

REVIEW PAPER

Setaria viridis and *Setaria italica*, model genetic systems for the Panicoid grasses

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Abstract

Setaria italica and its wild ancestor *Setaria viridis* are diploid C₄ grasses with small genomes of ~515 Mb. Both species have attributes that make them attractive as model systems. *Setaria italica* is a grain crop widely grown in Northern China and India that is closely related to the major food and feed crops maize and sorghum. A large collection of *S. italica* accessions are available and thus opportunities exist for association mapping and allele mining for novel variants that will have direct application in agriculture. *Setaria viridis* is the weedy relative of *S. italica* with many attributes suitable for genetic analyses including a small stature, rapid life cycle, and prolific seed production. *Setaria* sp. are morphologically similar to most of the Panicoideae grasses, including major biofuel feedstocks, switchgrass (*Panicum virgatum*) and *Miscanthus* (*Miscanthus giganteus*). They are broadly distributed geographically and occupy diverse ecological niches. The cross-compatibility of *S. italica* and *S. viridis* also suggests that gene flow is likely between wild and domesticated accessions. In addition to serving as excellent models for C₄ photosynthesis, these grasses provide novel opportunities to study abiotic stress tolerance and as models for bioenergy feedstocks.

Key words: Abiotic stress tolerance, biofuels, C₄ photosynthesis, domestication, *Setaria italica*, *Setaria viridis*.

Introduction

The Panicoideae subfamily is one of the most agronomically important grass groups. It includes the most economically important C₄ cereal in the USA, maize, as well as several important grains grown throughout the world including sorghum, pearl millet, and foxtail millet. It also includes the major biofuel, sugarcane, as well as emerging bioenergy feedstocks *Miscanthus* and switchgrass. Both *Setaria italica* (common name: foxtail millet) and *Setaria viridis* (common name: green foxtail) are members of the tribe Paniceae that is the sister to the Andropogoneae. The Andropogoneae tribe includes maize, sorghum, *Miscanthus*, and sugarcane (Fig. 1). Of plants with sequenced genomes, *S. viridis* and *S. italica* (<http://www.phytozome.net/foxtailmillet.php>) are the closest relatives of the biofuel feedstock switchgrass and the invasive weed guinea grass (*Panicum maximum*). As a genetic model, *S. viridis* has many desirable traits, including small size (10–15 cm), a short life cycle (6–9 weeks

depending on photoperiod conditions), and prolific seed production (~13,000 seeds per plant). Plants are self-compatible and can be transformed by *Agrobacterium*-mediated methods (Brutnell *et al.*, 2010). *Setaria italica* is a cereal crop with excellent drought tolerance and an extensive germplasm collection, providing opportunities to study the domestication process and to mine for novel allelic variation. Collectively, these traits suggest that *S. viridis* and *S. italica* are ideally suited for studies of C₄ evolution, comparative grass genomics, and use as models for biofuel feedstocks (Doust *et al.*, 2009; Brutnell *et al.*, 2010).

As whole-genome sequencing projects progress at an impressive rate (Schnable *et al.*, 2009; Vogel *et al.*, 2010), it is likely that new model organisms will emerge to address specific challenges in agriculture, for example biotic and abiotic stress tolerance. Currently, *Arabidopsis thaliana* serves

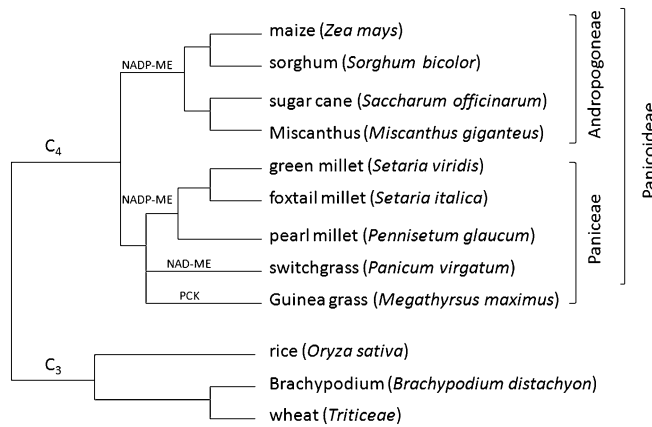


Fig. 1. Phylogenetic relationships of grass family members. The tree was adapted from Brutnell *et al.* (2010). Branch length does not reflect time of evolutionary divergence.

as the community standard to investigate many developmental, disease, and abiotic stress responses. However, monocots and eudicots diverged 127–175 million years ago (Bell *et al.*, 2010; Smith *et al.*, 2010), providing ample opportunity for divergence of gene structure and function. In this review, the potential of *S. italica* and *S. viridis* as models to investigate the mechanisms driving domestication and to understanding photosynthetic differentiation using a systems biology approach is discussed. In addition, the *Setaria* sp. provides novel opportunities to address one of the most pressing agronomic challenges in world agriculture today, namely abiotic stress tolerance.

Distribution of *Setaria italica* and *Setaria viridis*

Setaria italica is one of the oldest crops in the world; cultivation probably began 5900 BP in Gansu Province, Northwestern China (Barton *et al.*, 2009). Currently, *S. italica* is a minor food crop in China and India, and in the USA, Canada, and Australia is grown primarily for fodder or bird seed (Baltensperger, 2002; Diao, 2005, 2007; Doust *et al.*, 2009). Some cultivars of *S. italica* are extremely drought tolerant, making them major crops in the arid regions in the North of China where annual rainfall amounts can be as low as 100–400 mm (Diao, 2005, 2007).

Although *S. viridis* originated around latitude 45°N in Eurasia, it has since spread to a wide range of habitats in temperate, tropical, and subtropical regions (Dekker, 2003). Its successful history of invading, colonizing, and adapting to the local environment, together with simple growth requirements and large seed yield probably contributed to its domestication, and it is largely accepted that *S. viridis* is the wild ancestor of cultivated *S. italica* (Li *et al.*, 1945; de Wet, 1975). This hypothesis has been supported by isozyme and phylogenetic analyses using ribosomal and chloroplast sequence markers as well as through chromosomal fluorescent *in situ* hybridization (Wang *et al.*, 1995; Benabdelmouna *et al.*, 2001; Doust and Kellogg, 2002). The

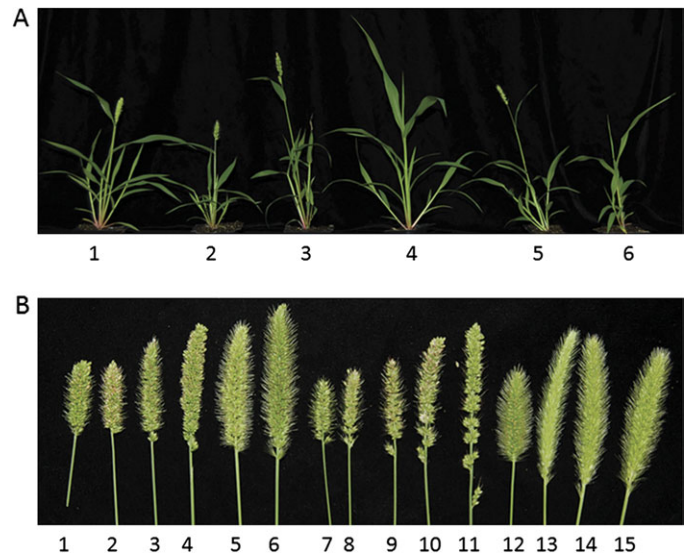


Fig. 2. Phenotypic variation within *S. viridis* accessions. (A) Plant height and flowering time variation among accessions. The numbers 1–6 are representatives of accessions: 1, A10.1; 2, Ames 21519; 3, Ames 21520; 4, PI 202407; 5, PI 204624; and 6, PI 204625. (B) Variation in inflorescence architecture. The numbers 1–15 are representatives of accessions: 1, A10.1; 2, PI 408810; 3, PI 204727; 4, PI 408811; 5, PI 230134; 6, PI 202407; 7, Ames 21519; 8, PI 230135; 9, PI 223677; 10, Ames 21520; 11, PI 204624; 12, PI 204625; 13, PI 221960; 14, PI 204730; and 15, PI 204628.

remarkable adaptation of *S. viridis* to a host of environments has contributed to its dubious honour as one of the worst weeds in world agriculture, successfully competing with many cereal crops (Dekker, 2003).

Genetic diversity of *Setaria italica* and *Setaria viridis*

Surveys of *S. italica* and *S. viridis* have revealed a germplasm rich with phenotypic variation. A comparison of 1535 *S. italica* accessions collected from 26 countries at the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) in India identified large variations in plant height, flowering time, inflorescence architecture, and seed morphology (Reddy *et al.*, 2006). A sample of a few *S. viridis* accessions also reveals large variations in plant height, flowering time, and inflorescence structure (Fig. 2). In contrast to the many accessions of *S. italica* available (Doust *et al.*, 2009), few accessions of *S. viridis* are available in the USA (GRIN: <http://www.ars-grin.gov/cgi-bin/npgs/html/taxon.pl?430573>). Clearly, additional collections of *S. viridis* from around the world would be of great value in exploring the diversity of this important plant group.

Despite the phenotypic diversity, the genetic diversity underlying this variation does not appear to be as great. A comparison of isozyme variation among 164 accessions of *S. viridis* suggests low genetic diversity within *S. viridis* populations, similar to estimates of genetic diversity in other self-pollinating weeds. However, this diversity is

distributed in discrete populations, suggesting strong local adaptation and regional differentiation (Wang *et al.*, 1995). More recent amplified fragment length polymorphism (AFLP) and single nucleotide polymorphism (SNP) marker analyses support these findings of low genetic variation within accessions and high variation between populations (d'Ennequin *et al.*, 2000). The silent nucleotide variation of *S. viridis* ($\theta_{\text{sil}}=0.0059$) is more than double that found in *S. italica* ($\theta_{\text{sil}}=0.0027$), suggesting a loss of diversity during domestication (Wang *et al.*, 2010a). This drop in diversity is similar to what has been observed in other self-pollinating grasses such as wild ($\theta_{\text{sil}}=0.0077\text{--}0.0095$) and domesticated rice ($\theta_{\text{sil}}=0.0024$) (Zhu *et al.*, 2007). A pairwise SNP analysis across nine loci indicated an increase of linkage disequilibrium corresponding to a loss of genetic diversity in *S. italica* relative to *S. viridis* and identified a potential selective sweep at one locus during the domestication process (Wang *et al.*, 2010a). However, F_{st} estimates between *S. viridis* and *S. italica* varied from 0.025 to 0.41 among the nine genes sampled (Wang *et al.*, 2010a). This broad range in F_{st} estimates suggests that a deeper sampling of both genes and accessions is necessary to reveal the degree of population differentiation between *S. viridis* and *S. italica*. It is also worth noting that the close physical proximity in which *S. viridis* and *S. italica* are often found, together with a lack of apparent hybridization barriers, probably permits cross-hybridization and gene flow between weedy and domesticated relatives. The estimated rate of outcrossing varies from 0.3% to as high as 4%, suggesting ample opportunity for gene flow between *Setaria* species, particularly from crop to weedy species (Wang *et al.*, 2010a). One caveat to all current diversity studies is the relatively small sample size of *S. italica* and *S. viridis* accessions taken from the putative centre of origin in Gansu province. Increasing the richness of the current germplasm collections to include multiple accessions closer to the centre of origin and domestication of *S. italica* (Barton *et al.*, 2009) and sampling a much broader segment of the genome should facilitate studies of genetic diversity and gene flow between populations.

Model genetic systems for understanding the domestication process

Most of the morphological and physiological differences between *S. italica* and *S. viridis* correspond to effects of human selection or the 'domestication syndrome' (Doebley *et al.*, 2006). Compared with its wild ancestor, many *S. italica* accessions have reduced vegetative branching, an enlarged inflorescence, synchrony of flowering, loss of seed dormancy, and loss of disarticulation. Much of our understanding of domestication in Panicoideae grasses has come from comparisons of maize with its wild progenitor, teosinte (Doebley and Stec, 1991; Lukens and Doebley, 1999). However, due to morphological and physiological differences between maize and other grasses, it is likely that different suites of genes have been targeted during the

domestication of maize relative to other Panicoideae crops. For example, *tb1*, a major domestication quantitative trait locus (QTL) underlying tillering in maize, does not appear to be a major control point of branching variation differentiating *S. italica* from *S. viridis* (Doust *et al.*, 2004). Although several loci appear to regulate inflorescence architecture in *Setaria* (Doust *et al.*, 2005), a comparative analysis of maize and *Setaria* QTLs that regulate branch morphology will be necessary to reveal both common and unique targets of domestication. Thus, the study of domestication traits in *S. italica* and *S. viridis*, which are morphologically most similar to the majority of Panicoideae grasses including many of the emerging bioenergy feedstocks, could identify novel targets of selection and suggest loci for marker-assisted selection.

Flowering time is an important trait in cereals, which directly influences grain yield (Jung and Muller, 2009) and is the result of breeding selection for adaptation to specific photoperiod and temperature environments (Colasanti and Coneva, 2009). Comparative studies between *Arabidopsis*, rice, and *Brachypodium* have revealed that many aspects of photoperiod control of flowering are conserved between eudicot and monocot lineages (Higgins *et al.*, 2010); however, homologous genes may be under very different regulation. For instance, the *CONSTANS* gene acts as a positive regulator of flowering under long days in *A. thaliana*, but, in rice, the homologous gene functions to repress flowering under long days (Hayama and Coupland, 2004). Furthermore, the vernalization pathways are significantly different between eudicot and monocot lineages (Higgins *et al.*, 2010). For instance, *FLC* acts as a central regulator of vernalization in *A. thaliana*, but no functional homologue is present in monocot lineages (Cockram *et al.*, 2007). Conversely, in wheat, a zinc-finger CCT domain protein *ZCCT1* (also known as *VRN2*) acts as a dominant repressor of flowering, but a clear homologue is absent in *Arabidopsis* (Yan *et al.*, 2004). Since flowering time is such an important trait related to grain yield, it is perhaps not surprising that significant variation exists in the mechanisms to alter the timing of flowering in crop species. Thus, even in closely related crops such as wheat and rice, there is significant divergence in the genes that influence flowering time (Cockram *et al.*, 2007). Study of the genes regulating flowering in pairs of cultivated and wild species may be more insightful in understanding the evolution of this important domestication trait rather than making wide cross-species comparisons. As *S. viridis* has one of the widest global distributions of any weed (Dekker, 2003) and *S. italica* is cultivated under both long-day and short-day conditions, there is probably substantial genetic variation in genes regulating photoperiod- and vernalization-dependent flowering time. In summary, comparative studies between *S. viridis* and *S. italica* will probably contribute much to our understanding of how flowering time can be manipulated during the domestication process.

An alternative approach to identify domestication genes is through whole-genome scans, which can be used to compare DNA polymorphisms between wild and domesticated species

to define the regions with reduced variation consistent with selection (Storz, 2005). Next-generation (NextGen) sequencing techniques provide more power for this approach (Wang *et al.*, 2010b), which can efficiently map genome-wide SNP variation (McNally *et al.*, 2009; Huang *et al.*, 2010). These techniques could be applied to characterize *S. italica* and *S. viridis* germplasm to identify selective sweeps, and determine markers for future breeding efforts. In addition to targeting genes for food production, it should also be possible to exploit these data for engineering bioenergy feedstocks, for example by identifying genes from *Setaria* sp. that repress inflorescence formation and delay senescence and thus increase biomass production. Because *S. italica* and *S. viridis* are close relatives to bioenergy feedstocks such as switchgrass, it is likely that these findings will be relatively easy to translate from *Setaria* into less tractable genetic systems such as switchgrass, *Miscanthus*, or sugarcane.

Model genetic systems for understanding C₄ photosynthesis

Despite the economic value of C₄ grasses, the regulatory networks that initiate and maintain C₄ photosynthesis are largely unknown (Brutnell *et al.*, 2010). This gap in our understanding is a major roadblock in current attempts to bioengineer C₄ traits into C₃ crops such as rice (Mitchell and Sheehy 2007; Sheehy *et al.*, 2007; Zhu *et al.*, 2010). The recent sequencing of the sorghum and maize genomes (Paterson *et al.*, 2009; Schnable *et al.*, 2009) coupled with a detailed analysis of the maize leaf transcriptome (Li *et al.*, 2010) provides new opportunities to investigate gene networks underlying C₄ photosynthesis. However, due to the long life cycle, large plant size, and lack of efficient transformation systems for these crop plants, neither sorghum nor maize is ideal for the genetic dissection of C₄ traits. Thus, it is time to explore *Setaria* sp. as potential models for understanding C₄ photosynthetic development.

C₄ photosynthesis evolved at least 17 times independently within the grass family (Duvall *et al.*, 2003; Christin *et al.*, 2009b). Thus, by using a comparative genomics approach, it should be possible to identify both common and unique signatures of C₄ evolution. For instance, a comparison of several C₄ enzymes, including phosphoenolpyruvate carboxylase (PEPC), phosphoenolpyruvate carboxykinase (PCK), and NADP-dependent malic enzyme (NADP-ME), among multiple lineages has revealed signatures of strong positive selection during C₄ evolution (Christin *et al.*, 2007, 2009a, c). Although much less is known about common *cis*-regulatory elements, it is likely that both shared and unique regulatory elements were recruited to drive high levels of gene expression in either bundle sheath or mesophyll cells (Yanagisawa and Sheen, 1998). One obvious difference among the grasses is in the choice of enzyme used to decarboxylate the major C₄ acid in the bundle sheath cells. There are three primary decarboxylase enzymes that were recruited to C₄ photosynthesis, NADP-ME, NAD-dependent malic enzyme (NAD-ME), and PCK (Leegood, 2002). In this

regard, the Paniceae clade is particularly interesting as all three subtypes exist. For instance, switchgrass uses NAD-ME for decarboxylation, guinea grass uses PCK, and *Setaria* uses NADP-ME. Thus, by comparing the *cis*-regulatory regions of these decarboxylases among these three lineages, it may be possible to discover common features that typify changes that accompany the cell-specific control of gene expression in C₄ photosynthesis.

As mentioned before, one emerging technology that promises to reshape the analysis of plant genomes is NextGen sequencing (Lister *et al.*, 2009; Wang *et al.*, 2010b). The recently released whole-genome sequences of two Andropogonea C₄ grasses, sorghum and maize, has already provided some insight into the mechanisms of gene duplication that are likely to drive C₄ differentiation (Wang *et al.*, 2009). Furthermore, with the decreasing cost and increasing throughput from emerging sequencing technologies, it will soon be possible to deeply profile gene expression across numerous tissues and developmental time points. For instance, a comparative analysis of *S. italica* and *S. viridis* genomes in the Paniceae with maize and sorghum in the Andropogoneae will allow the comparison of two independently derived NADP-ME subtypes of C₄. Additional sequencing of both C₃ and C₄ grasses distributed throughout the grass group will enable a much broader examination of the genome dynamics that has accompanied C₄ evolution. This will include comparisons of not only coding regions, *cis*-regulatory elements, and splice sites, but also large-scale and small-scale genome rearrangements and methylation profiles. Furthermore, as additional grass genomes are sequenced, comparative genomic approaches will become increasingly powerful. Thus, over the next few years, ultra high throughput sequencing technologies promise to provide an unprecedented opportunity to explore the evolution and diversification of C₄ photosynthesis in the grasses. As one of the smallest, fastest growing grasses with an efficient transformation protocol, *S. viridis* is well positioned as a model for functional genomic dissection of C₄ photosynthesis.

Model grasses for abiotic stress tolerance study

The range of environments in which *Setaria* sp. are found suggests that the germplasm may also be a rich source of genetic variation for genes controlling abiotic stress tolerance. For example, some *S. italica* cultivars are remarkably drought tolerant; for 1 g of dry biomass, maize requires 470 g of water, wheat 510 g, and *S. italica* a modest 257 g (Diao, 2005, 2007). This high water use efficiency (WUE) has helped establish *S. italica* as a major crop in the semi-arid and arid regions in north China (e.g. Gansu, Shanxi, and Neimengu) and India. Several morphological and physiological adaptations are associated with increased WUE in *S. italica*, including small leaf area, thickening of the cell walls, and ability to form a dense root system (Li, 1997). To identify genes underlying these traits, Zhang and colleagues

and Lata and colleagues examined differential gene expression among drought-tolerant and susceptible accessions of *Setaria* following stress treatment and identified hundreds of significant changes in expression that corresponded to genes involved in metabolism, proteolysis, and signalling (Zhang *et al.*, 2007; Lata *et al.*, 2010). Although a wide range of salt (Sreenivasulu *et al.*, 2004; Jayaraman *et al.*, 2008; Puranik *et al.*, 2010) and cold (PL, unpublished results) tolerance is evident in the *Setaria* germplasm, the mechanistic basis of this variation is unknown.

Although abiotic stress tolerance and photosynthesis are often studied in isolation, it is important to consider the effects of interaction of abiotic stress on photosynthetic activities. In particular, plants that maintain high photosynthetic rates under abiotically stressed conditions (e.g. high salt, drought, and poor soil nutrition) will be particularly important as more marginal lands are incorporated into agriculture, especially for bioenergy feedstocks. The sequencing of the *S. italica* and *S. viridis* genomes and the characterization of transcriptomics changes associated with abiotic stress treatments will provide new and exciting opportunities to investigate these networks.

Genomics resource development

Given the relatively small size of the *S. italica* and *S. viridis* genomes, surprisingly little progress has been made in establishing genetic tools for these plants. To date, only two QTL mapping populations have been published (Devos *et al.*, 1998; Wang *et al.*, 1998). One population was generated as an intraspecific cross between two *S. italica* accessions (Longgu 25×Pagoda Flower Green). The other was the result of an interspecific cross between *S. italica* and *S. viridis* accessions (*S. italica* B100×*S. viridis* A10). An additional recombinant inbred line population has also recently been generated between *S. italica* accession Yugu1 and *S. viridis* accession A10 (Doust *et al.*, 2009). Although many QTL studies have been performed in *Setaria*, few are publicly accessible. The Gramene database indicates that at least 65 QTLs have been defined in screens of the B100×A10 population (http://www.gramene.org/species/setaria/foxtailmillet_intro.html). As recently discussed, efforts are now underway to generate a high density map of the Yugu1×A10 population which should help to better define intervals in QTL studies (Doust *et al.*, 2009).

The recent whole-genome sequencing of *S. italica* (JGI, <http://www.phytozome.net/foxtailmillet.php>) and ongoing sequence analysis of *S. viridis* will greatly accelerate the genetic analysis of these two species as well as facilitate comparative analyses within the grasses. By combining genetics maps, QTL studies, and genome sequencing, it will soon be possible to identify candidate genes that underlie QTLs in *Setaria*. In addition, the establishment of transient and stable *Agrobacterium*-mediated transformation in *S. viridis* (Brutnell *et al.*, 2010), virus-induced gene silencing in *S. italica* (Doust *et al.*, 2009), and mutagenized populations of *S. viridis* will provide valuable tools for the molecular characterization of gene function in *Setaria*. Thus, through

the development of new genomics tools and technologies, it will soon be possible to add *Setaria* to the growing list of model organisms in the grasses that will aid in comparative genomic approaches to characterize the world's most important feed, fuel, and bioenergy feedstocks.

Conclusion

The weedy *S. viridis* and domesticated *S. italica* species promise to serve as excellent model systems for the study of C₄ photosynthesis, artificial selection, abiotic stress tolerance, and biomass production in the Panicoid grasses. Importantly, the completion of the *Setaria* genome sequence as well as the development of efficient transformation methods will rapidly advance these studies. What is needed now is a detailed anatomical, physiological, and biochemical exploration of these two species to provide the framework for the molecular genetic dissection of agronomically and economically important traits in the grasses.

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