Minor millets as model system to study C$_4$ photosynthesis - A review

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ABSTRACT

C$_4$ photosynthesis is the primary mode of carbon capture and drives productivity in several major food crops and bio-energy grasses. Gains in productivity associated with C$_4$ photosynthesis include improved water and nitrogen use efficiencies. Within grasses rice and brachypodium are used as model species. Since these two crops are using C$_3$ photosynthesis for their growth and development, it cannot be used as model for to study C$_4$ photosynthesis. In order to characterize the evolutionary innovations and to provide genomic insight into crop improvement for the many important crop species, a new genomic and genetic model species is required. Minor millets have small diploid genomes, shorter life cycles, self pollination and prolific seed production. Due to these characteristics it gains importance over major C$_4$ species which lack all of these traits. Within Minor millets, *Setaria italica* and *Setaria viridis* are used as model systems since these crops fulfils all the traits responsible to be a model species. Importantly, *Setaria* species uses NADP-Malic enzyme subtype C$_4$ photosynthetic system to fix carbon and therefore is a potential powerful model system for dissecting C$_4$ photosynthesis. C$_4$ grasses have a shorter distance between longitudinal veins in the leaves than C$_3$ grasses. The C$_4$ grasses have denser transverse and small longitudinal veins than the C$_3$ grasses. It indicates that C$_4$ grasses have a structurally superior photosynthate translocation and water distribution system by developing denser networks of small longitudinal and transverse veins. *Setaria* has high vein density and kranz anatomy that helps to concentrate CO$_2$ in the bundle sheath cells. This minimizes photorespiration thereby prevents the loss of energy.

Key words: C$_4$ photosynthesis, Minor millets, *Setaria italica*, *Setaria viridis*.

C$_4$ photosynthesis is the primary mode of carbon capture for some of the world's most important food, feed, and fuel crops *viz.*, maize (*Zea mays*), sorghum (*Sorghum bicolor*), sugarcane (*Saccharum officinarum*), millets (e.g. *Panicum miliaceum, Pennisetum glaucum, and Setaria italica*), Miscanthus x giganteus, and switchgrass (*Panicum virgatum*).

In contrast with C$_3$ plants, C$_4$ plants first fix CO$_2$ into a C$_4$ acid before delivering the CO$_2$ to the Calvin cycle (Hatch, 1971). For example, in maize and sorghum leaves, CO$_2$ entering mesophyll cells (MC) is first fixed into oxaloacetate, which is then reduced to malate in the MC chloroplasts. The malate then diffuses into the inner bundle sheath (BS) cells and is transported into the BS chloroplast. There, malate is decarboxylated by NADP-malic enzyme, releasing CO$_2$ close to ribulose 1,5-bisphosphate carboxylase /oxygenase (Rubisco). This carbon shuttle greatly lowers rates of photorespiration as Rubisco is both isolated from the site of O$_2$ evolution (oxygen evolving complex of photosystem -II) and also maintained in a CO$_2$ -rich environment. Indeed, in mature maize or sorghum leaves, rates of photorespiration are at the limits of detection under conditions where C$_3$ plants lose up to 30 % of their photosynthetic capacity due to photorespiration (Zhu *et al.,* 2008).

Accompanying this partitioning of photosynthetic activities are several anatomical adaptations. This includes close vein spacing and large numerous plastids of the inner BS. Together, these characters enable C$_4$ plants to thrive in environments that induce high rates of photorespiration in C$_3$ plants, such as the tropics or grassland savannas (Sage and Pearcy, 2000). An added benefit of the C$_4$ syndrome is improved nitrogen and water use efficiencies that have likely contributed to their global distribution and high rates of productivity (Sage, 2004; Edwards and Smith, 2010).

Characteristics of C$_4$ plants

➤ C$_4$ leaves are characterized by Kranz-type anatomy, in which the vascular bundle is surrounded by organelle-rich bundle sheath (BS) cells, and this tissue layer is further surrounded by radially arranged mesophyll cells(MC).

➤ BS cells are closely link with mesophyll cells through Plasmodesmata.

➤ Both cells contain Chloroplast called as Dimorphic Chloroplast.

➤ C$_4$ photosynthesis consists of morphological and biochemical novelties that create a CO$_2$ pump that concentrates CO$_2$ around Rubisco which decreases photorespiration and the resulting energy waste.

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Importance of C$_4$ photosynthesis

➢ C$_4$ photosynthesis drives productivity in several major food crops and bio-energy grasses, including maize (*Zea mays*), sugarcane *(Saccharum officinarum)*, sorghum (*Sorghum bicolor),* Miscanthus x giganteus, and switchgrass (*Panicum virgatum*).

➢ Gains in productivity associated with C$_4$ photosynthesis includes improved water and nitrogen use efficiencies. Thus, engineering C$_4$ traits into C$_3$ crops is an attractive target for crop improvement.

➢ Many grass species have also evolved to use the C$_4$ photosynthetic cycle, which is more energetically productive and uses both nitrogen and water more efficiently than the C$_3$ photosynthetic cycle found in most green plants (Sage, 2004).

➢ Within the grasses, rice and Brachypodium (Brachypodium distachyon) are also being developed as model systems for the grasses. However, rice and Brachypodium both are C$_3$ plants, so they are not suitable for many C$_4$-related functional analyses.

➢ In order to both characterize the evolutionary innovations responsible for C$_4$ photosynthesis and provide genomic insight into crop improvement for the many important crop species found within the panicoid grasses, a new genomic and genetic model species is required.

C$_4$ Pathway
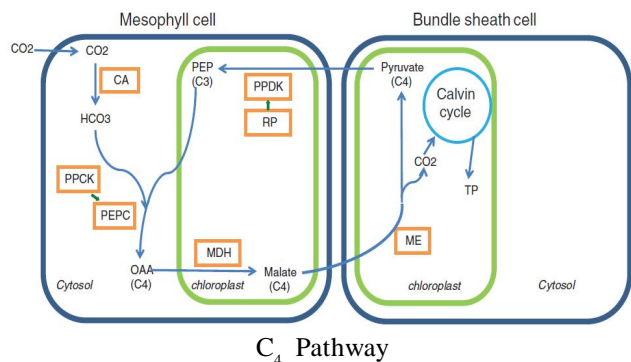
The leading role of model organisms

Model organisms are significant in enabling new scientific discovery and the development of new technology. Discoveries in early genetic models such as, pea *(Pisum sativum),* fruit fly (*Drosophila melanogaster*) and maize *(Zea mays),* laid the foundation for modern genetics. Research with these species has been responsible for the discovery of everything from the basic laws of genetics and heritability to the exceptions to those laws, such as transposable elements, and to the pioneering of the wholly new field of cytogenetics. This early understanding of genetics and inheritance led to the establishment of dramatically improved breeding methods using hybridization and single line selection. In the genomics era, new models such as Arabidopsis and rice were used to discover genetic regulatory networks, such as the ABC model of floral development. The technological progress of the genomics era, first elaborated in model species, has enabled novel approaches to breeding including marker assisted selection and breeding by molecular design.

Arabidopsis has been developed extensively as a model for plant molecular genetics and functional genomics, and this organism was especially suitable for answering questions about fundamental mechanisms that are conserved across all plant species. However, flowering plants are an extremely diverse group of species, with many traits and systems of particular importance to humans that are found only in specific plant lineages, such as C$_4$ photosynthesis. Characterizing these lineage-specific systems requires the identification and development of new model species.

Cereal crops, members of the grass family, contribute close to 70 % of the calories consumed by humans around the world, and are only distantly related to the eudicot clade, which contains Arabidopsis. Many grass species have also evolved to use the C$_4$ photosynthetic cycle, which is more energetically productive and uses both nitrogen and water more efficiently than the C$_3$ photosynthetic cycle found in most green plants (Sage, 2004). Within the grasses, rice and Brachypodium *(Brachypodium distachyon)* are also being developed as model systems for the grasses. However, rice and Brachypodium are both C$_3$ plants, so they are not suitable for many C$_4$-related functional analyses. In order to both characterize the evolutionary innovations responsible for C$_4$ photosynthesis and provide genomic insight into crop improvement for the many important crop species found within the panicoid grasses, a new genomic and genetic model species is required.

Drawback of major C$_4$ plants: The lack of a small, rapid cycling genetic model system to study C$_4$ photosynthesis has limited progress in dissecting the regulatory networks underlying the C$_4$ syndrome. The most extensively characterized C$_4$ plants include maize, sorghum, Flaveria sp. (Asteraceae), Amaranthus (Chenopodiaceae), and Cleome (Cleomaceae); All lack efficient transformation systems, are large in stature, and have relatively long generation times, often of several months (Brown *et al.,* 2005). The species group of the Panicoideae is the biggest subfamily in the grasses, and includes many C$_4$ species and a large number of important crop species: maize, sorghum, sugarcane, pearl millet, foxtail millet, common millet, switchgrass, napiergrass and some candidate biofuel crops.

Belonging to this subfamily of the grasses with close evolution relationships, a Setaria model will certainly facilitate functional genomics for these crops. Maize is a widely grown species of great economic importance, and a history of genetic investigation going back more than a century. However, in the genomics era, the large genome size of maize combined with its paleopolyploid evolutionary history and the shear size of adult maize plants present challenges for functional genomics research. Sorghum is also

considered as a model for C$_4$ functional genomics study, but its adult plant size and longer life cycle made it difficult for lab management. Switchgrass and napiergrass are considered putative biofuel crops, but their polyploid backgrounds make their functional genomics study difficult, so is the situation to common millet, an ancient cereal which is still widely grown in dry land area in the world. The establishment of Setaria as a model will assist in the genetic study of all these species, and many more Panicoid grasses.

C$_4$ species are characterized by the Kranz Structure of bundle sheath cells and C$_4$ acid shuttling-related enzymes such as malate dehydrogenase, phosphoenolpyruvate carboxylase and pyruvate orthophosphate dikinase. C$_3$ species, such as rice and Brachypodium, share the primary carbon shuttle enzymes with C$_4$ species of Setaria, sorghum and maize, albeit the amino acid identity of those enzymes between C$_4$ and C$_3$ might be low, and it is deduced those enzymes were recently recruited into the C$_4$ pathway from ancestral C$_3$ isoforms. (Bennetzen *et al.,* 2012) Understanding the genes necessary for Kranz structure development in C$_4$ species will be essential for the deciphering of C$_4$ genetic and molecular mechanisms, and identification of Kranz structural mutations in C$_4$ plants is one appropriate approach. The small stature and short life cycle of Setaria make it suitable for high throughput operations for the screening of related mutations.

Importance of minor millets in C$_4$ photosynthesis: *Setaria viridis* is a member of the Panicoideae clade and is a close relative of several major feed, fuel, and bio-energy grasses. It is a true diploid with a relatively small genome of ;510 Mb. Its short stature, simple growth requirements, and rapid life cycle will greatly facilitate genetic studies of the C$_4$ grasses. Importantly, S. viridis uses an NADP-malic enzyme subtype C$_4$ photosynthetic system to fix carbon and therefore is a potentially powerful model system for dissecting C$_4$ photosynthesis. 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). *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 C$_4$ evolution, comparative grass genomics, and use as models for biofuel feed stocks.

Phylogenetic position of important grass species
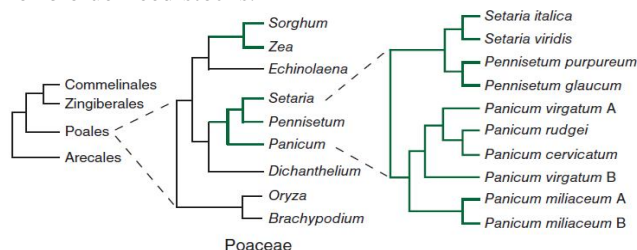
Distribution: Currently, is a minor food crop in China and India, and in the USA, Canada, and Australia is grown primarily for fodder or bird seed (Diao, 2005, 2007; Doust *et al.,* 2009). In India, Minor millets cultivation can be seen in the states of Karnataka, Madhya Pradesh, Andhra Pradesh, Uttar Pradesh, Maharashtra. In Tamil Nadu it is observed in the Districts of Dharmapuri, Madurai, Tirunelveli, Salem and Coimbatore.

Initiation of the setaria model: The grass genus Setaria, part of the tribe Paniceae in the subfamily Panicoideae of the family Poaceae, contains approximately 125 species world-wide. Species in this genus are found in tropical, sub-tropical and temperate regions, and include both crop and weed species with different life cycles and ploidy levels (Rominger, 1966). So far, seven genome types have been identified in the genus and their genetic and structural relationships have been investigated by genomic in situ hybridization (GISH) and molecular marker analyses (Zhao *et al.,* 2013).

Two species of Setaria, domesticated foxtail millet (*S. italica)* and its wild ancestor green foxtail *(S. viridis),* exhibit numerous properties that make them ideal models for functional genomics studies in the Panicoid grasses. Both species are diploid and have tractably small genomes, short generation times (50–90 days), small stature and prolific seed production. These two Setaria species show excellent genetic colinearity, because foxtail millet is believed to have been domesticated just within the last few thousand years from the weedy green foxtail(Bennetzen *et al.,* 2012). Given their hybrid fecundity, foxtail millet and green foxtail could be viewed as a single species with two botanical names. With a high quality reference genome sequence (Bennetzen *et al.,* 2012) and a high-density haplotype map of genome variation (Jia *et al.,* 2013) and other genomic data, the accumulated genomic information for foxtail millet and green foxtail has reached the level where this species pair can now truly be considered a novel model system.

Several publications, foxtail millet: a sequence driven grass model system by Doust *et al*. Setaria viridis: a model for C$_4$ photosynthesis by Brutnell *et al*. and two review papers of using foxtail millet and green foxtail as model (Li and Brutnell, 2011) have helped foster enthusiasm and forward momentum that is leading to the development of Setaria as a model system. A note on terminology: although Setaria is the name of a genus, Setaria is used here to refer to either the domesticated species, foxtail millet, or the wild ancestor, green foxtail, following the common convention.

**Model genetic systems for understanding C$_4$ photosynthesis**

Despite the economic value of C$_4$ grasses, the regulatory networks that initiate and maintain C$_4$ photosynthesis are largely unknown (Brutnell *et al.,* 2010). This gap in our understanding is a major roadblock in current attempts to bioengineer C$_4$ traits into C$_3$ crops such as rice (Mitchell and Sheehy 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 C_4 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 C_4 traits. Thus, it is time to explore Setaria sp. as potential models for understanding C_4 photosynthetic development. C_4 photosynthesis evolved at least 17 times independently within the grass family (Christin *et al.,* 2009b). Thus, by using a comparative genomics approach, it should be possible to identify both common and unique signatures of C_4 evolution.

A comparison of several C_4 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 C_4 evolution (Christin *et al.,* 2009a, c). Although much less is known about common cisregulatory 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 C_4 acid in the bundle sheath cells. There are three primary decarboxylase enzymes that were recruited to C_4 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 NADME 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 C_4 photosynthesis. One emerging technology that promises to reshape the analysis of plant genomes is NextGen sequencing (Wang *et al.,* 2010b). The recently released whole-genome sequences of two Andropogonea C_4 grasses, sorghum and maize, has already provided some insight into the mechanisms of gene duplication that are likely to drive C_4 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 C_4. Additional sequencing of both C_3 and C_4 grasses distributed throughout the grass group will enable a much broader examination of the genome dynamics that has accompanied C_4 evolution. 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 C_4 photosynthesis.
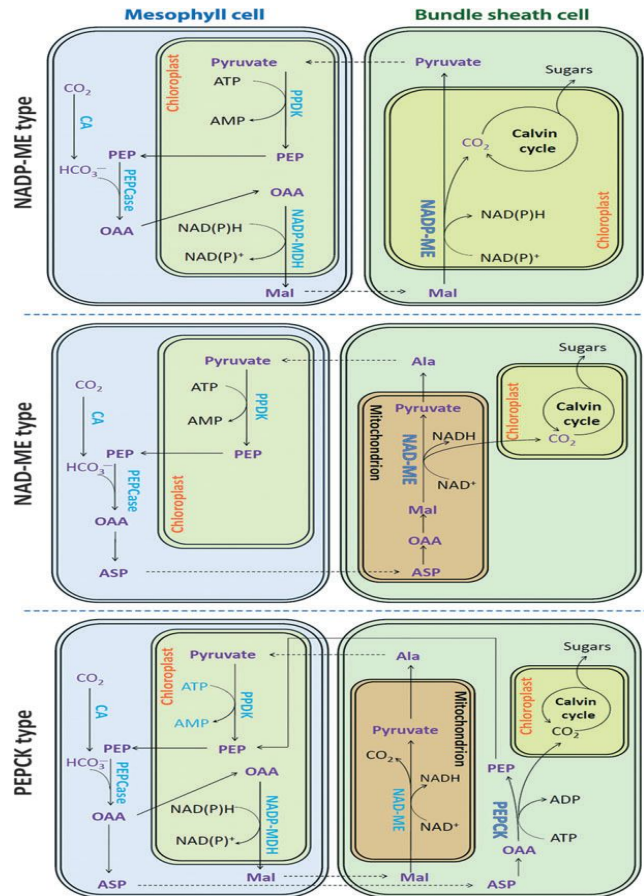
Important anatomical traits in *Setaria* associated with C_4 photosynthesis: *Setaria* being a C_4 species, has high vein density, so it could also be used to identify elements controlling the vein density trait. In C_4 species, the veins are closer giving them high vein density and they have kranz anatomy that helps to concentrate CO_2 in the bundle sheath cells. This minimizes photorespiration thereby prevents the loss of energy (Rizal *et al.,* 2012).

C_4 plants are divided into three C_4 subtypes differing in the process of decarboxylation of C_4 acids: the NADP-malic enzyme (NADP-ME), NAD-malic enzyme (NAD-ME) and phosphoenolpyruvate carboxykinase (PCK) types (Hatch, 1987). The difference in biochemical function is associated with that in structural features of leaves. In C_4 grasses, in general, the NADP-ME grasses have the BS that originated from the mestome sheath, whereas both the NAD-ME and PCK grasses have the BS that originated from the parenchyma sheath (Dengler and Nelson, 1999). The BS cells of the C_4 subtypes also differ in the structure,

intracellular position and amount of the chloroplasts and mitochondria (Hatch, 1987; Yoshimura *et al.,* 2004). The quantitative balance of photosynthetic tissues (Ohsugi and Murata, 1986; Dengler *et al.,* 1994) and organelles (Yoshimura *et al.,* 2004) between the M and BS cells reflects the difference in biochemical function of the photosynthetic subtypes. For the efficient operation of C_4 photosynthesis, a short distance between the M and BS cells is an prerequisite for rapid diffusion of photosynthetic metabolites (Hatch, 1987). Leaf veins show a hierarchical order and have different structures.
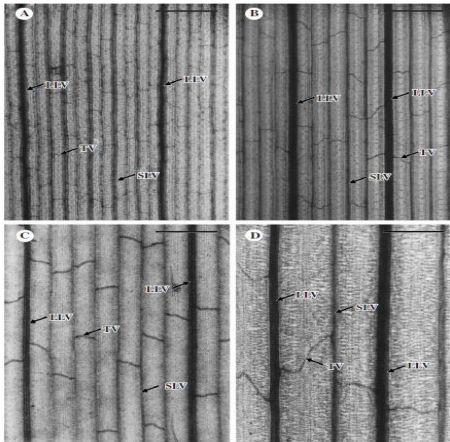
The large longitudinal veins serve primarily in longitudinal transport of photosynthate outside the leaf blade. The small longitudinal veins serve primarily in collecting photosynthate from nearby photosynthetic cells. The transverse veins connect the longitudinal veins, and play important roles in the lateral transport of photosynthate from the small to the large longitudinal veins (Altus and Canny, 1982).The transverse veins and small longitudinal veins play a vital role in lateral transport of photosynthate from the small to the large longitudinal veins (Altus and Canny, 1982).

Thus, denser development of transverse veins will probably be effective for movement of photosynthate from the small to the large longitudinal veins. C_4 grasses have acquired a superior photosynthate translocation system by developing denser networks of small longitudinal and transverse veins, while keeping a constant density of large longitudinal veins. C_4 grasses have a denser system of small longitudinal veins than C_3 grasses, as reported in previous work (Dengler *et al.,* 1994). The transverse veins and small longitudinal veins play a vital role in lateral transport of photosynthate from the small to the large longitudinal veins (Altus and Canny, 1982). Thus, denser development of transverse veins will probably be effective for movement of photosynthate from the small to the large longitudinal veins. C_4 grasses have acquired a superior photosynthate translocation system by developing denser networks of small longitudinal and transverse veins, while keeping a constant

density of large longitudinal veins. Reflecting the different conducting roles, the large longitudinal veins contain vessels of larger diameter than the small longitudinal veins.

Physiological traits
Water use efficiency: The yield potential of a crop depends on its to obtain resources from the environment and to use these resources to fix carbon dioxide into biomass. For grain crops, this also depends on the proportions of its biomass partitioned to grain (Long *et al.,* 2006). Desirable traits of crops in drought-susceptible and nitrogen- limited environments include efficient use of limited resources (Shangguan *et al.,* 2000). Water use efficiency provides an indicator of the performance of a crop growing under any environmental constraint. An improvement in water use efficiency not only improves crop yield and profitability but also reduces environmental impacts on groundwater recharge. Efficient photosynthetic nitrogen use is believed to increase the fitness of plants because high photosynthetic nitrogen use efficiency (PNUE) is associated with high relative growth rate (Hikosaka 2004). Low soil nitrogen can limit both the leaf area intercepting light and the capacity of plants to fix CO_2 photosynthetically. Improved crop water and nitrogen use efficiencies can therefore reduce the detrimental effects of drought and low soil nitrogen on leaf photosynthesis and crop yield.

Maize has higher Asat (light-saturated CO_2 assimilation rate), AQY (apparent quantum yield) and CE (Carboxylation efficiency) than in millet in Middle and late growth stages, indicating that maize used light and CO_2 more efficiently to reach a higher photosynthetic rate (Wang and Shangguan, 2014). The high carbon assimilation rate in maize could result in higher yields than in millet. Both maize and millet had high WUE in both growth stages, reflecting a relatively high Photosynthetic rate and low Transpiration rate. To obtain a higher Photosynthetic rate , plants must maintain a certain stomatal aperture, but any increase in stomatal aperture can lead to greater stomatal water loss. In later growth period, maize had higher Photosynthetic rate than millet, but due to the higher stomatal conductance it led to higher Transpiration rate, resulting in slightly lower WUE than millet. (Wang and Shangguan, 2014)

Photosynthetic nitrogen use efficiency: The parameter of PNUE is the ratio of the CO_2 assimilation rate to leaf nitrogen content and represents the economy of leaf photosynthesis (Hikosaka 2004). The observed PNUE tends to be higher in C_4 species than in C_3 plants (Anten *et al.,* 1995). Generally species with a high PNUE tend to have higher growth rates and higher productivity than those with a low PNUE (Hikosaka 2004). In this study, maize had much higher PNUE than millet in the two growth stages, indicating stronger resource capture and utilization capacities, especially with regard to nitrogen use. The proportion of nitrogen distributed between leaf photosynthetic organs and

nonphotosynthetic organs indicates a plant's strategy of increasing PNUE to improve photosynthetic efficiency versus increasing LMA to enhance defence capabilities.

Resource use efficiency: Resource utilization efficiency is the capacity of a plant to capture and use light, warmth, water, soil nutrition, etc. during growth. Plants with high resource use efficiency often have high productivity and are able to adapt well to environmental conditions in this case, enabling maize to have higher yields than millet in suitable conditions. In addition to inherent genetic factors, the environment of a plant also greatly affects its resource use efficiency. The higher leaf mass per area (LMA) of millet at the two growth stages indicated that millet is better than maize at tolerating stress (Wang and Shangguan, 2014). Therefore, stable yields of millet can be produced even in an extreme drought year. Zhang (2007) showed that millet had modest inter-annual variation in terms of yield compared with maize. This reliability probably is the main reason why millet has been cultivated so widely despite its lower yield.

Photosynthetic efficiency of *Setaria* under adverse conditions: Drought has a serious effect on plant growth and yield in many regions of the world. Environmental and endogenous factors interact to trigger the onset and progression of leaf senescence. During plant aging, drought stress usually induces the accumulation of reactive oxygen species (ROS) (Foyer and Noctor, 2005; Turkan *et al.,* 2005). If not effectively and rapidly removed from plants, ROS can damage a wide range of cellular macromolecules, such as lipids, enzymes and DNA. Plants combat oxidative damage by synthesizing antioxidative enzymes, such as catalase(CAT), ascorbate peroxidase (APX), superoxide dismutase (SOD), and glutathione reductase, as well as nonenzymatic compounds, such as glutathione and ascorbate, Malondialdehyde (MDA), a decomposition product of polyunsaturated fatty acid hydroperoxides, can result in oxidative damage and has frequently been used as a biomarker for lipid peroxidation (Turkan *et al.,* 2005). The rate of photosynthesis decreased mainly due to stomata closure (Sigaud-Kutner *et al.,* 2002). As the stress progressed, biochemical constraints might limit photosynthetic CO_2 fixation more directly (Dai *et al.,* 2011b). As limitation of CO_2 assimilation preceded inactivation of electron transfer reactions, an excess of reducing power is frequently generated in water-stressed plants (Chaves *et al.,* 2003).

Conclusion

The weedy S. viridis and domesticated S. italica species promise to serve as excellent model systems for the study of C_4 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. At present the requirement 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.

Present challenges and future work

Roundtable discussions at the ISGC 2014 indicated several key needs for development of Setaria as a model system. The first essential need for a successful Setaria model is the establishing of a stable and efficient transformation protocol. The efficient transformation systems available in Arabidopsis and rice greatly contributed to the maturation of these two species as model systems. Although there are reports of transformation of foxtail millet (Brutnell *et al.,* 2010), many scientists still find transformation to be challenging. Fortunately, there were four papers presented at ISGC 2014 describing the establishment of efficient protocols for transforming of Setaria, and one report of very high transformation rate of 29% (Martins *et al.,* 2014). Testing different components for optimizing transformation efficiency, such as screening diverse genotypes, as well as testing different types of explants, culture media and conditions, is currently being undertaken. The exchange of progress and discoveries from different efforts will provide a chance to establish a stable and efficient Setaria transformation system.

A second urgent need for development of Setaria as model is for a well-organized online database devoted to Setaria genetic and genomic information, along the lines of TAIR for Arabidopsis (www.arabidopsis.org) or Maize GDB for maize (www.maizegdb.org). This website could also be used to provide a searchable interface for a sequence-tagged mutant library constructed using t-DNA insertions or alternatively a set of resequenced EMS/Nnitrosomethylurea (NMU) /fast neutron mutant lines that would provide an equivalent reverse genetics resource. Although there are reports describing the initial generation of EMS mutant populations of Setaria (Diao *et al.,* 2014),and other mutant populations are in development, it will take a concerted effort to index the known mutations in these lines and make these data widely available to the community.

A third critical need for the Setaria community is the establishment of a core set of cultivars and populations that will be studied by individual laboratories. Several reports describe research investigating genetic diversity in Setaria (Jia *et al.,* 2013; Wang *et al.,* 2012), while others at ISGC 2014 discussed tool development in a key set of lines (for instance, S. Viridis Accession A10). Other groups described the construction of key mapping populations (Zhi *et al.,* 2014). Once a core set of genetic materials are settled upon by the Setaria community, comparison of genetic results and tool development will be much more efficient and productive.

REFERENCES

Altus, D. P. and Canny M. J. (1982). Loading of assimilates in wheat leaves. I. The specialization of vein types for separate activities. *Aust. J. Plant Physiol.* **9:** 571–581.

Anten, N. P. R., Schieving, F. and Werger, M. J. A. (1995). Patterns of light and nitrogen distribution in relation to whole canopy gain in C_3 and C_4 mono-and dicotyledonous species. *Oecologia* **101:** 504-513.

Bennetzen, J. L., Schmutz, J., Wang, H., Percifield, R., Hawkins, J., Pontaroli, A. C., Estep, M., Feng, L., Vaughn, J. N., Grimwood, J., Jenkins, J., Barry, K., Lindquist, E., Hellsten, U., Deshpande, S., Wang, X., Wu, X., Mitros, T., Triplett, J., Yang, X., Ye, C. Y., Mauro-Herrera, M.,Wang, L., Li, P., Sharma, M., Sharma, R., Ronald, P. C., Panaud,O., Kellogg, E. A., Brutnell, T. P., Doust, A. N., Tuskan, G. A., Rokhsar, D., Devos, K. M. (2012). Reference genome sequence of the model plant Setaria. *Nat. Biotechnol*. **30:** 555-561.

Brown, N. J., Parsley, K., and Hibberd, J. M. (2005). The future of C_4 research–Maize, Flaveria or Cleome? *Trends Plant Sci.* **10:** 215–221.

Brutnell, T. P., Wang, L., Swartwood, K., Goldschmidt, A., Jackson, D., Zhu, X. G., Kellogg, E., Van Eck, J. (2010). Setaria viridis: a model for C_4 photosynthesis. *Plant Cell* **22:** 2537–2544

Chaves, M., Maroco, J. and Pereira, J. (2003). Understanding plant responses to drought - from genes to the whole plant. *Funct. Plant Biol.* **30:** 239–264.

Christin, P. A., Petitpierre, B., Salamin, N., Buchi, L. and Besnard, G. (2009a). Evolution of C phosphoenolpyruvate carboxykinase in grasses, from genotype to phenotype. *Mol. Biol. Evol.* **26:** 357–365.

Christin, P. A., Salamin, N., Kellogg, E.A., Vicentini, A. and Besnard, G. (2009b). Integrating phylogeny into studies of C_4 variation in the grasses. *Plant Physiol.* **149:** 82–87.

Christin, P.A., Samaritani, E., Petitpierre, B., Salamin, N. and Besnard, G. (2009c). Evolutionary insights on C_4 photosynthetic subtypes in grasses from genomics and phylogenetics. *Genome Biol. Evol.* **1:** 221–230.

Dai, H. P., Zhang, P. P., Lu, C., Jia, G. L., Song, H., Ren, X. M., Chen, J., Wei, A. Z., Feng, B. L., Zhang, S. Q. (2011b). Leaf senescence and reactive oxygen speciesmetabolism of broomcorn millet (*Panicum miliaceum* L.) under drought condition. *Australian Journal of Crop Sci.* **5:**1655-1660.

Dengler, N.G., Dengler, R. E., Donnelly, P.M. and Hattersley, P. W. (1994). Quantitative leaf anatomy of C_3 and C_4 grasses (Poaceae): bundle sheath and mesophyll surface area relationships. *Ann. Bot.* **73:** 241–255.

Dengler NG, Nelson T. (1999). Leaf structure and development in C_4 plants. In: Sage RF, Monson RK, eds. C_4 plant biology. San Diego: Academic Press, 133–172.

Diao, X., Li, W., Zhi, H., Jia, G., Ge, Y., Chai, Y. and Li, J. (2014). Construction of an EMS mutation library for foxtail millet functional genomics. The first international Setaria genetics conference abstracts, Beijing, 56

Diao, X. (2005). Advances in foxtail millet biotechnology and its future directions. *J. Hebei Agricul. Sci.* **9:** 61–68.

Diao, X. (2007). Foxtail millet production in China and its future development tendency. In: Chai, Y. Wan, F. S, eds. The industrial development of China special crops. Beijing. Chinese Agricultural Science and Technology Press, 32–43.

Doust, A. N., Kellogg, E. A., Devos, K.M. and Bennetzen, J. L. (2009). Foxtail millet: a sequence-driven grass model system. *Plant Physiol.* **149:** 137–141

Edwards, E. J. and Smith, S. A. (2010). Phylogenetic analyses reveal the shady history of C_4 grasses. *Proc. Nat. Acad. Sci. USA* **107:** 2532–2537.

Foyer, C. H. and Noctor, G. (2005). Oxidant and antioxidant signalling in plants: a re-evaluation of the concept of oxidative stress in a physiological context. *Plant Cell Environ,* **28:**1056–1071.

Hatch, M. D. (1971). The C_4-pathway of photosynthesis: Evidence for an intermediate pool of carbon dioxide of the donor C_4-dicarboxylic acid. *Biochemistry* **125:** 425–432.

Hatch, M. D. (1987). C_4 photosynthesis: a unique blend of modified biochemistry, anatomy and ultrastructure. *Biochim. Biophys. Acta.* **895:** 81–106.

Hikosaka, K. (2004). Interspecific difference in the photosynthesis_nitrogen relationship: patterns, physiological causes, and ecological importance. *J. Plant Res.* **117:** 481- 494.

Jia, G., Huang, X., Zhi, H., Zhao ,Y., Zhao, Q., Li, W., Chai, Y., Yang, L., Liu, K., Lu, H., Zhu, C., Lu, Y., Zhou, C., Fan, D., Weng, Q., Guo, Y., Huang, T., Zhang, L., Lu, T., Feng, Q., Hao, H., Liu, H., Lu, P., Zhang, N., Li, Y., Guo, E., Wang, S.,Wang, S., Liu, J., Zhang, W., Chen, G., Zhang, B., Li, W., Wang, Y., Li, H., Zhao, B., Li, J., Diao, X. and Han, B. (2013). A haplotype map of genomic variations and genome-wide association studies of agronomic traits in foxtail millet (Setaria italica). *Nature Genetics* **45:** 957– 961

Jia, G., Shi, S., Wang, C., Niu, Z., Chai, Y., Zhi, H. and Diao, X. (2013). Molecular diversity and population structure of Chinese green foxtail [Setaria viridis (L.) Beauv.] revealed by microsatellite analysis. *J. Exp. Bot.* **64:** 3645–3656

Leegood, R. C. (2002). C$_4$ photosynthesis: principles of CO$_2$concentration and prospects for its introduction into C(3) plants. *J. Exp. Bot.* **53:** 581–590

Li, P. and Brutnell, T. P. (2011). Setaria viridis and Setaria italica, model genetic systems for the Panicoid grasses. *J. Exp. Bot.* **62:** 3031–3037

Li, P., Ponnala, L., Gandotra, N. *et al.*, (2010). The developmental dynamics of the maize leaf transcriptome. *Nature Genet.* **42:** 1060–1067.

Long, S.P., Zhu, X.G., Naidu, S.L, Ort, D.R. and Usda, A. R. S. (2006). Can improvement in photosynthesis increase crop yields? *Plant Cell. Environ.* **29:** *315- 330.*

Martins, P. K., Dias, B. B. A., Ribeiro, A. P., Kobayashi, A. K., Molinari, H. B. C. (2014). *Setaria viridis*: a tool for functional gene analysis in sugancane. The first international Setaria genetics conference abstracts, Beijing, 19

Mitchell, P. L. and Sheehy, J. E. (2007). The case for C$_4$ rice. In Charting New Pathways to C$_4$ Rice, J.E. Sheehy, P.L. Mitchell, and B. Hardy, eds (Los Banos, Philippines: International Rice Research Institute), pp. 27–36.

Ohsugi, R. and Murata, T. (1986). Variations in the leaf anatomy among some C$_4$ Panicum species. *Ann. Bot.* **58:** 443–453.

Paterson, A.H., Bowers, J. E. and Bruggmann, R. (2009). The Sorghum bicolor genome and the diversification of grasses. *Nature* **457 :** 551–556.

Rizal, G., Karki, S. and Thakur, V. (2012). "Towards a C$_4$ rice," *Asian J. Cell Biol.* **7:** 13–31

Rominger, J. M. (1962). Taxonomy of Setaria (Gramineae) in North America. In: Illinois Biol Monogr, volume 29. Edited by Horsfall WR, Delevoryas T, De Moss RD, Kruidenier FJ, and Taylor AB. Urbana: University of Illinois Press; 100–108

Sage, R. F. (2004). The evolution of C$_4$ photosynthesis. *New Phytol.* **161:** 341–370

Sage, R.F. and Pearcy, R.W. (2000). The physiological ecology of C$_4$ photosynthesis. In Photosynthesis: Physiology and Metabolism, T.D. Sharkey and S. von Caemmerer, eds (Dordrecht, The Netherlands: Kluwer Academic Publishers), pp. 497–532.

Schnable, P.S., Ware, D., Fulton, R. S. et al., (2009). The B73 maize genome: complexity, diversity, and dynamics. *Science* **326:** 1112–1115.

Shangguan, Z. P., Shao, M. A. and Dyckmans, J. (2000). Nitrogen nutrition and water stress effects on leaf photosynthetic gas exchange and water use efficiency in winter wheat. *Environ. Exp. Bot.* **44:** 141- 149.

Sigaud-Kutner, T. C. S., Pinto, E., Okamoto, O. K., Latorre, L. R. and Colepicolo, P. (2002). Changes in superoxide dismutase activity and photosynthetic pigment content during growth of marine phytoplankters in batch-cultures. *Physiol. Plant.* **114:**566–571.

Turkan, I., Bor, M., Ozdemir, F. and Koca, H. (2005). Differential responses of lipid peroxidation and antioxidants in the leaves of drought-tolerant *P. acutifolius* Gray andmdrought-sensitive *P. vulgaris* L. subjected to polyethylene glycol mediated water stress. *Plant Sci.* **168:** 223–231.

Wang, C. F., Jia, G. Q., Zhi, H., Niu, Z. G., Chai, Y., Li, W., Wang, Y. F., Li, H. Q., Lu, P., Zhao, B. H. and Diao, X. M. (2012). Genetic diversity and population structure of Chinese foxtail millet [Setaria italica (L.) Beauv.] land races. G3 (Bethesda), **2(7):** 769–777.

Wang, K. and Shangguan, Z. (2010). Photosynthetic characteristics and resource utilization efficiency of maize (Zea mays L.) and millet (Setaria italica L.) in a semi-arid hilly loess region in China N. *Z. J. Crop Hortic. Sci.* **38:** 247-254

Wang, L., Li, P. and Brutnell, T. P. (2010b). Exploring plant transcriptomes using ultra high-throughput sequencing. *Briefings Funct. Genomics* **9:** 118–128.

Wang, X., Gowik, U., Tang, H., Bowers, J.E., Westhoff, P. and Paterson, A. H. (2009). Comparative genomic analysis of C$_4$ photosynthetic pathway evolution in grasses. *Genome Biol.* **10:** R68.

Wang, K. and Shangguan, Z. (2014). Photosynthetic characteristics and resource utilization efficiency of maize (*Zea mays* L.) and millet (*Setaria italica* L.) in a semi-arid hilly loess region in China. *N. Z. J. Crop Hortic. Sci.* **38(4):** 247-254

Yanagisawa, S. and Sheen, J. (1998). Involvement of maize Dof zinc finger proteins in tissue-specific and light-regulated gene expression. *The Plant Cell* **10:** 75–89.

Yoshimura, Y, Kubota F, Ueno O. (2004). Structural and biochemical bases of photorespiration in C$_4$ plants: quantification of organelles and glycine decarboxylase. *Planta* **220:** 307–317.

Zhang, X. (2007). Drought adaptability of main minor crops in Loess Plateau. *J. Arid Land Resources Environ.* **21:** 111-115.

Zhao, M., Zhi, H., Doust, A. N., Li, W., Wang, Y., Li, H., Jia, G., Wang, Y., Zhang, N. and Diao, X. (2013). Novel genomes and genome constitutions identified by GISH and 5S rDNA and knotted1 genomic sequences in the genus Setaria. *BMC Genomics* **14:** 244

Zhi, H., Jia, G., Niu, Z., Liu, X., Ge, Y., Chai, Y. and Diao, X. (2014). Construction of an RIL population and segment introgression lines via interspecific cross between Setaria italica and S. viridis. The first international Setaria genetics conference abstracts, Beijing, 50

Zhu, X. G., Shan, L., Wang, Y. and Quick, W. P. (2010). C_4 rice - an ideal arena for systems biology research. *J. Integrative Plant Biol.* **52:** 762–770.

Zhu, X. G., Long, S. P. and Ort, D. R. (2008). What is the maximum efficiency with which photosynthesis can convert solar energy into biomass? *Curr. Opin. Biotechnol.* **19:** 153–159.