 3 | 3 | 18 | Large | Hard |
| B274 $\times$ B104 | 6 | 1 | 1 | 18 | Large | Hard |

B. sylvaticum $(2 n=18) \times$ B. glaucovirens $(2 n=16)$

| $\mathrm{B9O} \times \mathrm{B} 226$ | 12 | 8 | 6 | 17 | Large | Hard |
| :--- | ---: | ---: | ---: | ---: | ---: | :--- |
| $\mathrm{B} 364 \times \mathrm{B} 226$ | 6 | 3 | 3 | 17 | Large | Hard |

B. glaucovirens $(2 n=16) \times$ B. sylvaticum $(2 n=18)$

B224 x B48, B61, 27(3) 181716 Large Hard B134, B261
B. glaucovirens/sylvaticum intermediate $(2 n=17) \times$ B. sylvaticum $(2 n=18)$

| B249 $\times$ B7 | 2 | 1 | 1 | 17 | Large | Hard |
| :--- | :--- | :--- | :--- | :--- | :--- | :--- |
| B249 $\times$ B253 | 2 | 2 | 1 | 17 | Large | Hard |

B. glaucovirens/sylvaticum intermediate $(2 n=17) \times$ B. glaucovirens ( $2 n=16$ )
B249 x B226 216 Large Hard

## APPENDIX 3 CONTINUED


B. pinnatum variant $(2 n=18) \times$ B. pinnatum variant $(2 n=36)$

| B144 $\times$ B115 | 8 | 0 | 0 | - | Small | Soft |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| B. pinnatum variant ( $2 n=18$ ) $\times$ B. sylvaticum $(2 n=18)$ |  |  |  |  |  |  |
| B144 $\times$ B257 | 6 | 6 | 4 | - | Medium | Hard |
| B. pinnatum variant $(2 n=18) \times$ B. pinnatum $(2 n=28)$ |  |  |  |  |  |  |
| B144 $\times$ B8 | 7 | 2 | 2 | - | Large | Soft |
| B. pinnatum variant $(2 n=36) \times$ B. pinnatum $(2 n=28)$ |  |  |  |  |  |  |
| B115 $\times$ B20 | 2(7) | 0 | 0 | - | Small | Soft |
| B. sylvaticum $(2 n=18) \times$ B. pinnatum variant $(2 n=18)$ |  |  |  |  |  |  |
| $\begin{aligned} & \mathrm{B} 29 \times \mathrm{B} 144 \\ & \mathrm{~B} 102 \times \mathrm{B} 144 \end{aligned}$ | $\begin{aligned} & 5 \\ & 5 \end{aligned}$ | $5$ | 2 1 | - | Medium Medium | Soft Soft |
| B. sylvaticum $(2 n=18) \times$ B. pinnatum variant $(2 n=36)$ |  |  |  |  |  |  |
| B257 $\times 1115$ | 4(1) | 0 | 0 | - | Small | Soft |
| B. pinnatum $(2 n=28) \times$ B. pinnatum variant ( $2 n=18$ ) |  |  |  |  |  |  |
| B20 $\times$ B144 | 10 | 10 | 7 | - | Large | Hard |

B. pinnatum $(2 n=28) \times$ B. pinnatum variant $(2 n=36)$

| B20 $\times$ B115 | 8 | 1 | 0 | - | Small | Soft |
| :--- | :--- | :--- | :--- | :--- | :--- | :--- |
| B36 $\times$ B115 | 7 | 5 | 0 | - | Medium | Soft |
| B220 $\times$ B248 | 5 | 4 | 2 | - | Large | Hard |

B. pinnatum $(2 n=28) \times$ B. phoenicoides $(2 n=28)$

| B34 $\times \mathrm{B} 88, \mathrm{B91}$, | 8 | 8 | 6 | 28 | Large | Hard |
| :--- | :--- | :--- | :--- | :--- | :--- | :--- |
| B186 |  | $\cdot$ |  |  |  |  |
| B36 $\times$ B88 | $9(1)$ | 7 | 7 | 28 | Large | Hard |
| B220 $\times$ B181 | 6 | 5 | 3 | 28 | Medium | Hard |

B. sylvaticum $(2 n=18) \times$ B. phoenicoides $(2 n=28)$

| B7 $\times$ B181 | 16 | 12 | 5 | 23 | Large | Hard |
| :--- | :---: | :---: | :---: | :---: | :---: | :---: |
| B28 $\times$ B2 | 7 | 2 | 1 | 23 | Large | Soft |
| B41 $\times$ B39 | $10(12)$ | 4 | 4 | 23 | Large | Soft |
| B93 $\times$ B55 | 5 | 2 | 0 | - | Large | Soft |
| B150 $\times$ B785 | 22 | 1 | 0 | - | Small | Soft |
| B250 $\times$ B91 | $11(10$ | 0 | 0 | - | Small | Soft |
| B284 $\times$ B55 | 46 | 1 | 0 | - | Large | Soft |
| B286 $\times$ B39 | 5 | 2 | 1 | - | Large | Soft |

## APPENDIX 3 CONTINUED

B. phoenicoides $(2 n=28) \times$ B. phoenicoides $(2 n=28)$

| B18 $\times$ B125 | $2(2)$ | 2 | 1 | 28 | Large | Soft |
| :--- | :---: | :---: | :---: | :---: | :--- | :--- |
| B71 $\times$ B55 | 16 | 11 | 7 | 28 | Nedium | Hard |
| B91 $\times$ B279 | 4 | 2 | 1 | 28 | Large | Hard |
| B279 $\times$ B55 | $5(2)$ | 2 | 1 | 28 | Large | Hard |

B. phoenicoides $(2 n=28) \times$ B. sylvaticum $(2 n=18)$

| $\mathrm{B} 2 \times \mathrm{B75}, \mathrm{~B} 264$ | $8(8)$ | 0 | 0 | - | Small | Soft |
| :--- | ---: | ---: | ---: | ---: | :--- | :--- |
| $\mathrm{B} 55 \times \mathrm{B} 4$ | $26(5)$ | 10 | 9 | 23 | Small | Soft |

B. phoenicoides $(2 n=28) \times$ B. pinnatum $(2 n=28)$

| B55 $\times$ B35 | 2 | 2 | 2 | 28 | Large | Hard |
| :--- | :--- | :--- | :--- | :--- | :--- | :--- |
| B88 $\times$ B36 | 4 | 3 | 3 | 28 | Large | Hard |

B. phoenicoides $(2 n=28) \times$ B. pinnatum/sylvaticum intermediate $(2 n=28)$

| B91 $\times$ B5 | 9 | 0 | 0 | Small Soft |
| :--- | :--- | :--- | :--- | :--- | :--- | :--- |

B. pinnatum/sylvaticum intermediate $(2 n=28) \times$ B. phoenicoides $(2 n=28)$

| B5 $\times$ B55 | 13 | 3 | 2 | 28 | Large | Hard |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| B5 $\times$ B88 | 6 | 4 | 3 | 28 | Large | Hard |
| B. retusum $(2 n=32) \times$ B. sylvaticum $(2 n=18)$ |  |  |  |  |  |  |
| B173 $\times$ B250 | 9 | 0 | 0 | - | - - | - |

B. retusum $(2 n=32) \times$ B. pinnatum $(2 n=28)$
B173×B60 $21(7) 17 \quad 13$ - Large Hard
B. mexicanum $(2 n=42) \times$ B. sylvaticum $(2 n=18)$

| B347 $\times$ Bl50 | $2(1) \quad 0 \quad$ Small |  |
| :--- | :---: | :---: | :---: | :---: | :---: |
| B. mexicanum | $(2 n=42) \times$ B. phoenicoides | $(2 n=28)$ |


| B347 $\times$ Bl86 | 1 | 0 | - Medium Soft |
| :--- | :--- | :--- | :--- | :--- |

B. distachyon $(2 n=30) \times$ B. sylvaticum $(2 n=18)$

| B199 $\times$ B104 | $4(1)$ | 2 | 0 | - | Medium | Soft |
| :--- | :--- | :--- | :--- | :--- | :--- | :--- |
| B201 $\times$ B253 | 1 | 1 | 0 | - | Medium | Soft |
| B201 $\times$ B153 | 3 | 3 | 0 | - | Large | Soft |

B. distachyon $(2 n=30) \times$ B. pinnatum $(2 n=28)$

| B199 $\times$ B8 | $2(1)$ | 2 | 2 | 29 | Large | Soft |
| :--- | :--- | :--- | :--- | :--- | :--- | :--- |
| B199 $\times$ B36 | $6(1)$ | 4 | 3 | 29 | Large | Soft |
| B201 $\times$ B118 | 1 | 1 | 1 | 29 | Large | Soft |
| B266 $\times$ B8 | $2(1)$ | 2 | 2 | 29 | Large | Soft |

## APPENDIX 3 CONTINUED



Allard, R. W. \& Kannenberg, L. W. (1968). Population studies in predominently self-pollinated species, XI. Genetic divergence among the members of the Festuca microstachys complex. Evol., 22, 517-528.

Anderson, E. (1949). Introgressive hybridization. John Wiley and Sons, New York. 109pp.

Anderson, E. (1953). Introgressive hybridization. Biol. Rev., 28, 280-307.

Anderson, S. (1931). Graes-hybrider 1 Danmark. Botanisk Tiddskrift, 41, 424-430.

Arber, A. (1934). The Gramineae: A study of cereal, bamboo and grass. Cambridge University press, New York.

Ascherson, P. \& Graebner, P. (1901). Brachypodium, in Synopsis der Mitteleuropäischen Flora, 2, 631-640. Leipzig.

Auquier, P. (1971). Le problème de Festuca rubra L. subsp. arenaria ( Osb .) Richt. et de ses relations avec $F$. juncifolia St Amans. Lejeunia, N.S., 57, 1-24.

Avdulov, N. P. (1931). Karyo-systematische Untersuchungen der Gramineen. Bull. Appl. Bot. Genot. Pl. Breed., Suppl., 44, 1-428.

Barker, C. M. (1980). Investigation into the relationships and ancestry of Vulpia and Festuca. Ph.D. Thesis, University of Leicester.

Barker, C. M. \& Stace, C. A. (1982). Hybridization in the genera Vulpia and Festuca. The production of artificial $F_{1}$ plants. Nord. J. Bot., 2, 435-444.

Beauvois, A. M. F. J. Palisot de (1812). Essai d'une nouvelle agrostographie; ou nouveaux genres des Graminées, avec figures representant les caractères de tous les genres. Fain, Paris.

Bebyakin, V. M. \& Kumakov, A. V. (1981). Gliadin composition in Triticum durum varieties of different grain quality and in their $F_{1}$ hybrids. Tsitol Genet, 15(1), 39-42.

Beetle, A. A. (1955). The four subfamilies of the Gramineae. Bull. Torrey bot. Club, 82, 196-107.

Bentham, G. (1881). Notes on Gramineae. J. Linn. Soc. Bot., 19, 14-134.

Bews, T. G. (1929). The world's grasses: their differentiation, distribution, economics and ecology. Longmans Green, London.
Bolkhavskikh, Z. et al. (1969). Chromosome numbers of flowering plants. Academy of sciences of tho U.S.S.R., Leningrad.

Bluff, M. J., Nees, C. -G. \& Schauer, J. C. (1836). Compendium florae Germaniae, ed. alt., 1(1). Nürnberg.

Borrill, M. (1961). Dactylis marina Borrill, sp. nov., a natural group of related tetraploid forms.
J. Linn. Soc. Bot., 56, 431-440.

Borsos, S. (1974). Notes on the leaf anatomy of the Brachypodium pinnatum complex. Acta Botanica

Academiae Scientiarum Hungaricae, 20(1-2), 13-21.
Bowden, W. M. (1958). Natural and artificial X
Elymordeum hybrids. Can. J. Bot., 36,101-123. Bremer, G. (1923). A cytological investigation of some species and species hybrids within the genus Saccharum. Genetica, 5, 97-148, 273-326. Bremer, G. (1924). The cytology of the sugarcane, 2. A cytological investigation of some cultivated hybrids and their parents. Genetica, 6, 497-525. Bremer, G. (1925). The cytology of the sugarcane,
3. The chromosomes of primitive forms in the genus Saccharum. Genetica, 7, 293-322.

Brown, R. (1810). Prodomus florae Novae Hollandiae,

1. I. R. Taylor, London.

Brown, W. V., Harris, W. F. \& Graham, J. D. (1959). Grass morphology and systematics, 1. The internode. Southw. Nat., 4(3), 115-125. Brown, W. V., Heimsch, C. \& Emery, W. H. P. (1957). The organization of the grass shoot apex and systematics. Amer. J. Bot., 44, 590-595.

Brown, W. V., Pratt, C. A. \& Mobley, H. M. (1959). Grass morphology and systematics, $1 d, 2$. The nodal pulvinus. Southw. Nat., 4(3), 126-130.

Bruns, E. (1892). Der Grasembryo. Flora, 76, 1-33.
Burr, S. M. \& Turner, D. M. (1933). British economic
grasses: their identification by their leaf
anatomy. E. Arnold \& Co., London.
Camus, A. (1957). Bromus hybrides de la flore française.
Bull. Jard. bot. Etat Brux., 27, 479-485.
Camus, A. (1958). Graminées hybrides de la flore française (Genre Bromus excepté). Bulletin du Jard. Bot. Etat Brux., 28, 337-374.
Carnahan, H. L. \& Hill, H. D. (1961). Cytology and genetics of forage grasses. Bot. Rev.. 27, 1-162.

Carroll, C. P. \& Borrill, M. (1965). Tetraploid hybrids from crosses between diploid and tetraploid Dactylis and their significance. Genetica, 36, 65-82.

Cenci, C. A. (1974). Miglioramento genetico del Brachypodium pinnatum P. B. Ricerche sulla sua possibile utilizzazione nell' impianto dei pascoli. Genetica Agraria, 28(2), 204-218.

Church, G. L. (1949). A taxonomic study of Glyceria and Puccinellia. Amer. J. Bot., 36, 155-165.
Clark, J. (1960). Preparation of leaf epidermis for topographic study. Stain Technol., 35, 35-39.
Claustres, G. \& Huon, A. (1965). Sur la valeur des caractères épidermiques dans la taxinomio des Festuca rubra L. du littoral armoricain. C. R. Acad. Sc. (Paris), 260, 4241-4244.

Clayton, W. D. (1978). Poales. In: Flowering plants of the world. (ed. V. H. Heywood). Oxford University Press.

Clifford, H. T. \& Goodall, D. W. (1967). A numerical contribution to the classification of the Poaceae. Aust. J. Bot., 15, 499-519.
Cockerell, T. D. A. (1908). The fossil flora of Florissant, Colorado. Amer. Mus. Natur. Histor. Bu11., 24, 71-110.
Cotton, R. (1974). Cytotaxonomy of the genus Vulpia. Ph.D. thesis, University of Manchester.

Crane, M. B. \& Lawrence, W. J. C. (1952). The genetics of garden plants, 4 th ed. Macmillan, London.
Cugnac, A. de (1931). Recherches sur les glucides des Gramineae. Ann. Sci. Nat. Bot., Sér. 10, 13, 1-29.

Darlington, C. D. \& La Cour, L. F. (1969). The handling of chromosomes, 5 th ed. London.
Dumortier, B. C. J. (1824). Observations sur les Graminées de la flore belgique. Tournay. Duval-Jouve, J. (1875). Histotaxie des feuilles do Graminées. Ann. Sci. Nat., b, Sér. 6, 1, 294-371. Ellis, R. P. (1976). A procedure for standardizing comparative leaf anatomy in the Poaceae, 1. Leaf-blade as viewed in transverse section. Bothalia, 12(1), 65-109.
Ellis, W. M. (1973). The breeding system and variation in populations of Poa annua L. Evolution, 27, 656-662.

Evans, G. (1926). Chromosome complements in grasses. Nature, 118, 841.

Fagerlind, F. (1937). Embryologische, zytologische und bestäubangungsexperimentelle Studien in der Familie Rubiaceae nebst Bemerkungen über einige Polyploidista̋tsprobleme. Acta Horti Bergiani, 11, 195-470.

Fairbrothers, D. E. \& Johnson, M. A. (1961). The precipitin reaction as an indication of relationships in some grasses. Recent advances in botany, 1, 116-120. University of Toronto Press.

Fries, E. (1846). Summa Vegetabilium Scandinaviae, pp. 75-76, 247-248. Uppsala.

Goossen, A. P. (1938). A study of South African species of Sporobolus with special reference to the leafanatomy. Trans. R. Soc. S. Afr., 26, 173-223. Gould, F. W. (1968). Grass systematics. Mc GrawHill, New York.

Grant, V. (1971). Plant speciation. Columbia University Press, New York \& London.

Grob, A. (1896). Beiträge zur Anatomie der Epidermis der Gramineenblätter. Bibl. Bot., 7 (36), 1-107. Gunzel, F. (1912). Blattanatomie Südwestafrikanischon Gräser. Bot. Jb., 49, Beiblatt 108.
Gunzel, F. (1921). Weitere Beiträge zur Kenntnis dor Blattanatomie afrikanischer Gräser. Bot. Jb., 57, Beiblatt 126, l-26.

Hall, O. (1959). Immuno-electrophoretic analysis of allopolyploid rye-wheat and its parental species.

Hereditas, 45, 495-504.

Hansen, A. (1959). Die Gras-Hybriden in der Flora Frankreichs. Kritik und Ergänzungen. Bull. Jard. bot. Etat Brux., 29, 61-68.

Harrison, B. J. \& Darky, L. (1955). Unilateral hybridization. Nature (Lond.), 176, 982.

Harz, C. O. (1880). Beiträge zur Systematik der Gramineen. Linnaea, 43, 1-30.

Hatch, M. D. \& Slack, C. R. (1966). Photosynthesis by sugarcane leaves. A new carboxylation reaction and the pathway of sugar formation. Biochem. J., 101, 103-111.

Hilu, K. W. \& Wright, K. (1982). Systematics of Gramineae: A cluster analysis study. Taxon, 31, 9-36.

Hisu, C. C. (1971). A guide to the Taiwan grasses, with keys to subfamilies, tribes, genera and species. Taiwania, 16(2), 199-341.

Hubbard, C. E. (1935). Brachypodium serpentini Hubbard. Hooker's I cones Plantarum, t. 3280.

Hubbard, C. E. (1948). Gramineae. In Hutchinson, J., British flowering plants, pp. 284-348. Gawthorn, London.

Hubbard, C. E. (1954). Grasses. Penguin Books, Harmondsworth.

Hubbard, C. E. (1966). Gramineae, in Willis, J. C. A dictionary of the flowering plants, 7 th ed. by Shaw, H. K. A., pp.492-496. Cambridge University Press.

Huon, A. \& Redon, G. (1967). Quelques types épidermiques de l'espèce collective Festuca rubra L. C.R. $91^{\text {e }}$ Congr. Soc. Sav. (Rennes), 1966, Sect. Sc., 3, 243-252.

Hutchinson, J. (1934). Gramineae. In Families of flowering plants, 2, 199-229. MacMillan and Co. London.

Hylander, N. (1950). Some ideas regarding the systematics of Scandinavian grasses. Proc. Seventh Inter. Bot. Cong., pp. 854-855.

Isaiah ( 1000 BC ). Old testament. Chapter 40, verse 6. Jacques-Félix, H. (1962). Les Graminées d'Afrique Tropicale, 1. Généralités, classification, description des genres. Bull. Sci. Inst. Rech. Agron. Trop., 8, 1-345.

Jenkin, T. J. (1933). Interspecific and intergeneric hybrids in herbage grasses. Initial crosses. J. Genetics, 28, 205-264.

Jenkin, T. J. (1955). Interspecific and intergeneric hybridizations in herbage grasses. Some of the breeding interactions of Festuca gigantea. J. Genet., 53, 94-99.

Jenkin, T. J. (1959). Fescue species (Festuca L.); The rye grasses (Lolium L.). Handbuch der Pflanzenzüchtung., 4, 418-452.

Johnson, B. L. (1972). Seed-protein profiles and the origin of the hexaploid wheats. Amer. J. Bot., 59, 952-960.

Johnson, B. L. \& Hall, O. (1965). Analysis of phylogenetic affinities in the Triticinae by protein electrophoresis. Amer. J. Bot., 52, 506-513.

Kaneta, M. \& Sugiyama, N. (1973). Identification of flavone compounds in eighteen Gramineae species. Agr. Biol. Chem., 37(11), 2663-2665.

Kannenberg, L. W. \& Allard, R. W. (1967). Population studies in predominently self-pollinated species, VIII. Genetic variability in the Festuca microstachys complex. Evol., 21, 227-240.

Kattermann, S. (1931). Chromosomenuntersuchungen bei Gramineen. Planta, 12, 19-37.

Kinges, H. (1961). Merkmale des Graminees embryo. Bot. Jahrb., 81, 50-93.

Kiss, A. \& Rajhathy, T. (1956). Investigations on crossability within the subtribe Triticinae. Züchter, 26, 127-136.

Kliphuis, E. \& Wieffering, J. H. (1972). Chromosome numbers of some angiosperms from the south of France. Acta bot. neerl., 2l, 598-604.

Knobloch, I. W. (1968). A check list of crosses in the Gramineae. Mich. State Univ., East Lansing. Knobloch, I. W. (1972). Intergeneric hybridization in flowering plants. Taxon, 21, 97-103.
Kožuharov, S. I. \& Nicolova, T. (1975). Cytotaxonomic studies on Bulgarian Gramineae. Bulgarian Academy of Sciences, in honour of Acad. Daki Jordanov, pp.69-77. Sofia.

Kožuharov, S. I., Petrova, A. V. \& Stoeva, M. (1974). Chromosomal variation in the Brachypodium genus in Bulgaria with regard to its evolution and taxonomy. Genet. Pol., 15, 13-23.

Kumada, Y. (1919). Die Chromosomenzahl von Zea mays L. J. Coll. Sci. Imp. Univ. Tokyo, 39, 1-48.

Kunth, C. S. (1833). Enumeratio plantarum.
Lange, W. \& Wojcieckowska, B. (1976). The crossing of common wheat (Triticum aestivum L.) with cultivated rye (Secale cereale L.), I. Crossability, pollen grain germination and pollen tube growth. Euphytica, 25, 609-620.

Larsen, K. (1963). Cytology of the endemic Canarian elements, II. Bot. Notiser, 116, 409-424.

Lawrence, H. M. (1969). Taxonomy of vascular plants. MacMillan, New York.

Lawton, J. R. (1980). Observations on the structure of epidermal cells, particularly the cork-cells and the silica-cells, from the flowering stem internode of Lolium temulentum L., Gramineae. Bot. J. Linn. Soc., 80, 161-177.
Lepage, E. (1952). Etudes sur quelques plantos américaines, II. Hybrides intergénériques: Agrohordeum et Agroelymus. Naturaliste Canadien, 79, 241-266.
Lepage, E. (1953). Nouvelles notes sur des hybrides de Graminées. Naturaliste Canadien, 80, 189-199.

Levan, A. Fredga, K. \& Sandberg, A. A. (1965). Nomenclature for centromeric position on chromosomes. Hereditas, 52, 201-220.

Lewis, D. \& Crowe, L. K. (1958). Unilateral
interspecific incompatibility in flowering plants. Heredity, 12, 233-256.

Lewton-Brain, L. (1904). On the anatomy of the leaves of British grasses. Trans. Linn. Soc. Lond. Bot. Ser. 2, 6, 315-359.

Link, J. H. F. (1827). Hortus regius botanicus Berolinensis descriptus. Berlin.

Linnaeus, C. (1753). Species plantarum. Stockholm.
Lohauss, L. (1905). Der anatomische Bau der Laubblätter der Festucaceen und dessen Bedeutung für die Systematik. Bibl. Bot., 13(63), 114.

Longely, A. E. (1924). Chromosome in maize and maize hybrids. J.Agr. Res., 28, 673-682. Lőve, A. \& Lőve, D. (1961). Some nomenclatural changes in the European flora. Bot. Notiser, 144, 33-47. Macleod, A. M. \& McCorquodale, H. (1958). Watersoluble carbohydrates of seeds of the Gramineac. New Phytol., 57, 178-182.

Maekawa, F. (1964). Geohistory and differentiation of species. Nat. Sci. Mus., Tokyo, 31, 2-16. Maire, D. R. \& Weiller, M. (1955). Flore de L'Afrique du Nord, 3, Lechevalier, Paris.

Mehra, P. N. \& Sunder, S. (1969). Cytological studies in the North Indian grasses, II. Res. Bull. Punjab. Univ., 20(3-4), 503-539.

Melderis, A. (1950). Genetic problems within the tribe Hordeae. Proc. Inter. Bot. Cong., 853854.

Melderis, A. (1978). Taxonomic notes on the genus Festucopsis (C. E. Hubbard) Melderis. Bot. J. Linn. Soc., 76, 316-320.

Metcalfe, C. R. (1960). Anatomy of the Monocotyledons,

1. Gramineae. Oxford University Press, London.

Meusel, H. (1965). Vergleichende Chorologie der Zentraleuropäischen Flora, I, 42-43. Jena.

Mimeur, G. (1950). Contribution au catalogue chromosomique des Graminées prairiales. Bull. Mus. natn. Hist. nat., Paris, 22 (Sér. 2), $1,130$.

Morrison, J. W. \& Rajnathy, T. (1959). Cytogenetic studies in the genus Hordeum, III. Pairing in some interspecific and intergeneric hybrids. Can. J. Genet. Cytol., $1,65-77$.

Murbeck, A. (1891). Beiträge zur Kenntniss der Flora von Südbosnien und der Hercegowina. Acta. Univ. Lund., 27, 22.

Myers, W. M. (1947). Cytology and genetics of forage grasses. Bot. Rev., 13, 319-421.

Natarjan, G. (1978). I. O. P. B. chromosome number reports. Taxon, 27, 527.

Nettancourt, D. de (1977). Incompatibility in angiosperms. Monographs on theoretical and applied genetics, 3. Springer-Verlag, Berlin.

Nevski, S. A. (1933). On the systematics of the tribe Hordeae Benth. (In Russian). Trudy bot. Inst. Akad. Nauk S.S.S.R., Ser. 1, 9-32.

Nevski, S. A. (1934). Brachypodium and Trachynia. In Komarov, V. L. (ed.), Flora of the U.S.S.R., 2, 593-597. Academy of Sciences of the U.S.S.R., Leningrad.

Niles, C. D. \& Chase, A. (1925). Contrib. U.S. National Herbarium, 24, 196.

Nilsson, F. (1935). Amphidiploidy in the hybrid Festuca arundinacea $x$ gigantea. Hereditas, 20, 181-198.

Nixon, R. W. (1925). Immediate influence of pollen in determining the size and time of the fruit of the date-palm. J. Hered., 19, 241-255.

Nixon, R. W. (1936). Metaxenia and interspecific pollinations in Phoenix. Proc. Amer. Soc. Hort. Sci., 33, 21-26.

Parodi, L. R. (1961). La taxonomia de las Gramineae Argentinas a la luz de las investigaciones mas recentes. Recent Advances in Bot., 1, 125-129. University of Toronto Press.

Pée-Laby, E. (1898). Etude anatomique de la feuille des Graminées de la France. Ann. Sci. Nat., Bot., Sér. 8, 8, 227-346.

Pilger, R. (1954). Das System der Gramineae. Bot. Jahrb., 76, 281-384.

Potztal, E. (1964). Engler's Syllabus der Pflanzenfamilien, 12th ed., 2, 561-579. Berlin.
Prat, H. (1932). L'épiderme des Graminées. Etude anatomique et systématique. Ann. Sci. nat., Bot., Sér. 10, 14, 117-324.
Prat, H. (1936). La systématique des Graminées. Ann. Sci. nat., Bot., Sér. 10, 18, 165-258.
Prat, H. (1948). General features of the epidermis in Zea mays. Ann. Mo. Bot. Gdn., 35, 341-351.

Prat, H. (1951). Histophysiological gradients and plant organogenesis, Part I. Bot. Rev., 14, 603-643.

Prat, H. (1960). Revue d'agrostologie, vers une classification naturelle des Graminées. Bull. Soc. Bot. France, 107, 32-79.

Reeder, J. R. (1953). Affinities of the grass genus Beckmania Host. Bull. Torrey Bot. Club, 80, 187-196.

Reeder, J. R. (1957). The embryo in grass systematics. Amer. J. Bot., 44, 756-768.

Reeder, J. R. (1961). The grass embryo in systematics. Recent advances in Botany, 91-96. University of of Toronto Press.

Reeder, J. R. (1962). The bambusoid embryo. A reappraisal. Amer. J. Bot., 49, 639-641.
Reeder, J. R. \& Maltzahn, K. von (1953). Taxonomic
significance of root-hair development in the
Gramineae. Proc. U.S. natn. Acad. Sci., 39, 593-598.

Renvoize, S. A. (1981). The subfamily Arundinoideae and its position in relation to a general classification of the Gramineae. Kew Bulletin, 36, 85-102. Riley, R. \& Chapman, V. (1967). The inheritance in wheat of crossability with rye. Genet. Res., 9, 259-267.

Robertson, I. H. (1981). Chromosome numbers in Brachypodium Beauv (Gramineae). Genetica, 56, 55-60.

Romero Lopez, C. et al. (1979). A phylogenetic interpretation of chromosomal and electrophoretic data in Columbiformes. Cytologia, 44, 39-47.

Row, H. C. \& Reeder, J. R. (1957). Root-hair development as evidence of relationships among genera of Gramineae. Amer. J. Bot., 44, 596-601.
Saint Yves, A. (1934). Contribution à l'étude des Brachypodium (Europe et région méditerranéenne). Candollea, 5, 427-493.

Sangster, A. G. (1970). Intracellular silica deposition in immature leaves in three species of the Gramineae. Ann. Bot., 34, 245-257.

Saunders, T. B. \& Hamrick, J. L. (1980). Variation in the breeding system of Elymus canadensis. Evolution, 34, 117-122.

Scholz, H. (1968). Die Artbestimmung Im Brachypodium pinnatum - Complex. Wildenowia, 5 , 133-118.

Scheuchzer, J. (1708). Agrostographiae Helveticae Prodromus. Zürich.

Schwenderer, S. (1890). Die Mestomscheiden der Gramineenblätter. Sitzungsberichte de Königlich Preussischen Akademie der Wissenschaften zu Berlin, 22, 405-426.

Sennen, F. (1911). Brachypodium paui (ramosum $x$ distachyon?). Plantes d'Espagne: notes et diagnoses. Bull. de Geogr. Bot., 20(133), 53.

Sharma, A. K. \& Sharma, A. (1965). Chromosome technique. Theory and practice. London.

Sharma, M. L. (1979). Some considerations on the phylogeny and chromosomal evolution in grasses. Cytologia, 44, 679-685.

Sinnott, E. W. (1939). Growth and differentiation in living plant meristems. Proc. U.S. nat. Acad. Sci., 25, 55-58.

Sinnott, E. W. \& Block, R. (1939). Cell polarity and the differentiation of root hairs. Proc. U.S. Nat. Acad. Sci., 25, 248-252.

Smith, P. M. (1969). Serological relationships and taxonomy in certain tribes of the Gramineae. Ann. Bot., 33, 591-613.

Smith, P. M. (1971). The taxonomy and nomenclature of the brome-grasses. Notes R. Bot. Gdn Edinb., 30, 361-375.

Smith, P. M. (1972). Serology and species relationships in annual bromes (Bromus L. sect. Bromus). Ann. Bot., 36, 1-30.
Smith, P. M. (1976). The chemotaxonomy of plants. Edward Arnold, London.

Smith, P. M. (1980). Brachypodium Beauv. In Tutin, T. G. et al.. eds, Flora Europaea. 5, 189-190. Cambridge University Press.

Sokal, R. R. \& Sneath, P. H. A. (1963). Principles of numerical taxonomy. Freeman, San Francisco.

Sokolovskaya, A. P. \& Probatova, N. S. (1978). Chromosome numbers of some grasses (Poaceae) of the U.S.S.R. flora, II. Bot. Zhurn. SSSR, 63(9), 1247-1257. (In Russian).

S申rensen, T. (1953). A revision of the Greenland species of Puccinellia Parl. Medd. om Gronl., 136, 1-180.

Stace, C. A. (1975). Hybridization and the flora of British Isles. Academic Press, London.

Stace, C. A. \& Cotton, R. (1974). Hybrids between Festuca rubra $L$. sensu lato and Vulpia membranacea (L.) Dumort. Watsonia, 10, 119-138.

Stebbins, G. L. (1950). Variation and evolution in plants. Columbia University Press, New York.

Stebbins, G. L. (1956). Cytogenetics and evolution of the grass family. Amer. J. Bot., 43, 890-905.

Stebbins, G. L. (1959). Genes, chromosomes and evolution. In Turrill, W. B., ed., Vistas in botany, pp. 258-290. London.

Stebbins, G. L. (1971). Chromosomal evolution in higher plants. Edward Arnold, London. Stebbins, G. L. (1972). The evolution of the grass family. In Youngner, V. B. \& McKell, C. M. (eds.), The biology and utilization of grasses, pp. 1-17. Academic Press, New York and London.

Stebbins, G. L. \& Crampton, B. (1961). A suggested revision of the grass genera of temperate North America. In: Recent Advances in Botany, 1, 133-145. University of Toronto Press.

Strecker, W. (1913). Erkennen und Bestimmen der Wiesengräser im Blüten und blütenlosen Zustande, sowie ihr Wert und ihre Samenmischungen für Wiesen und Weiden. Berlin.

Strid, A. (1983). I.O.P.B. chromosome number reports, 78. Taxon, 32, 138-139.

Sustar, F. (1976). Horoloskka in Taksonomska Problematika Kompleksa Brachypodium pinnatum. Biol. vestn. (Ljubljana), 24(1), 1-11.

Tateoka, T. (1955). Karyotaxonomic studies in Poaceae, II. Ann. Rep. Natl. Inst. Genet. (Japan), 5, 63-69. Tateoka, T. (1956). On morphological convergence between Brachypodium sylvaticum and Agropyron yezoense. Cytologia, 21, 146-152.

Tateoka, T. (1957). Proposition of a new phylogenetic system of Poaceae. Jour. Jap. Bot. 32, 275-287.

Tateoka, T. (1962). A cytological study of some Mexican grasses. Bull. Torr. Bot. Club., 89(2), 77-82.

Tateoka, T. (1968). Phytogeographical notes on the genus Brachypodium P. Beauv. (Gramineae). Boletin Sociedad Argentina de Botánica, 12, 44-56.
Tateoka, T., Inoue, S. \& Kawano, S. (1959). Notes on some grasses, 9. Systematic significance of bicellular microhairs of leaf epidermis. Bot. Gaz., 121, 80-91.

Tischler, G. (1950). Die Chromosomenzahlen der Gefässpflanzen Mitteleuropas. 'S-Gravenhage, Junk.

Tutin, T. G. (1952). Gramineae. In Clapham, A. R. Tutin, T. G. \& Warburg, E. G. Flora of the British Isles.. Cambridge.

Ullmann, W. (1936). Natural and artificial hybridization of grass species and genera. Herb. Rev., 4, 105-142.

Van Tieghem, P. (1897). Morphologie de l'embryon et de la plantule chez les Graminées et les Cypéracées. Ann. Sci. Nat., Bot., Sér. 8, 3, 259-309.

Vickery, J. W. (1935). The leaf anatomy and vegetative characters of the indigenous grasses of N.S. Wales. Proc. Linn. Soc. N.S.W., 60, 5-6, 340-373.

Willis, J. C. (1973). A dictionary of the flowering plants and ferns. Cambridge University Press.

Yakovlev, M. S. (1950). Structure of endosperm and embryo in grasses as a systematic feature. Morf. Anat. Trudy Bot. Akad. Nauk. S.S.S.R., Ser. 7, 1, 121-218.

Zarco, C. R. \& Devesa, J. A. (1983). Numeros chromosomicos para la flora española. Lagascalia, 12(1), 124-125.

Ziegenspeck, H. (1938). Die Phylogenie der Glumiflorae. Bot. Archiv., 29, 177-205.

Zimmermann, R. (1979). The influence of controlled burning on rocks head-semidry meadows and succession associations in southwestern West Germany. Phytocoenologia, 5(4), 447-524.


[^0]:    $\frac{\text { B. pinnatum }}{(2 n=28)}(2 n=28) \times$ B. pinnatum
    $\frac{\text { B. pinnatum }}{(2 n=18)}(2 n=28) \times$ B. sylvaticum $\frac{B . \text { pinnatu }}{(2 n=28)}$

[^1]:    B. glaucovirens/sylvaticum intermediate x

[^2]:    Plate 3.7/10: Leaf sections of synthetic Brachypodium hybrids (female parent first)

    A - B. pinnatum $\times$ B. phoenicoides
    B - B. phoenicoides $\times$ B. pinnatum

