

Foxtail Millet: A Sequence-Driven Grass Model System^[W]

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Foxtail millet (*Setaria italica*) is a small diploid C₄ panicoid crop species, whose genome is being sequenced by the Joint Genome Institute (JGI) of the Department of Energy. The rationale for sequencing foxtail millet is that it is closely related to the bioenergy grasses switchgrass (*Panicum virgatum*), napiergrass (*Pennisetum purpureum*), and pearl millet (*Pennisetum glaucum*), yet is a more tractable experimental model because of its small diploid genome (1C genome size = 490 Mb) and inbreeding nature. This compares to the larger genomes of the outbreeding species pearl millet (diploid, 1C = 2,352 Mb), napiergrass (tetraploid, 1C = 2,254 Mb), and switchgrass (tetraploid, 1C = 1,372–1,666 Mb, octaploid 1C = 2,352–3,136 Mb; Bennett et al., 2000). Switchgrass is largely self-incompatible, which significantly complicates forward or reverse genetics, trait mapping, genetic engineering, and genome sequencing. Foxtail millet is self-compatible and has a highly conserved genome structure relative to the ancestral grass lineage (Devos et al., 1998). Some genetic resources such as genetic maps (Devos et al., 1998; Wang et al., 1998) and a small collection of ESTs (Zhang et al., 2007) are already available for foxtail millet, but most of the tool development and genetic research in foxtail millet will be sequence driven.

Foxtail millet will be useful as an experimental crop to investigate many aspects of plant architecture, genome evolution, and physiology in the bioenergy grasses. Foxtail millet, pearl millet, napiergrass, maize (*Zea mays*), and sorghum (*Sorghum bicolor*) differ from switchgrass in the decarboxylation enzyme used during C₄ photosynthesis (NADP-malic versus NAD-malic; Watson and Dallwitz, 1992), but are otherwise similar in C₄ photosynthesis, making foxtail millet of value in understanding this important trait. Last, but not least, the draft foxtail millet genome will provide an assembly guide for any future switchgrass sequencing project, as ongoing work in switchgrass indicates that there is good colinearity between switchgrass and foxtail millet (C. Tobias, personal communication).

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ORIGIN AND RELATIONSHIPS OF FOXTAIL MILLET

The earliest archeological remains of foxtail millet are found in northern China, in the Cishan and Peiligang ruins in the Yellow River Valley, approximately 7,400 years before present (BP) and 7,935 years BP, respectively (Li and Wu, 1996). A succession of sites in the Yiluo valley of northern China show that foxtail millet was the dominant grain for 4 millennia, before becoming part of the current North China farming tradition of millets, wheat (*Triticum aestivum*), and legume rotations by 3,600 to 3,300 years BP (Lee et al., 2007). Foxtail millet is also known from archeological remains in Europe and the Middle East, but these are younger, dating from around 4,000 years BP (Austin, 2006).

Phylogenetic analyses using both chloroplast and nuclear genes show foxtail and green millet (*Setaria viridis*) as close relatives (Giussani et al., 2001; Doust et al., 2007), consistent with the hypothesis that foxtail millet is a domesticated version of green millet (Li et al., 1944, 1945; de Wet and Harlan, 1975; Wang et al., 1995; Le Thierry d'Ennequin et al., 1999). The two species are part of a larger monophyletic clade of approximately 300 species that all share the characteristic of inflorescences with both spikelets and sterile branches (bristles; Zuloaga et al., 2000). Pearl millet and napiergrass also belong to this group, as do the sand burrs (*Cenchrus* spp.).

Isozyme and DNA analyses of green and foxtail millet have shown a high level of diversity (Wang et al., 1995; Le Thierry d'Ennequin et al., 2000), and revealed that the genetic distance between green and foxtail accessions from the same region are sometimes smaller than the genetic distance between two foxtail millet accessions from different geographical locations. This has been interpreted as supporting multiple domestications of foxtail millet (Kawase and Sakamoto, 1984, 1987; Jusuf and Pernes, 1985; Fukunaga et al., 1997, 2002, 2005, 2006; Li et al., 1998; Nakayama et al., 1998; Le Thierry d'Ennequin et al., 2000; Benabdelmouna et al., 2001; Kawase et al., 2005), followed by episodes of genetic introgression from green millet (Darmency et al., 1987; Wang et al., 1995; Jarvis and Hodgkin, 1999). Green millet is a serious weed, not only of foxtail millet, but of wheat and other temperate cropping systems worldwide (Defelice, 2002; Dekker, 2003).

Foxtail millet and switchgrass last shared a common ancestor about 13 ± 3 million years ago (Vicentini

et al., 2008), about the same degree of divergence as seen between maize and sorghum (Swigonova et al., 2004). This is much less than the approximately 60 million years since the divergence of switchgrass and *Brachypodium*, a grass model species being sequenced by the JGI for bioenergy traits (Garvin et al., 2008; Vicentini et al., 2008). Relative divergence time may be an important consideration when choosing model species for the genetic dissection of traits such as vegetative branching or C_4 photosynthetic traits in bioenergy grasses, as closer phylogenetic relationships may indicate more genetic, genomic, and physiological similarity. However, the very small size and fast generation time of *Brachypodium* make it the grass system of choice for the genetic dissection of traits that are more highly conserved, such as genes involved in cell wall synthesis (Garvin, 2007).

MORPHOLOGY

Foxtail millet has a relatively small stature, with plants of different accessions varying from 20 to 215 cm tall (Reddy et al., 2006). Foxtail millet has a short generation time (depending on the accession, approximately 5–8 weeks from planting to flowering, 8–15 weeks from planting to seed maturity) and can produce hundreds of seeds per inflorescence (Fig. 1). Seed of foxtail millet are generally not dormant and can easily be cultivated in the glasshouse at densities of up to 100 plants/m² or in the field in temperate or tropical regions. A recent review details many of the growing requirements and other biology of foxtail and other millets (Dekker, 2003).

Foxtail millet has a typical domesticated plant architectural form consisting of a single stalk or a few tillers, with large inflorescences that mature more or less at the same time (Fig. 1). Its wild progenitor, green millet, has tillers and axillary branches that appear over the life of the plant, and inflorescences that readily shatter to release the seed at maturity (Defelice, 2002). Segregating populations of crosses between the two species produce a wide range of plant architecture, suggesting that an understanding of the genetic control of plant architecture in foxtail millet may provide additional insights into branching patterns, an important component of biomass production (Doust et al., 2004; Doust and Kellogg, 2006).

Foxtail millet varieties can also be remarkably drought and salt tolerant, and further breeding for these characteristics can increase the usefulness of foxtail millet for semiarid and marginal lands (Li and Wu, 1996; Dekker, 2003).

GERMPLASM RESOURCES

Collection of foxtail millet landraces in China started in the 1920s and more than 26,000 accessions, 90% of which are landraces, are currently preserved at the



Figure 1. A, Habit, foxtail millet variety Yugu1 (scale = 6 cm). B, Yugu1 mature inflorescence (scale = 4 cm). C, Green millet variety A10 (scale = 4 cm).

Chinese National Genebank in Beijing. Smaller germplasm collections are maintained at the International Crops Research Institute for the Semi-Arid Tropics, Patancheru, India (1,534 accessions from 26 countries), the National Institute of Agrobiological Sciences, Tsukuba, Japan (1,279 accessions), and the Plant Genetic Resources Conservation Unit of the U.S. Department of Agriculture-Agricultural Research Service (766 accessions; Supplemental Table S1). Phenotypic characterization of the collected materials has indicated that foxtail millet germplasm is highly diverse (Li and Wu, 1996; Reddy et al., 2006).

GENETIC MAPS

Two complete genetic maps were created in the late 1990s using RFLP markers (Devos et al., 1998; Wang et al., 1998). The first was generated from an intervarietal cross within foxtail millet, whereas the second was generated in an interspecific cross between foxtail millet and green millet. Although wide crosses often lead to a reduction in recombination, map lengths were very similar in the intra- and interspecific crosses

(Wang et al., 1998). The interspecific map used foxtail millet markers plus 111 rice (*Oryza sativa*) and five wheat RFLPs. The comparison of the position of common markers on the foxtail millet and rice genetic maps showed that the two genomes were highly colinear, with six foxtail millet chromosomes that were orthologous to single rice chromosomes and three foxtail millet chromosomes that were each orthologous to two rice chromosomes (Devos et al., 1998).

CURRENT DEVELOPMENTS

Yugu1, a highly inbred commercial line from China, was selected for genomic sequencing. This was instead of the accession of foxtail millet used to generate the interspecies genetic map, because that accession showed partial male cytoplasmic infertility (Wang et al., 1998). Genomic sequence of foxtail millet inbred Yugu1 will be generated with a shotgun approach by the JGI, and the first (approximately 4 \times) draft is projected to be available soon after April, 2009. An approximately 11 \times bacterial artificial chromosome library has recently been constructed from Yugu1 by the Clemson University Genomics Institute (C. Sasaki and J.L. Bennetzen, unpublished data), and sequences of Yugu1 bacterial artificial chromosome ends will help assemble and orient scaffolds of the shotgun sequence. In addition, several hundred thousand ESTs are being sequenced with RNA from >15 different developmental stages, tissues, or treatment types, including whole seedling, inflorescence, root, and drought-stressed material. These sequences will help annotate the genomic sequence and provide the necessary genes for microarray chip construction.

The genome of green millet inbred A10 will be sequenced using next generation sequencing technology and compared with the Yugu1 sequence to identify a set of >1,500 single nucleotide polymorphism markers for the construction of a high-density genetic map in a newly generated cross between Yugu1 and green millet accession A10. The single nucleotide polymorphism-based genetic map will be used to anchor the genomic sequence, facilitate comparative genomic analysis, and allow quantitative trait loci (QTL) analyses of biomass traits. An approximately 8.1 \times fosmid library from green millet inbred A10 has recently been constructed, and five randomly selected clones from this library have been sequenced (A.C. Pontaroli and J.L. Bennetzen, unpublished data). One of the clones contained tandem ribosomal DNA repeats, while the other four clones contained numerous nuclear genes that exhibited excellent colinearity with the rice genome (A.C. Pontaroli and J.L. Bennetzen, unpublished data).

Foxtail millet has been adopted as a high-throughput platform for virus-induced gene silencing to model gene inhibition results that have been technically more difficult in switchgrass (R. Nelson, personal

communication). Progress is also being made in developing an efficient transformation system for foxtail millet, necessary for testing mechanistic hypotheses of gene action. Embryogenic callus cultures (Rao et al., 1988; Vishnoi and Kothari, 1996) and direct somatic embryos (J. Zale, personal communication) have been established in vitro for foxtail millet. However, only one lab has reported successfully regenerating transgenic plants, using *Agrobacterium*, and with an efficiency of 6.6% (Liu et al., 2005). In a comprehensive publication, Liu et al. (2007) investigated the parameters affecting *Agrobacterium*-mediated transformation of foxtail millet and reported that plant genotype, explant source, time of inoculation, and length of cocultivation influenced gene transfer (Liu et al., 2007).

CONCLUSION

For the familiar model systems such as *Arabidopsis* (*Arabidopsis thaliana*), rice, or maize, a fully sequenced genome was the culmination of decades of genetic study, and was the tool that laced together many disparate strands of research. In contrast, for foxtail millet, as for *Brachypodium* and other novel model systems, the fully sequenced genome is the foundation on which genetic resources will be built. Foxtail millet and *Brachypodium* illustrate a novel way to create a model, in which the basic genome structure is given and can be interpreted by comparison to the other grasses. QTL studies, forward genetics, and reverse genetics can then proceed rapidly, and in a comparative context from the outset.

Foxtail millet provides a valuable tool for investigating the C_4 grasses, particularly those that are being developed as biomass sources for biofuel production. It was chosen as a target model system as much for its phylogenetic position and its photosynthetic pathway as for its ease of growth and small genome size. Such choices will become increasingly common in the future, as sequencing costs continue to drop. Model systems can be chosen for their ability to address particular questions or to represent particular phylogenetic groups. This approach is especially promising in grasses, where the presence of several completed genomes (and the promise of more) provides an evolutionary genomic context for each new species sequenced. Multiple sequenced genomes, coupled with the ease of comparative analysis among the highly colinear chromosomes of grasses, allow new genomic sequences to be successfully annotated and related to genetic information from other grass species.

The foxtail millet genome will be useful not only for understanding the genetics of biomass production with application to biofuel grasses, but also for the favorable agronomic characteristics of foxtail millet itself. Although foxtail millet is a minor North American grain, it is grown extensively in northern China, Mongolia, India, and Russia, and is also grown for hay in the northern and western Great Plains in the United

States (Baltensperger, 1996, 2002). The genome sequence will advance foxtail millet breeding as well as gene discovery (Diao, 2005, 2007).

However, even with the planned genomic sequence analyses, EST discovery process, genetic map improvements, and QTL analyses, foxtail millet lacks some essential tools needed to become an ideal model system. Mutagenized populations are few, as are reverse genetics tools for characterization of gene function. An expression array has been constructed to examine gene expression under drought stress, but, to our knowledge, neither additional expression arrays nor metabolomic and proteomic tools are yet under development. These will be needed to provide comprehensive analyses of gene function. However, the presence of a completed genome sequence and the current perception of an urgent need to develop herbaceous sources of biomass for biofuel production will provide a powerful impetus to create a full genetic and genomic toolkit in foxtail millet. In response to this challenge, researchers from the United States, China, France, and Japan are beginning to collaborate to fast track tool development in this promising model species.

Supplemental Data

The following materials are available in the online version of this article.

Supplemental Table S1. Foxtail millet germplasm resources.

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