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Andrew Doust
Xianmin Diao *Editors*

Genetics and Genomics of Setaria

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Andrew Doust • Xianmin Diao
Editors

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Editors
Andrew Doust
Department of Plant Biology, Ecology, and Evolution
Oklahoma State University
Stillwater, OK, USA

Xianmin Diao
Institute of Crop Sciences
Chinese Academy of Agricultural Sciences
Haidian District, Beijing, China

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Preface

Setaria is a genus of panicoid grasses that utilizes the highly efficient C_4 photosynthetic pathway and is related to other C_4 grasses such as switchgrass, pearl millet, maize, and sorghum. The Setaria system comprises two species (considered subspecies by some authors), namely, wild green foxtail (*S. viridis*), one of the most widespread weeds on the planet, and its domesticated cousin, foxtail millet (*S. italica*), a drought-hardy and nutritious cereal important in China, India, and Africa. Together, the two species make up a remarkable system for investigating different aspects of plant biology, ranging from the processes of ecological differentiation, domestication, morphological and developmental change, genetic regulation, C_4 photosynthesis, breeding, and genome evolution. The aim of this book is to introduce the Setaria system to a wider audience, explore current research in Setaria, and provide protocols and guidance for crossing, mutant production, creation of genetic resources, transformation, and genetic analysis.

The wide latitudinal and ecological range of green foxtail and foxtail millet has led to population divergence and local adaptation to a variety of conditions. The model *S. viridis* accession, A10.1, is consistent in its growth form under controlled conditions but very sensitive to growth environment, making it ideal for examining the molecular basis of abiotic stress. Accession A10.1 is a small variety of green foxtail that can be grown in growth chamber, greenhouse, and field trials and needs no special growth conditions. In particular, the small physical size and rapid life cycle of A10.1, coupled with a small diploid genome, lend itself to genetic analyses such as those that have commonly been performed in *Arabidopsis*. Further model accessions are being developed that combine attributes of foxtail millet, especially non-seed shattering, with other desirable traits.

Multiple chapters in this volume speak to the utility of Setaria as a model system for C_4 grass biology, including C_4 photosynthesis, cell wall regulation, root and shoot regulation, root-microbe interactions, herbicide tolerance, and drought stress. These studies are enabled by genetic and genomic resources, including multiple genome sequences for foxtail millet and green foxtail, multiple sequenced diversity lines for population genetics, and genome-wide association studies, a renewed interest in creating mapping populations and mutant collections, and efficient

transformation techniques, including the promise of a spike dip protocol analogous to the floral dip protocol for transformation that revolutionized *Arabidopsis* genetic research. High-throughput sequencing (HTS) has been an important component in the development of several of these resources, including genome by sequencing and whole-genome sequencing of diversity lines, and rapid identification of candidate loci underlying quantitative trait loci (QTL) and mutant phenotypes. Coupled with HTS, new gene editing techniques are allowing rapid and efficient reverse genetic approaches, including testing candidate loci generated from GWAS, QTL mapping, and fine mapping in mutant populations.

An important aspect of the Setaria system is that it is part of a larger set of genetic model species in the grasses that allow inferences about gene and genome evolution that is simply not possible in any other family. These models include rice, maize, sorghum, and *Brachypodium* (*B. distachyon* and *B. stacei*), a pooid C_3 model grass much like Setaria in its small size and ease of use. In addition, there is the tetraploid genome of switchgrass (*Panicum virgatum*), the diploid genome of its close relative *Panicum hallii*, and multiple draft genomes in progress in other species. These genomes span the vast majority of grass diversity and allow unparalleled opportunities for investigating genome evolution and the genetic basis of morphological and physiological evolution. In addition, grass synteny allows basic research in these model systems to be quickly and efficiently translated into agronomically important crops such as maize, rice, and wheat.

Setaria is unique among these grass model systems because it encompasses both an important domesticated cereal and an emerging model system. The drought hardiness of Setaria makes it an attractive crop in parts of China, India, and Africa and an alternative to other cereals such as pearl millet and sorghum. The use of Setaria as a model system to understand drought stress will rapidly be translated into both better Setaria varieties and the possibility of better drought-hardy cereals in general. The small size and ease of genetic analysis in Setaria also make it the model of choice for understanding the genetics and physiology of C_4 photosynthesis, and Setaria is a key tool in the grand challenge of converting C_3 photosynthetic grasses like rice into highly efficient C_4 cereals to feed an ever-growing human population.

Finally, a note on nomenclature. Setaria is used in this volume to denote the Setaria system (both *Setaria italica* and its wild progenitor *Setaria viridis*), written without italicization and starting with a capital letter. The two species names are written in italics as is normal for Latin binomials. In addition, *S. viridis* has been referred to in past literature as both green millet and green foxtail, but we advocate the use of green foxtail as its correct common name, as it is not a millet cereal grain.

Stillwater, OK — Andrew Doust
Haidian, Beijing, P.R. China — Xianmin Diao

Contents

Contributors

Beverly Jose Agtuca Divisions of Plant Science and Biochemistry, Christopher S. Bond Life Sciences Center, University of Missouri, Columbia, MO, USA

Fernanda Plucani do Amaral Divisions of Plant Science and Biochemistry, Christopher S. Bond Life Sciences Center, University of Missouri, Columbia, MO, USA

Jeffrey L. Bennetzen Department of Genetics, University of Georgia, Athens, GA, USA

Eduardo Blumwald Department of Plant Sciences, University of California, Davis, CA, USA

Christopher W. Brown University of Newcastle, Newcastle, NSW, Australia

Thomas P. Brutnell Donald Danforth Plant Science Center, St Louis, MO, USA

Caitlin S. Byrt School of Agriculture, Food and Wine, Waite Research Institute, University of Adelaide, Urrbrae, SA, Australia

Carla Coelho Donald Danforth Plant Science Center, St Louis, MO, USA

Henri Darmency Agroécologie, AgroSup Dijon, INRA, Univ. Bourgogne Franche-Comté, Dijon, France

Christophe Délye Agroécologie, AgroSup Dijon, INRA, Univ. Bourgogne Franche-Comté, Dijon, France

Katrien M. Devos Institute of Plant Breeding, Genetics and Genomics (Dept. of Crop and Soil Sciences), and Dept. of Plant Biology, University of Georgia, Athens, GA, USA

Xianmin Diao Institute of Crop Sciences, Chinese Academy of Agricultural Sciences, Beijing, People's Republic of China

José R. Dinneny Department of Plant Biology, Carnegie Institution for Science, Stanford, CA, USA

Andrew N. Doust Department of Plant Biology, Ecology, and Evolution, Oklahoma State University, Stillwater, OK, USA

Joyce Van Eck Boyce Thompson Institute, Ithaca, NY, USA

Maximillian Feldman Donald Danforth Plant Science Center, St. Louis, MO, USA

Kenji Fukunaga Prefectural University of Hiroshima, Shobara, Japan

Robert T. Furbank Australian Research Council Centre of Excellence for Translational Photosynthesis, Plant Science Division, Research School of Biology, The Australian National University, Acton, ACT, Australia

Christopher P. L. Grof University of Newcastle, Newcastle, NSW, Australia

John G. Hodge Department of Plant Biology, Ecology, and Evolution, Oklahoma State University, Stillwater, OK, USA

Pu Huang Donald Danforth Plant Science Center, St Louis, MO, USA

Guanqing Jia Institute of Crop Sciences, Chinese Academy of Agricultural Sciences, Beijing, People's Republic of China

Hui Jiang Donald Danforth Plant Science Center, St. Louis, MO, USA

Elizabeth A. Kellogg Donald Danforth Plant Science Center, St. Louis, MO, USA

Kazuhiro Kikuchi Donald Danforth Plant Science Center, St. Louis, MO, USA

Christopher J. Lambrides School of Agriculture and Food Sciences, The University of Queensland, St Lucia, QLD, Australia

Anthony P. Martin University of Newcastle, Newcastle, NSW, Australia

Kimberly Maxson-Stein Donald Danforth Plant Science Center, St. Louis, MO, USA

Mehanathan Muthamilarasan Plant Molecular Genetics and Genomics, National Institute of Plant Genome Research (NIPGR), New Delhi, Delhi, India

Duc Q. Nguyen University of Newcastle, Newcastle, NSW, Australia

William M. Palmer University of Newcastle, Newcastle, NSW, Australia

Minkyu Park Department of Genetics, University of Georgia, Athens, GA, USA

Kaitlin Pidgeon Boyce Thompson Institute, Ithaca, NY, USA

Manoj Prasad Plant Molecular Genetics and Genomics, National Institute of Plant Genome Research (NIPGR), New Delhi, Delhi, India

Peng Qi Institute of Plant Breeding, Genetics and Genomics (Dept. of Crop and Soil Sciences), and Dept. of Plant Biology, University of Georgia, Athens, GA, USA

Prasenjit Saha Department of Plant Sciences, University of California, Davis, CA, USA

Jose Sebastian Department of Plant Biology, Carnegie Institution for Science, Stanford, CA, USA

Christine Shyu Donald Danforth Plant Science Center, St. Louis, MO, USA

Gary Stacey Divisions of Plant Science and Biochemistry, Christopher S. Bond Life Sciences Center, University of Missouri, Columbia, MO, USA

Kerry Swartwood Boyce Thompson Institute, Ithaca, NY, USA

Hao Wang Department of Genetics, University of Georgia, Athens, GA, USA

TianYu Wang Institute of Crop Sciences, Chinese Academy of Agriculture Sciences, Beijing, China

Xiaomei Wu Institute of Plant Breeding, Genetics and Genomics (Dept. of Crop and Soil Sciences), and Dept. of Plant Biology, University of Georgia, Athens, GA, USA

Center for Plant Environmental Sensing, and College of Life and Environmental Sciences, Hangzhou Normal University, Hangzhou, China

Hongye Zhou Department of Genetics, University of Georgia, Athens, GA, USA

Editors Biographies

Andrew Doust is an Associate Professor of Plant Biology in the Department of Plant Biology, Ecology, and Evolution at Oklahoma State University. After obtaining his Ph.D. from the University of Melbourne, Australia, he served as a postdoctoral fellow both at the University of Missouri-St. Louis and the University of Florida. His general interests revolve around the genetic regulation of development and how that changes over evolutionary time, with a particular concentration on domestication in grasses.

Xianmin Diao is a Professor of Plant Genetics in the Institute of Crop Sciences at the Chinese Academy of Agricultural Studies. He holds a Ph.D. in plant physiology from the Institute of Botany at the Chinese Academy of Sciences and formerly served as a visiting scholar at the University of California, Berkeley. As the leading scientist for the foxtail and proso millet section of China's agricultural research system, he runs a lab focused on foxtail millet germplasm management, breeding, genetics, and genomics.

Part I
Evolution

Chapter 1
Evolution of *Setaria*

Elizabeth A. Kellogg

Abstract The genus *Setaria* includes almost 100 species of panicoid grasses. Within the subfamily Panicoideae it falls in the tribe Paniceae, subtribe Cenchrinae. Members of the subtribe are characterized by the presence of sterile branches in the inflorescence, often known as "bristles." Major clades of *Setaria* are geographically localized, with the African species falling in a distinct clade from the South American ones, which are in turn distinct from the Asian ones. Many species have become weedy and are distributed widely in warm areas throughout the world. Nearly all members of the genus share a chromosome base number of $x = 9$, similar to most other members of Paniceae, and polyploidy is common, with some species including tetraploid, hexaploid, and octoploid members. The crop species *S. italica* was domesticated from the weed *S. viridis*, and both share a genome designated as A. A second diploid weed, *S. adhaerens*, is genomically distinct, with a genome designated as B. The tetraploid *S. verticillata* includes both A and B genomes, while tetraploid *S. faberi* appears to be derived from two A-like ancestors.

Keywords *Setaria* • Paniceae • Panicoideae • Poaceae • Polyploidy • Bristle clade

1.1 Relationships of *Setaria* to Other Monocots

The genus *Setaria* is a member of subtribe Cenchrinae, tribe Paniceae, subfamily Panicoideae, family Poaceae, order Poales, in the commelinid clade of monocots (Soreng et al. 2015; Kellogg 2015). Because *Setaria* inherits the characters of each of these larger clades, we will consider each in turn, progressing from the most to the least inclusive.

E.A. Kellogg, Ph.D. (✉)
Donald Danforth Plant Science Center, 975 North Warson Road, St. Louis, MO 63132, USA
e-mail: ekellogg@danforthcenter.org

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1.1.1 Characteristics of Setaria Shared with the Commelinid Clade, Poales, and Poaceae

1.1.1.1 Commelinids

The commelinid clade includes Poales, Zingiberales (gingers and bananas), and Commelinales (spiderworts and their relatives). All members of this clade have endosperm that is well developed and persists in the seed (Stevens 2012). In addition, the commelinid monocots have unique cell walls. The hemicelluloses in the primary walls are largely arabinoxylans, specifically glucuronoarabinoxylans (Carpita 1996; Withers et al. 2012); the latter appear to be unique to the commelinids. The amount of pectin and protein is fairly low in comparison to eudicots (Carpita 1996). In the secondary walls, major lignin subunits are *p*-coumaric and ferulic acid (Harris and Hartley 1980; Harris and Tretheway 2009). Much of the information on the biochemistry of the commelinid secondary wall comes from grasses (Withers et al. 2012; Petrik et al. 2014; Molinari et al. 2013) but is presumed to apply to all commelinids. All commelinids accumulate silica in their leaves (Stevens 2012). Thus, *Setaria* could be a useful model for studies of endosperm, cell walls, and silica accumulation, with the results of such studies applying not only to grasses, but to other members of the commelinid clade.

1.1.1.2 Poales

The Poales in its current broad sense includes 16 families (Angiosperm Phylogeny Group 2009; Givnish et al. 2010), all of which accumulate silica specifically in the leaf epidermis (Stevens 2012). Silica accumulation protects the plant from pathogenic bacteria and fungi (Isa et al. 2010; Ma and Yamaji 2006), and also appears to reduce insect herbivory (Massey and Hartley 2009; Garbuzov et al. 2011). In addition, deposition of silica provides structural support, reduces the uptake of toxic metals, and regulates water loss (Isa et al. 2010; Ma and Yamaji 2006). One popular theory suggested that production of silica was selected as a defense against mammalian grazers because it would wear down their teeth (Simpson 1951; Baker et al. 1959). However, this idea is not supported by data (Sanson et al. 2007; Strömberg 2006).

Endosperm development in all Poales is unique among angiosperms, with multiple rounds of nuclear division before cell walls form (nuclear endosperm) (Stevens 2012). Few other morphological characters are shared by members of Poales, although many are wind pollinated, often occur in nutrient-poor habitats, and are often fire adapted (Linder and Rudall 2005). Flowers generally occur in tiny clusters called “spikelets” in both Cyperaceae and Poaceae, but the structure of these is quite different and nonhomologous in the two families.

Within Poales, Poaceae fall into the graminid clade, a well-supported group that also includes Flagellariaceae, Restionaceae (which now includes members of the former Centrolepidaceae), Anarthriaceae, Joinvilleaceae, and Ecdeiocoleaceae.

Members of the graminid clade have monoporate pollen, with a raised ring or annulus around the pore (Stevens 2012), a character that is retained in *Setaria*. The functional significance of this pollen form is unknown. Nearly all graminids have two-ranked (distichous) sheathing leaves. The endothecium of the anther has girdle-like thickenings. Stigmas are generally plumose, with receptive cells on multicellular branches. The graminids also all share the ability to produce flavones.

The immediate sister group of Poaceae is uncertain. Possible candidates are *Joinvillea*, the sole genus in Joinvilleaceae, or Ecdeiocoleaceae, a family with two genera, *Ecdeiocolea* and *Georgeantha*. Current data suggest that the two families are sisters, and that the clade is then sister to Poaceae (McKain et al. 2016). Members of both families have conventional monocot flowers with two whorls of perianth parts, and thus their structure sheds little light on the homologies of the grass spikelet (but see (Preston et al. 2009; Kellogg 2015)). Like *Setaria* and other grasses, both Joinvilleaceae and Ecdeiocoleaceae have dumbbell-shaped stomatal guard cells. This guard cell shape is thought to enhance the speed of pore opening (Haworth et al. 2011; Franks and Farquhar 2007). Also Joinvilleaceae shares with the grasses the pattern of alternating long and short cells in the epidermis (Campbell and Kellogg 1987). As with many such morphological characteristics, the genetic controls and functional significance of this character are unknown.

1.1.1.3 Poaceae

Poaceae, or Gramineae (both names are correct), is the most speciose of the families in Poales. It includes ca. 12,000 species (Clayton et al. 2006 onwards; Kellogg 2015) and is clearly monophyletic (Kellogg and Campbell 1987; Kellogg and Linder 1995; Vicentini et al. 2008; GPWG 2001; GPWG II 2012).

The grasses all share a distinctive embryo and fruit (GPWG 2001; Kellogg 2015). The seed coat is generally fused to the inner epidermis of the pericarp, forming a single seeded fruit or caryopsis (Fig. 1.1a). Unlike all other commelinid monocots (and indeed most monocots), the grass embryo is highly differentiated (Kellogg 2000; Campbell and Kellogg 1987; Rudall et al. 2005), with a well-developed shoot apical meristem surrounded by a sheath-like structure, the coleoptile, and bearing two or more leaves (Sylvester et al. 2001) (Fig. 1.1a). The root apical meristem is also differentiated and surrounded by a coleorhiza. Attached to the embryo is a large shield-shaped haustorial organ, the scutellum. Together the coleoptile and scutellum appear to represent the sheath and blade, respectively, of a highly modified cotyledon (Takacs et al. 2012).

Also characteristic of Poaceae is the formation of tiny trichomes (microhairs) from the short cells of the leaf epidermis. Microhairs are two-celled, with an elongate apical cell (Johnston and Watson 1976). The functional significance of microhairs is unknown, although it is possible that they could be secretory in some instances. While the ability to produce microhairs is clearly ancestral in the grasses (GPWG 2001) and occurs in *Setaria* as well as all other panicoid species, the cool-season grasses in subfamily Pooideae do not produce them so they are not universal in grasses.

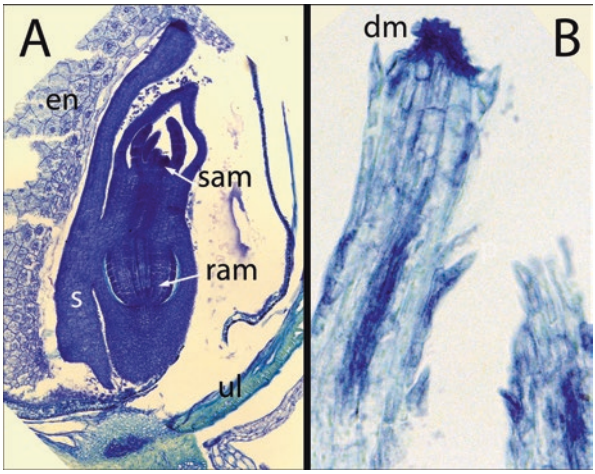

Fig. 1.1 (**a**) Embryo of *Setaria viridis*. As in all other grasses, the *Setaria* embryo has well-developed shoot and root meristems and a clear scutellum. The deep cleft between the base of the scutellum and the coleorhiza is common in most grasses except for the Pooideae. en, endosperm; sam, shoot apical meristem; ram, root apical meristem; s, scutellum. (**b**) Apex of young bristle showing collapsed cells. Bristles appear to lose their meristems early in development. dm, degenerating meristem. Photos by John G. Hodge

Poaceae genomes have been studied extensively because genomic information is so essential for breeding efforts. A whole genome duplication occurred in the common ancestor of all grasses, so that many loci are retained in duplicate (Goff et al. 2002; Yu et al. 2002; Paterson et al. 2004, 2009; McKain et al. 2016).

Poaceae is divided into 12 subfamilies (Fig. 1.2) (Kellogg 2015; Soreng et al. 2015). The ones that diverged early in the evolution of the family include only a handful of species (Anomochlooideae, four species, Pharoideae, 12, and Puelioideae, 11) (Kellogg 2015). The vast majority of species fall in the BOP and PACMAD clades, the names of which are acronyms for the included subfamilies. BOP includes Bambusoideae, Oryzoideae, and Pooideae, whereas PACMAD includes Panicoideae, Arundinoideae, Chloridoideae, Micrairoideae, Aristidoideae, and Danthonioideae.

The grass spikelet, which is a tiny spike delimited by two bracts (glumes) and with one or more flowers, characterizes all grasses except Anomochlooideae. The precise timing of origin of the spikelet, however, is unclear. Either the spikelet was present in the common ancestor of all grasses and was then highly modified in Anomochlooideae, or the spikelet originated in the common ancestor of Pharoideae and all remaining grasses (GPWG 2001; Preston et al. 2009; Kellogg 2015) (node 1, Fig. 1.2). In either case, *Setaria* is like all but about four species of grasses in having flowers borne in spikelets.

Other widespread aspects of grasses characterize Puelioideae plus the BOP+PACMAD clades (i.e., descendants of node 2, Fig. 1.2), and not

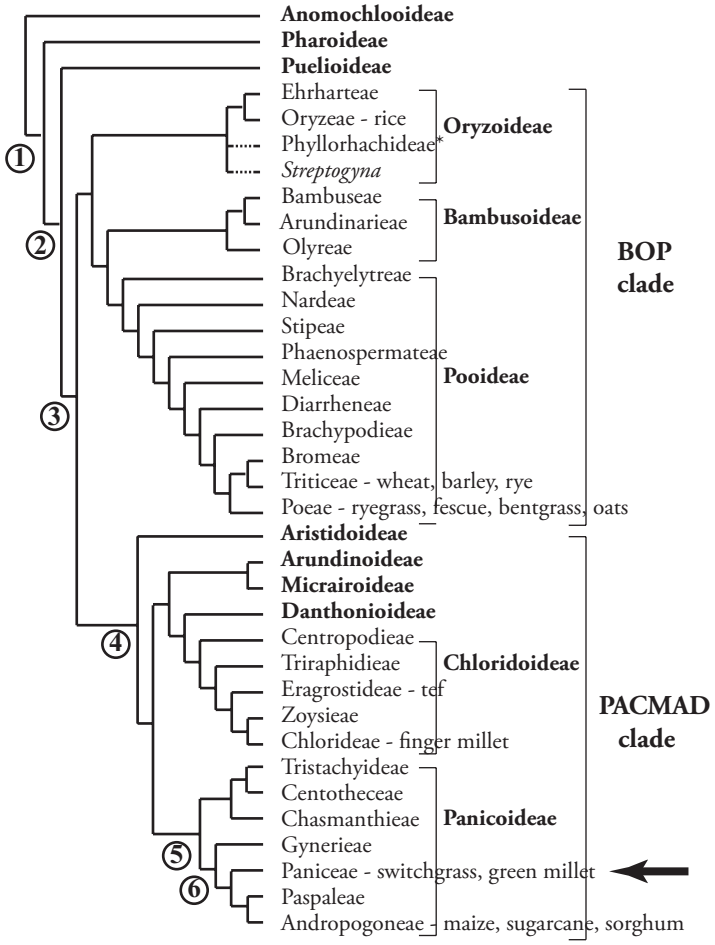

Fig. 1.2 Phylogeny of the grasses, based largely on GPWG II (2012) and redrawn from Kellogg (2015). Arrow points to subfamily Panicoideae, tribe Paniceae, which includes *Setaria*

Anomochlooideae or Pharoideae. For example, style branches and stigmas are reduced to two in this group (although the character reverses in some taxa), and the stigmas have two orders of branching (GPWG 2001). Spikelets each have multiple flowers (another character that reverses frequently). Anther walls have a middle layer that breaks down during development, and the inner walls of the endothecial cells become fibrous at maturity.

The female gametophyte in the grasses is fairly conventional in early development, with an egg, two synergids, a binucleate central cell, and antipodal cells. However, in all investigated species other than the early diverging genera *Streptochaeta* and *Pharus* (Sajo et al. 2007, 2008), the antipodals continue to divide (Anton and Cocucci 1984; Evans and Grossniklaus 2009; Shadowsky 1926). The function of these extra divisions is unknown.

The BOP+PACMAD clade (descendants of node 3, Fig. 1.2) has no obvious morphological synapomorphy. The Grass Phylogeny Working Group (GPWG 2001) suggested that lack of a pseudopetiole in the leaves, reduction of lodicule number to 2 and stamen number to 3 might be synapomorphic. Although these characters reverse in a number of lineages, they all characterize *Setaria*.

Members of the PACMAD clade (node 4, Fig. 1.2) have nothing obvious in common. They are thought to share an elongated mesocotyl internode in the embryo, but relatively few species have actually been investigated for this character and it is unclear how reliable or consistent it is (GPWG 2001). The clade also includes all 24 origins of the C_4 photosynthetic pathway in grasses (GPWG II 2012).

1.1.2 Characteristics of Setaria Shared with Subfamily Panicoideae, Tribe Paniceae, and Subtribe Cenchrinae

1.1.2.1 Panicoideae

Nearly 1/3 of the species of Poaceae are in subfamily Panicoideae. Panicoideae s.s. (node 6, Fig. 1.2) was one of the earliest subfamilies to be recognized as distinct. In 1810, Robert Brown noted that the group (which he called Paniceae) mostly has spikelets with exactly two flowers, with the upper one bisexual and the lower one staminate or sterile (Brown 1810, 1814). Recent phylogenetic work has shown that Panicoideae s.s. is part of a larger clade which now bears the name Panicoideae (node 5, Fig. 1.2), in which the spikelet morphology is more variable (Sánchez-Ken and Clark 2007, 2010).

Spikelets in Panicoideae s.s. are dorsiventrally compressed. The glumes and lemmas are generally not folded and are borne ab- and adaxially in relation to the spikelet-bearing axis. This pattern of compression contrasts with that of most other grasses such as rice, tef, and *Brachypodium*, in which the glumes and lemma are both folded along the midrib, a pattern known as lateral compression. In these taxa, the glumes and lemmas initiate at right angles to the spikelet-bearing axis. As with many such morphological characters, the significance of this highly consistent difference is unknown.

Spikelet development in the panicoids is basipetal, with the distal flower maturing before the proximal one (Bess et al. 2005; Doust and Kellogg 2002; Malcomber and Kellogg 2004). This pattern is similar to that in rice, but distinct from what is found in Pooideae, Chloridoideae, and other major groups.

Silica bodies in the leaf epidermis of panicoid grasses are generally bilobed in surface view and symmetrical in cross section (Piperno 2006; Piperno and Pearsall 1998). Nothing is known about deposition of silica in the grass epidermis and the mechanism by which silica body shape is defined.

Early phylogenetic work in subfamily Panicoideae found that the phylogeny reflected chromosome numbers rather than photosynthetic pathway as had been thought

previously (Gómez-Martinez and Culham 2000; Aliscioni et al. 2003; Giussani et al. 2001). The ancestral base chromosome number of the subfamily is unknown but most likely to be 11 or 12, and the number was then reduced in the common ancestor of Panicoideae s.s. One descendant of this ancestor acquired a base number of $x=9$, a number that now characterizes the tribe Paniceae, whereas the other descendant acquired a number of $x=10$, which is shared by the tribes Andropogoneae and Paspaleae.

1.1.2.2 Paniceae

Within Paniceae, major clades are strongly supported by both nuclear, chloroplast, and mitochondrial sequences (Vicentini et al. 2008; GPWG II 2012; Washburn et al. 2015). All analyses to date have identified clades corresponding to subtribes Cenchrinae, Melinidinae, Panicinae, Boivinellinae, Neurachninae, and Anthephorinae (Fig. 1.3). The genus *Dichanthelium* is monophyletic and could be placed in its own subtribe (Dichantheliinae (Soreng et al. 2015)). In addition, there is an unnamed clade made up of the genera *Sacciolepis*, *Trichanthecium*, and *Kellochoa* plus a number of species formerly placed in *Panicum* (node 4, Fig. 1.3) (Morrone et al. 2012; GPWG II 2012; Zuloaga et al. 2011; Nicola et al. 2015).

Cenchrinae, Melinidinae, and Panicinae form a robust group (the MPC clade) (Morrone et al. 2012; GPWG II 2012) (node 2, Fig. 1.3), with Cenchrinae and Melinidinae sisters (node 3, Fig. 1.3 (Washburn et al. 2015)). The clade was first identified as the "C_4 three subtypes" clade by Giussani et al. (2001) because all members are C_4, but each subtribe exhibits a different subtype of the C_4 pathway. Cenchrinae includes species that are NADP-ME subtype, Melinidinae members are PCK, and Panicinae are NAD-ME. Each C_4 subtype has characteristic leaf anatomy (Hattersley 1987; Hattersley and Watson 1992; Prendergast and Hattersley 1987; Prendergast et al. 1987). In Panicinae and Melinidinae, each vein is surrounded by an inner sheath of thick walled cells, the mestome sheath, and an outer sheath of parenchymatous cells. Carbon reduc-

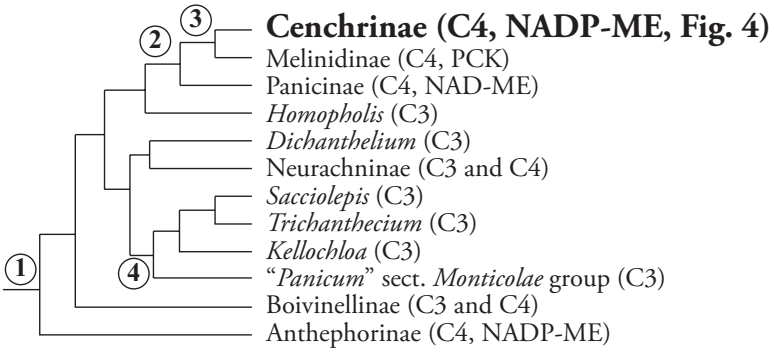

Fig. 1.3 Phylogeny of Paniceae based on chloroplast, mitochondrial, and nuclear rDNA gene sequences (Washburn et al. 2015; Nicola et al. 2015). The clade made up of Cenchrinae, Melinidinae, and Panicinae is found in all molecular phylogenies. Other relationships, particularly those surrounding node 1, are contradicted by other gene trees

tion occurs in the latter. In Cenchrinae, and thus in *Setaria*, as in most NADP-ME grasses, veins are surrounded by a single sheath, a derived condition in the subtribe.

Relationships among the other clades of Paniceae are unclear. Chloroplast data place *Dichanthelium* as sister to the MPC clade, whereas nuclear data place it sister to all other Paniceae (Vicentini et al. 2008) and combined chloroplast, mitochondrial, and nuclear RNA data place it sister to Neurachninae (Washburn et al. 2015). Conversely, Anthephorinae is placed sister to all Paniceae by chloroplast data (node 1, Fig. 1.3), but sister to the MPC clade by nuclear genes.

1.1.2.3 Cenchrinae

The Cenchrinae is also known as the "bristle clade," because almost all members of the clade form sterile branches ("bristles") in the inflorescence. These sterile branches originate as ordinary branches, but instead of forming spikelets, they grow out and terminate blindly (Doust and Kellogg 2002). The bristles may form a meristem at their apex, but this often simply aborts, leaving a small collapsed set of cells (Fig. 1.1b). Bristles may be restricted to the ends of branches, or a bristle may be paired with each spikelet, or individual spikelets may be surrounded by an involucre of bristles. In the latter case, the bristles may be terete or flattened.

Two species, *Zuloagaea bulbosa* and "*Panicum*" *antidotale*, lack bristles. (The latter species is unrelated to true *Panicum*, which is in Panicinae, but has not yet been transferred to another genus.) Developmental studies in *Zuloagaea* show that early development in that species is strikingly similar to that of *Panicum miliaceum* (*Panicum* s.s.) and there is no evidence of bristle formation at any point in development (Bess et al. 2005, 2006).

The phylogeny of the group is poorly resolved largely because no one has yet investigated it using a sufficient number of markers. Nonetheless, a few strong clades can be identified. The *Cenchrus* clade (*Cenchrus* sensu lato, Fig. 1.4) includes both *Cenchrus* and the former genus *Pennisetum*, plus the monotypic *Odontelytrum* (Chemisquy et al. 2010; Donadio et al. 2009; Morrone et al. 2012). All species of *Cenchrus* s.l. form an abscission zone at the base of the primary branch such that the spikelets fall from the plant surrounded by an involucre of bristles. Developmentally, the species are also distinct because the primary branch enlarges isodiametrically, rather than growing primarily in a proximo-distal direction (Doust & Kellogg 2002). While many species of *Cenchrus* s.s. are easily identified by their flattened bristles forming an involucre around the spikelets, others intergrade morphologically with the former *Pennisetum*. Thus, the boundary between the genera is not sharp, consistent with the pattern found in phylogenetic studies (Donadio et al. 2009; Chemisquy et al. 2010).

Primary branches of the inflorescence are spirally arranged in most species, whereas the secondaries and higher order branches are distichous (Bess et al. 2005; Doust and Kellogg 2002; Kellogg et al. 2004, 2013).

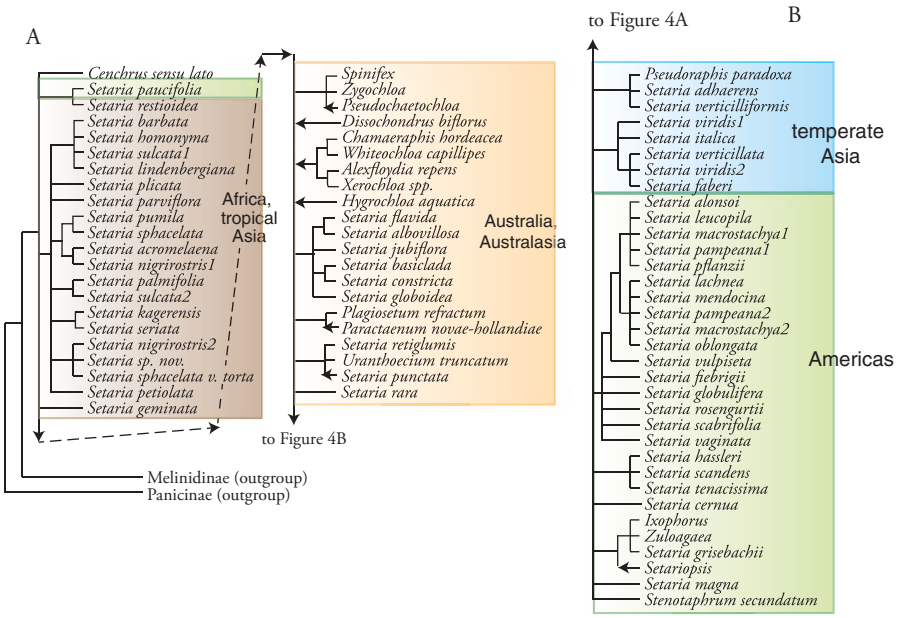

Fig. 1.4 Phylogeny of Cenchrinae based on the chloroplast gene *ndhF*, focusing on species in *Setaria*. Redrawn from Kellogg et al. (2009). *Arrows* show the approximate placement of additional taxa included in the study of GPWG II (2012). All branches shown have either parsimony or maximum likelihood bootstrap values >80, or Bayesian posterior probability >0.95, or both. *Brown* box indicates taxa native to Africa or tropical Asia, *yellow* is Australia and Australasia, *blue* is temperate Asia and *green* is the Americas, mostly South and Central America

1.2 Relationships Within the Genus *Setaria*

1.2.1 Phylogeny and Characteristics of the Genus

Species of *Setaria* are described in detail in two monographs, which together cover 99 species (Morrone et al. 2014; Pensiero 1999), although Clayton et al. (2006 onward) lists 103 names. The genus has no unique character and as currently defined is likely to be para- or polyphyletic. The current phylogeny shows a number of well-supported clades corresponding largely to geography, but relationships among them are unresolved, making generic circumscription impossible at the moment (Fig. 1.4). As currently circumscribed, *Setaria* includes the members of Cenchrinae that do not fall in the *Cenchrus* clade, have bisexual spikelets (i.e., are not *Spinifex* or *Zygochloa*), and lack the distinctive characters of the various oligotypic genera such as *Dissochondrus*, *Paractaenum*, or *Plagiosetum*. Almost certainly, *Setaria* will need to be expanded to include some of these elements, but without a solid phylogeny it is hard to find a good rationale for doing so.

Species of *Setaria* occur in warm regions throughout the world, and in diverse habitats (Morrone et al. 2014). Some species, such as *S. sulcata* and *S. palmifolia*, occur in

disturbed areas in moist forest shade. Others, such as *S. nigrirostris* and *S. sphacelata*, are found in damp grasslands, and still others, such as *S. rara* and *S. reflexa*, in dry open habitats. A handful of species, notably *S. viridis* and *S. pumila*, are weedy and have followed human activity to spread far beyond their original distribution.

The number and position of bristles in the inflorescence varies considerably among *Setaria* species (Morrone et al. 2014). In species such as *Setaria palmifolia*, each spikelet is accompanied by a single bristle. In other species, such as *S. parviflora* and *S. viridis*, each mature spikelet is surrounded by multiple bristles. The relationship between the number of spikelets and the number of bristles is developmentally complex however (Doust and Kellogg 2002). Bristle and spikelet identity are specified early in inflorescence development. In some cases, all spikelets develop to maturity so that the number of bristles per spikelet reflects meristem identity decisions. In other species, however, late forming spikelets fail to develop so that high numbers of bristles per spikelet reflect a process of spikelet abortion rather than branch identity specification.

In some species of *Setaria* the primary branches of the inflorescence are themselves unbranched (i.e., the spikelets are borne directly on the primary branches) and the branches end in a sharp bristle-like tip (Morrone et al. 2014). The inflorescence thus looks superficially similar to that in *Paspalum*, but the presence of the terminal bristle is diagnostic; species with this inflorescence morphology have often been placed in a genus *Paspalidium*. However, species occur in which some spikelets are associated with bristles in addition to the one at the branch tip and thus the morphology intergrades with that of *Setaria* sensu stricto. Recognizing this morphological intermediacy, all *Paspalidium* species have been transferred to *Setaria* (Veldkamp 1994; Webster 1993, 1995), and the transfer has been supported by phylogenetic data (Morrone et al. 2012; Kellogg et al. 2009; GPWG II 2012).

The species of *Setaria* vary widely in inflorescence architecture and leaf form (Morrone et al. 2014). Inflorescences may be narrow with short stiff lateral branches (the inflorescence thus shaped like a bottle brush, e.g., *S. pumila*, *S. sphacelata*, *S. nigrirostris*), broad and lax with spreading branches (shaped like a Christmas tree, e.g., *S. grandis*, *S. sulcata*, *S. lindenbergiana*), or sparse with few spreading primary branches (like an antenna, e.g., *S. jubiflora*, *S. flavida*). Each primary branch may produce spikelets directly (e.g., *S. jubiflora*, *S. flavida*, *S. rara*) or may rebranch up to six times (e.g., *S. parviflora*, *S. pumila*). Plants may be annual (e.g., *S. faberi*, *S. acromelaena*, *S. sagittifolia*, *S. viridis*) or perennial (most species), and may be a few cm (e.g., some specimens of *S. clementii*, *S. ustilata*) to over 1 m (e.g., *S. grandis*) tall. Spikelets are generally ovate but sometimes may be elongate or orbicular. Leaves are generally flat, but some species (e.g., *S. sulcata*, *S. palmifolia*) have striking folded leaves. The latter were once placed in their own section because the leaf morphology is so distinctive, but they do not form a clade in molecular phylogenies. Sagittate leaves are found in *S. sagittifolia* and *S. appendiculata*.

As in all groups of grasses, polyploids are common (Table 1.1). Except for polyploids involving *Setaria viridis* (see next section) the history of few of these has been disentangled, although it would be straightforward to do so using low-copy nuclear genes. Sequences of the nuclear gene *Knotted1* have shown that *S. flavida*

Table 1.1 Published chromosome numbers for species of *Setaria*

Species	Origin	*n*	Reference	2*n*	Reference
adhaerens	Asia	9	Gupta and Singh (1977)		
apiculata	Australia			36	Le Thierry d'Ennequin et al. (1998)[a]
barbata	Africa	18	Olorode (1975)	54	Gadella (1977)
		27	Christopher and Abraham (1976) and Dujardin (1978)	56	Sarkar et al. (1976)
		28	Sarkar et al. (1976)		
faberi	Asia			36	Probatova and Sokolovskaya (1983) and Warwick et al. (1987, 1997)
fiebrigii	South America	18	Oliveira Freitas-Sacchet (1980) and Oliveira Freitas-Sacchet et al. (1984)	36	Pensiero (1999)
flavida	Australia	18	Bir and Chauhan (1990)	44	Sharma and Sharma (1979)
		27	Mehra (1982), Bir and Sahni (1983) and Nadeem Ahsan et al. (1994)	54	Sinha et al. (1990)
		56	Bir and Chauhan (1990)		
geminata	Africa	9	Rao and Mwasumbi (1981) and Nadeem Ahsan et al. (1994)		
grisebachii	Central and South America			18	Reeder (1971)
homonyma	Africa	10	Singh and Gupta (1977)		
		18	Mehra and Sharma (1975)		

(continued)

Table 1.1 (continued)

Species	Origin	*n*	Reference	2*n*	Reference
italica	Asia	9	Khosla and Sharma (1973), Gupta and Singh (1977), Mehra (1982) and Sinha et al. (1990)	18	Christopher and Abraham (1976), Li and Chen (1985) and Sinha et al. (1990), Chikara and Gupta (1979), Frey et al. (1981), Zhou et al. (1989), Kozuharov and Petrova (1991), Li et al. (1996), Wu and Bai (2000), and Le Thierry d'Ennequin et al. (1998)[a]
				36	Li and Chen (1985)
kagerensis	Africa			18	Lakshmi and Yacob (1978)
lachnea	South America	18	Gupta and Singh (1977)	36	Bowden and Seen (1962), Manero de Zamelzú and Ochoa de Suárez (1991), Pensiero (1999), and Le Thierry d'Ennequin et al. (1998)[a]
leucopila	SW US, Mexico, South America			54, 68, 72	Emery (1957a)
longiseta	Africa	18	(Olorode 1975)	36	
macrostachya	North, Central, and South America	27	Gupta and Singh (1977)	54	Emery (1957b), Pensiero (1999), and Le Thierry d'Ennequin et al. (1998)[a]
				72	Gupta and Singh (1977)
magna	North, Central, and South America			36	Brown (1948)
nigrirostris	Africa	9	Gupta and Singh (1977)	18	Raman et al. (1959) and Le Thierry d'Ennequin et al. (1998)[a]
		18	Spies and duPlessis (1986)	36, 54	Spies and duPlessis (1986), Raman et al. (1959), and Le Thierry d'Ennequin et al. (1998)[a]
		27	Spies and duPlessis (1986)		

(continued)

Table 1.1 (continued)

Species	Origin	*n*	Reference	2*n*	Reference
oblongata	Argentina, Bolivia	18	Tiranti and Genghini (2000)		
palmifolia	Asia, Africa	27	Mehra and Sharma (1975), Mehra (1982) and Christopher and Abraham (1976)	54	Christopher and Abraham (1976)
				36	Le Thierry d'Ennequin et al. (1998)[a]
pampeana	Argentina			ca. 50	Pensiero (1999)
parviflora	North, Central, and South America	18	Gupta and Singh (1977), Oliveira Freitas-Sacchet (1980) and Mehra (1982)	36	Gould and Soderstrom (1967), Pohl and Davidse (1971), Norrmann et al. (1994), and Le Thierry d'Ennequin et al. (1998)[a]
				72	Gould and Soderstrom (1967) and Fernández and Queiróz (1969)
pflanzii	South America			36	Caponio and Pensiero (2002)
plicata	Asia	36	Mehra (1982)		
pumila	Africa, Asia	9, 18+0–2B, 27	Sahni (1989)	36	Sahni (1989), Kozuharov and Petrova (1991), Baltisberger (1988), Devesa et al. (1991), and Singh and Godward (1960)
		36	Sahni (1989) and Nadeem Ahsan et al. (1994)	54	Le Thierry d'Ennequin et al. (1998)[a]
restioidea	Africa	18	Dujardin (1978)		
rosengurtii	South America	54	Oliveira Freitas-Sacchet et al. (1984) and Oliveira Freitas-Sacchet (1980)		

(continued)

Table 1.1 (continued)

Species	Origin	*n*	Reference	2*n*	Reference
scabrifolia	South America	18	Oliveira Freitas-Sacchet et al. (1984) and Oliveira Freitas-Sacchet (1980)		
sphacelata	Africa	9	Gupta and Singh (1977), Dujardin (1978) and Rao and Mwasumbi (1981)	18	deWet (1958)
		18	Gupta and Singh (1977), Bir and Sahni (1986, 1987) and Sahni (1989)	36	deWet (1954), deWet and Anderson (1956), and Le Thierry d'Ennequin et al. (1998)[a]
		27	Gupta and Singh (1977)	54	Gupta and Singh (1977) and deWet (1958)
sulcata	Central and South America			18	Gupta and Singh (1977)
		16	Oliveira Freitas-Sacchet et al. (1984) and Oliveira Freitas-Sacchet (1980)	32	Oliveira Freitas-Sacchet (1980)
		18	Olorode (1975) and Dujardin (1978)	36	Quarín (1977), deWet and Anderson (1956), and Le Thierry d'Ennequin et al. (1998)[a]
				54	Moffett and Hurcombe (1949)
tenacissima	Central and South America			36	Sede et al. (2010)
vaginata	South America	18	Oliveira Freitas-Sacchet et al. (1984) and Oliveira Freitas-Sacchet (1980)		

(continued)

Table 1.1 (continued)

Species	Origin	*n*	Reference	2*n*	Reference
verticillata	Eurasia	9	Christopher and Abraham (1976)[b]	18	deWet (1954) and Wu and Bai (2000)[b]
		18	Gupta and Singh (1977), Sahni (1989), Bir and Sahni (1986) and Bala and Sachdeva (1989, 1990)	36	Váchová and Feráková (1980)
		27	Christopher and Abraham (1976), Gupta and Singh (1977), Mehra (1982), Sahni (1989), Bir and Sahni (1983), Sahni and Bir (1985), Bala and Sachdeva (1989, 1990) and Sinha et al. (1990)	54	Khosla and Sharma (1973), Gupta and Singh (1977), and Sinha et al. (1990)
		36, 54	Sahni (1989) and Bir and Sahni (1986)		
viridis	Asia	9	Christopher and Abraham (1976), Gupta and Singh (1977) and Koul and Gohil (1988, 1991)	18	Khosla and Sharma (1973), Chopanov and Yurtsev (1976), Magulaev (1976), Váchová (1978), Kliphuis and Wieffering (1979), Belaeva and Siplivinsky (1981), Löve and Löve (1981), Guzik (1984), Li and Chen (1985), Kozuharov and Petrova (1991), Xu et al. (1992), Moffett and Hurcombe (1949), Le Thierry d'Ennequin et al. (1998),[a] and (Layton and Kellogg 2014)[a]
		18	Löve and Löve (1981), Saxena and Gupta (1969) and Mulligan (1984)		

(continued)

Table 1.1 (continued)

Species	Origin	*n*	Reference	2*n*	Reference
vulpiseta	Central and South America	9	Sede et al. (2010)	36	Pensiero (1999)
		18	Oliveira Freitas-Sacchet et al. (1984) and Oliveira Freitas-Sacchet (1980)	54	Norrmann et al. (1994)

Table reproduced and updated from Kellogg et al. (2009). Names of species follow Morrone et al. (2014) and Pensiero (1999) and have been updated from those in the original publications
[a]Estimated by flow cytometry
[b]Likely misidentified specimens of *S. adhaerens*

and *S. jubiflora* are the products of a single polyploidization event (Doust et al. 2007). One of the genomes that produced the tetraploid ancestor of the two species also appears to be shared with *S. grisebachii* (diploid) and also with *Stenotaphrum secundatum* (a presumed diploid), *Ixophorus unisetus* (tetraploid), and *Zuloagaea bulbosa* (tetraploid). The origin of the other genome is less clear.

A handful of published chromosome counts appear to have a base number other than $x=9$ (Table 1.1). One accession of *S. homonyma* is reported to have $n=10$ (Singh and Gupta 1977), one accession of *S. sphacelata* is apparently $n=18+1B$ (Dujardin 1978); chromosome spreads are illustrated in both papers, and the counts appear accurate, although Singh and Gupta acknowledge that the *S. homonyma* count might be 9+1B. An accession of *S. sulcata* may have $n=16$ and $2n=32$ (Oliveira Freitas-Sacchet et al. 1984; Oliveira Freitas-Sacchet 1980), but the count is not documented photographically.

1.2.2 The Temperate Asian Clade

The annual species *Setaria italica*, *S. viridis*, *S. faberi*, and *S. verticillata* form a clade in chloroplast phylogenies (Kellogg et al. 2009; Layton and Kellogg 2014) and in phylogenies using the nuclear genes encoding *knotted1* and 5S rDNA (Zhao et al. 2013; Layton and Kellogg 2014; Doust et al. 2007) (Fig. 1.4). *S. viridis* is the wild ancestor of the cultivated species *S. italica*, as documented by data from many sources, and the two remain interfertile (Le Thierry d'Ennequin et al. 2000; Hunt et al. 2008; Hirano et al. 2011; Darmency et al. 1987; Shi et al. 2008; Huang et al. 2014). This relationship is discussed more extensively in the chapters by Jia (Chap. 2), Huang and Feldman (Chap. 3), and Diao and Jia (Chap. 4) in this book.

The diploid genome of *S. italica* was designated as the A genome by Li et al. (1945); diploid *S. viridis* shares the A genome with *S. italica*, as verified by hybrid fertility, and cytogenomic, enzymatic, and molecular markers (Li et al. 1945; Wang

et al. 1998; Benabdelmouna et al. 2001a, b; Darmency and Pernes 1987). The diploid genome of *S. adhaerens* is distinct from that of *S. viridis* and *S. italica* and has been designated as the B genome by Benabdelmouna et al. (2001b); this designation has been confirmed by Wang et al. (2009) and Zhao et al. (2013). In addition, sequence data show that *S. adhaerens* and *S. viridis* are not closely related (Fig. 1.4) (Layton and Kellogg 2014). The diploid genome of *S. grisebachii* from America was identified as genome C due to its poor hybridization signals with both the A genome of *S. viridis* and the B genome of *S. adhaerens* (Wang et al. 2009).

A combination of molecular phylogenetics and cytogenetics has identified the progenitors of several polyploid taxa. Chromosomes of the tetraploid species *S. pumila* (often erroneously known as *S. glauca* (Morrone et al. 2014)) and *S. parviflora* strongly cross-hybridized but no hybridization signal was detected when the chromosomes of these two species were hybridized with probes derived from the known A, B, and C genomes; thus *S. pumila* and *S. parviflora* were designated as having genome D (Zhao et al. 2013). Similarly, the lack of hybridization signals with A, B, C, and D donor genomes led to the recognition of the E genome from *S. palmifolia* and the F genome from *S. arenaria* (Zhao et al. 2013). (Note that *S. arenaria* is a dubious name according to Morrone et al. (2014) and thus it is unclear what material was used by Zhao et al. (2013).) GISH also identified an apparent A genome autotetraploid, *S. apiculata* (=*queenslandica*). Analysis of *kn1* and *5S rDNA* sequences was consistent with the GISH results (Zhao et al. 2013).

The tetraploid *S. faberi* formed from an A genome (*S. viridis*) plus another genome from an unknown source closely related to *S. viridis* (Layton and Kellogg 2014). *S. faberi* is morphologically similar to *S. viridis*, but in the former species the upper glume is slightly shorter, so that the upper 1/4–1/3 of the upper lemma is visible (Layton and Kellogg 2014). In contrast, the upper glume of *S. viridis* completely covers the upper lemma and often slightly overlaps the lower lemma at the apex of the spikelet. *S. faberi* also has macrohairs on the adaxial surface of the leaves, whereas the leaves of *S. viridis* are glabrous. In addition, *S. faberi* is less tolerant of drought than *S. viridis* is and often grows in more mesic habitats such as the margins of cultivated fields, whereas *S. viridis* occurs more frequently in poor soil and cracks in pavement (Layton and Kellogg 2014).

Tetraploids classified as *S. verticillata* and *S. verticilliformis* each have one genome from the diploid *S. adhaerens* and one from *S. viridis* (Benabdelmouna et al. 2001b). Phylogenetic data using the low-copy nuclear marker *knotted1* on the same plant accessions confirmed the cytogenetic results, showing that *S. verticillata* and *S. verticilliformis* each have two loci, consistent with their ploidy level (Layton and Kellogg 2014). One *kn1* locus is related to that of the diploid *S. adhaerens* and the other to that of the diploid S. *viridis*.

The phylogenetic and cytogenetic data settle confusion over the taxonomy of *S. verticillata*, *S. adhaerens*, and *S. verticilliformis*. Some authors have considered the three species as synonymous (Rominger 1962; Doust et al. 2007; Kellogg et al. 2009), others have distinguished *S. verticillata* and *S. verticilliformis* but synonymized *S. adhaerens* with *S. verticillata* (Morrone et al. 2014), and still others maintain all three species as distinct (Rominger 2003). Both *S. verticillata* s.s. and *S. adhaerens* have retrorse prickles on the bristles, a character that is distinctive within