Enhancing the productivity of grasses under high-density planting by engineering light responses: from model systems to feedstocks

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Abstract

The successful commercialization of bioenergy grasses as lignocellulosic feedstocks requires that they be produced, processed, and transported efficiently. Intensive breeding for higher yields in food crops has resulted in varieties that perform optimally under high-density planting but often with high input costs. This is particularly true of maize, where most yield gains in the past have come through increased planting densities and an abundance of fertilizer. For lignocellulosic feedstocks, biomass rather than grain yield and digestibility of cell walls are two of the major targets for improvement. Breeding for high-density performance of lignocellulosic crops has been much less intense and thus provides an opportunity for improving the feedstock potential of these grasses. In this review, we discuss the role of vegetative shade on growth and development and suggest targets for manipulating this response to increase harvestable biomass under high-density planting. To engineer grass architecture and modify biomass properties at increasing planting densities, we argue that new model systems are needed and recommend Setaria viridis, a panicoid grass, closely related to major fuel and bioenergy grasses as a model genetic system.

Key words: Biomass, far-red, high-density planting, phytochrome, red, shade avoidance.

Introduction

A global population that is expected to reach 9.3 billion by the year 2050 with a rising middle class will continue to drive both global food production and energy demand (Bruinsma, 2003). Determining the ideal combination of food, feed, and fuel-targeted bioproducts is not a simple task and has been the subject of much debate (Brutnell and Frommer, 2012), but a common goal of these efforts is to raise output with fewer inputs. This is particularly true for dedicated bioenergy grasses that must compete with sources of fuel that are currently much cheaper to produce such as natural gas and oil. Although they are more expensive, dedicated bioenergy grasses afford many benefits that are harder to quantify such as the potential to improve soil quality, prevent erosion of marginal lands, increase species diversity, improve water quality, and ultimately provide carbon neutral sources of energy (Adler et al., 2007; Meehan et al., 2010). To realize the full potential of these grasses, varieties that are high yielding under increased planting densities and that require minimum inputs of fertilizer and water must be developed.

An important determinant of plant yield is the light environment in which the plants grow. Both light quantity (incident radiation) and light quality (light spectrum) affect plant growth and development and ultimately determine plant yield (Chory, 2010; Kami et al., 2010; Zhu et al., 2010; Li et al., 2012a). Biomass accumulation is dependent on the radiation use efficiency and light interception and the latter is defined by the architecture of the canopy, as well as planting density (Byrt, 2011). The most promising biofuel feedstocks are undomesticated grasses. There is growing interest in understanding the signalling mechanisms regulating light-mediated
control of biomass, particularly in the context of high-density planting. Planting at increased densities has proven to be an effective mechanism to increase maize yields (Duvick, 1997), but has not been systematically applied to breeding bioenergy grasses. In this review, we discuss the current understanding of light signalling that is fundamental to shade response and suggest ways that these networks may be manipulated in grasses to help improve the output of bioenergy grasses.

**Responses to vegetative shade**

Environmental cues inform endogenous growth and developmental programmes through complex signal transduction networks. In response to light exposure, plants utilize a number of photoreceptors to modulate growth and development including ultraviolet B (UV-B), blue/ultraviolet A (B/UV-A), and red/far-red (R/FR) receptors (Chen et al., 2004). Plants grown in high densities are subject to vegetative shading and several classes of photoreceptors are known to regulate shade-induced changes in growth and development, such as phytochromes (Smith, 2000), cryptochromes, phototropins (Briggs and Christie, 2002), and UVR8 (Rizzini et al., 2011). The red (R)/far-red (FR)-absorbing phytochromes are the most extensively studied of the higher plant photoreceptors (Neff et al., 2000; Smith, 2000; Nagy and Schafer, 2002; Schepens et al., 2004; Franklin and Quail, 2010) and the phytochrome gene family in monocots is composed of three members: PHYA, PHYB, and PHYC (Mathews and Sharrock, 1996, 1997). The changes in R:FR ratios accompanied by vegetative shading are perceived primarily by phyB. R light triggers the photoconversion of phyB from the Pr to the Pfr form, while FR light photoconverts phyB from the Pfr to the Pr form. The Pfr form of phyB is able to interact physically with transcription factors that act downstream of R/FR perception (Casal, 2013). Vegetative shading is characterized by a R:FR ratio of about ~0.1 to 0.2 and the low R:FR ratio induces a host of developmental and physiological changes, collectively referred to as the shade avoidance response (SAR). The SAR is thought to provide an adaptive advantage by increasing a plant’s ability to compete for limited resources in natural settings (Smith, 1982; Ballare et al., 1989; Schmitt, 1997; Schmitt et al., 2003). Although this developmental plasticity may promote plant survival under competing vegetation, it is accompanied by potentially deleterious effects on a plant’s fitness, disease resistance, and/or yield (Libenson et al., 2002; Weijspedé et al., 2006; Weining et al., 2006; Robson et al., 2010). In crop plants, a robust SAR could divert resources to stem elongation at the cost of reproductive fitness (Sessa et al., 2005; Kebrom and Brutnell, 2007); and thus, domestication over the decades has likely favoured either the attenuation or refinement of SAR to maximize grain yields (Smith, 1992). Although targeted breeding and selection has helped in suppressing SAR, disabling responses to low R:FR ratios by genetic manipulation of phytochrome genes or downstream signalling components offers a potential alternative to increase the harvest index. This is particularly true with bioenergy crops that tend to have polyploid genomes and are recalcitrant to genetic analyses. While attenuation of responses to shade has the potential to lessen the competitive ability of plants for limited resources under high densities, the developmental plasticity of the SAR also offers an opportunity to re-engineer shade perception to human benefit when the canopy becomes dense. A better understanding of the molecular switch that occurs under shade may enable a synthetic biology approach to rewiring the response network that would effectively convert good competitors into good neighbours. These efforts to engineer the response network will maximize productivity on a canopy level rather than optimizing per plant performance.

**Enhancing productivity at high density**

Lignocellulosic biomass for cellulosic biofuel production is primarily obtained from vegetative tissues. Two approaches that have been used to obtain higher yield gains in food crops are planting at high densities (Dunwell, 2000; Maddonni et al., 2002; Boccalandro et al., 2009) and increasing photosynthetic efficiency through alterations of leaf architecture (Sakamoto and Matsuoka, 2004; Torney et al., 2007). However, unlike most breeding efforts where selection has been on seed production, enhancing biomass production will require partitioning of carbon to above ground vegetative tissues. Photosynthetic efficiency, shoot and tiller development, and primary and secondary cell-wall development are among the primary traits that govern biomass production and fermentability.

Non-food grasses such as Miscanthus and switchgrass are promising second generation lignocellulosic biofuels (Yuan et al., 2008; Somerville, 2010; Heaton et al., 2008, 2010; Dohleman and Long, 2009). However, breeding improvements in production varieties of Miscanthus (a sterile triploid) and switchgrass (an obligate outcrossing polyploid) will be hampered by the challenging genetics. Thus, model systems that are closely related to these target feedstocks offer the potential to accelerate the understanding of the mechanisms that regulate target traits and transfer the knowledge into crop improvement through transgenic manipulations or enhanced breeding efforts.

In the grasses, carbon is often partitioned between primary (main culm) and secondary (tillers) shoots. One of the primary environmental influences on this partitioning in grasses is light, in particular the ratio of R:FR perceived by phyB (Kebrom and Brutnell, 2007). Under vegetative shading where light quality is characterized by a low R:FR, apical dominance is enhanced (Smith and Whitelam, 1997) resulting in reduced tiller formation, reduced harvest index, and enhanced lodging (Kasperbauer and Karlen, 1994; Andreiu et al., 2006; Kebrom and Brutnell, 2007). Under shade conditions, the inactivation of phyB initiates a signal transduction cascade affecting both primary and secondary shoot growth and development. However, the plasticity of the SAR enables selection against ‘unfavourable’ phenotypes in a shaded environment and selection for ‘favourable’ phenotypes at increasing densities.
To manipulate SAR, phytochrome photoreceptors (i.e. \textit{PHYA} and \textit{PHYB}) have been overexpressed in several dicots and monocots (Boylan and Quail, 1989; Keller et al., 1989; Nagatani et al., 1991; Robson et al., 1996; Thiele et al., 1999; Shlumukov et al., 2001; Boccaletandro et al., 2003; Sawers et al., 2005). However, the beneficial effects of \textit{PHY} manipulation are often accompanied by negative effects on plant architecture and yield, and may also display dramatic cultivar- and species-specific variations in phenotypes (Keller et al., 1989; Nagatani et al., 1991; Clough and Vierstra, 1997; Shlumukov et al., 2001; Garg et al., 2006). To more precisely engineer plant responses to shade, it may be possible to alter the expression of effectors that act downstream of phyB-mediated perception of low R:FR allowing finer control of a subgroup of shade-induced responses.

To this date, little progress has been made in improving the biomass yields in bioenergy crops through genetic engineering, especially at higher planting densities (Fu et al., 2012). One approach to engineer biomass for higher yields would be to fine tune genetic responses to light signals. However, there remain a number of challenges to this approach as well. For instance, redundancies in the signalling networks may preclude the use of loss-of-function alleles in downstream effectors and require more sophisticated use of dominant negative and tissue-specific expression of transgenes. Furthermore, the desired architecture may vary with cultivation practices and end use. For example, tillers that develop later in the growing season could potentially increase net biomass per plant (Sakamoto and Matsuoka, 2004) by effectively extending the growing season without affecting lodging of the main culm. Currently, bioenergy feedstocks are planted at relatively low densities and under this scenario, plasticity for tiller production may enable plants to better utilize the light environment. However, under high-density planting, tiller production is likely counterproductive to yield enhancement. Under dense planting, Miscanthus and switchgrass are likely to respond to shade with elongated internodes and potentially repress axillary branching. In maize and rice, breeding has selected for stable tiller development and thus less plasticity of SAR. Thus, selecting for lines of Miscanthus or switchgrass with reduced tillers regardless of planting density may be a strategy for increasing yield at the expense of plasticity. A role for phyB in tiller development has been reported in sorghum (Kebram et al., 2006; Kebrum and Bratrull, 2007; Kebrum et al., 2010). Although a number of tillering-related negative and positive regulatory genes have been identified, including \textit{TEOSINTE BRANCHED1} (\textit{TBI}) in maize (Doebley et al., 1997), Os\textit{TBI} in rice (Takeda et al., 2003), \textit{HIGH TILLERING DWARF1} (\textit{HTD1}) (Zou et al., 2006), and \textit{MONOCULM1} (\textit{MOCl}) (Li et al., 2003), the mechanisms by which these genes interact with light signalling pathways and integrate shade signals perceived by phyB is poorly understood.

The initial period of vegetative development is characterized by the iterative production of leaves from the shoot meristem. The shoot meristem undergoes a change in fate and enters reproductive development, when flowering is initiated (Poethig, 1990; Henderson and Dean, 2004). In bioenergy grasses, the delay or inhibition of flowering is desirable as carbon assimilates are not shunted to seed production from vegetative growth. Light quality is among the several environmental signals that regulate the transition from vegetative growth to reproductive organ development (Henderson and Dean, 2004). A low R:FR ratio accelerates flowering (Halliday et al., 1994) and thus may be viewed as antagonistic to vegetative growth. The light-quality pathway mediating the acceleration of flowering in low R:FR has been shown to operate through the expression of the floral integrator \textit{FLOWERING TIME} (\textit{FT}) (Cerdan and Chory, 2003; Halliday and Fankhauser, 2003) and the activation of this floral integrator by the phyB-dependent light-quality pathway requires the transcriptional activator \textit{PHYTOCHROME AND FLOWERING TIME1} (\textit{PFT1}) (Cerdan and Chory, 2003). In \textit{Arabidopsis}, \textit{pft1} mutants display impaired flowering responses to end-of-day FR treatments suggesting a role in transducing shade signals to the meristem. Homologues of \textit{Arabidopsis PFT1} have not been characterized in grasses, so the conservation of this pathway in monocots is less clear. In barley, four recessive mutations are known to cause insensitivity to photoperiod (Gallagher et al., 1991) and wild-type genes are potential repressors of flowering (Laurie, 1997). Thus, overexpression of these genes may be useful to maintain plants in vegetative phase.

Photoperiodic induction of flowering in \textit{Arabidopsis} involves perception of light signals by phyA and cryptochrome 2 in the leaves (Percy, 2005; Zevaarta, 2006). The key regulator of the photoperiod pathway, \textit{CONSTANS} (\textit{CO}), activates \textit{FT} in the leaves where it moves via the phloem sap to the apical meristems. In the meristem, \textit{FT} interacts with the \textit{FLOWERING LOCUS D} (\textit{FD}) to trigger the floral transition (Halliday et al., 2003; Corbesier et al., 2007). \textit{SUPPRESSOR OF OVEREXPRESSION OF CONSTANS I} (\textit{SOC1}) and \textit{FRUITFULL} (\textit{FUL}) are MADS box genes that act downstream of \textit{FT} in the apical meristems. Mutations in \textit{SOC1}, \textit{FT}, or \textit{FUL} genes not only cause an indeterminate apical meristem activity but also an increase in vascular cambium activity (Demura and Ye, 2010). By increasing the vegetative meristem and vascular cambium activity, as well as delaying the switch from the vegetative to the reproductive phase (Salehi et al., 2005; Melzer et al., 2008; Demura and Ye, 2010), a plant is forced to remain in the vegetative phase and it results in more resource allocation to vegetative growth. Thus, genes involved in regulating flowering time and meristem activity could potentially be manipulated for higher biomass yields in lignocellulosic-based biofuel crops. However, an important caveat to these studies is that little is known of genes and networks regulating flowering time in grasses that have diverged substantially from their eudicot relatives (Laurie, 1997; Izawa et al., 2003; Colasanti and Coneva, 2009). Thus, a sophisticated engineering of flowering time response in bioenergy grasses will likely require a much deeper understanding of the factors that contribute to flowering in grasses (Colasanti and Coneva, 2009; Mauro-Herrera et al., 2013).

Transcription factors that interact with phytochromes within the primary SAR signalling cascade are potential targets for engineering of SAR. The primary signalling cascade
involves the physical interaction of phyB in the Pfr form with a subset of basic helix–loop–helix transcriptional factors, collectively known as the PHOTOTROPIC INTERACTING FACTORS (PIFs) (Duke and Fankhauser, 2005; Monte et al., 2007). PIFs and PIF-like transcription factors (PILs) can function as positive and negative regulators of their target genes (Martinez-Garcia et al., 2000; Kim et al., 2003). Among the seven PIFs that have been characterized in Arabidopsis, PIF3 (Sellaro et al., 2011; Leivar et al., 2012a,b), PIF4, PIF5 (Lorrain et al., 2008; Sellaro et al., 2011; Leivar et al., 2012a) and PIF7 (Li et al., 2012b) have been associated with the induction of SAR. Several reports indicate that proteins similar to PIFs and PILs are functional in monocots to mediate phytochrome or drought responses (Nakamura et al., 2007; Todaka et al., 2012). For example, in rice, OsPIL13/14 and OsPIL15/14 are homologues of Arabidopsis PIF4 and PIF3, respectively (Nakamura et al., 2007). PIF7 has been characterized as a major positive regulator of SAR. PIF7 directly binds to G-boxes of auxin biosynthetic genes to promote auxin biosynthesis and consequently enhances stem elongation under shade (Li et al., 2012b). Shade responses such as stem elongation, leaf hyponasty, and apical dominance are resultant phenotypes of high auxin levels and these phenotypes further confirm that a low R:FR ratio causes a change in auxin levels. PIF7 is a potential target for increasing plant height and taller plants will potentially have an adaptive benefit to harvest light energy maximally and flourish in dense canopies. Increased plant height is a characteristic morphological change associated with SAR and allows the plants to project out from the canopy shade. In cereal crops such as rice, maize, and sorghum, the elongation of internodes and leaf sheaths is observed under shade (Takano et al., 2001, 2005; Andrieu et al., 2006; Kebrom et al., 2006; Finlayson et al., 2007; Sheehan et al., 2007). However, increased plant height could also contribute to more lodging and less robust stands. Thus, the fine tuning of SAR will likely be required to maximize biomass gains with minimal yield drag.

A dynamic balance between positive effectors and negative effectors is crucial for regulating SAR (Sessa et al., 2005). While PIF3, 4, 5, and 7 are positive regulators of shade-induced responses, ATHB-2 (Steindler et al., 1999) and HFR1 (Sessa et al., 2005) act as negative regulators of SAR under prolonged shade by forming a negative feedback loop to prevent overresponsiveness to shade. Negative regulators of SAR can be expressed during later stages of plant development using developmental stage-specific promoters to alter the plasticity of SAR when the canopy becomes more dense. Based on transcriptomics analyses in shade, the early responsive genes to shade include ATHB2, ATHB4, PIL1, HFR1, PARI, PAR2, HAT1, HAT2, and HAT3 and these genes are collectively called PHOTOTROPIC RAPIDLY REGULATED (PAR) genes (Devin et al., 2003; Roig-Villanova et al., 2006; Jiao et al., 2007; Martinez-Garcia et al., 2010). Among the PAR genes, ATHB2, ATHB4, PIL1, and PARI genes are directly regulated by phytochrome activity and thus may mediate early responses to SAR (Roig-Villanova et al., 2006). PAR genes may integrate shade signals perceived by phytochromes to regulate the transcriptional network that control physiological and developmental responses to shade. Again, the genetic dissection of PAR genes in grasses may provide opportunities to engineer plant architecture for favourable traits in response to shade.

Another potential target for manipulation of vegetative growth is cell elongation. Alterations in biosynthesis and signalling of gibberellin (GA) and brassinosteroid can alter cell elongation (Wang et al., 2012). DELLA, a group of growth inhibitory proteins, function as key repressors of GA-responsive growth, by inhibiting GA-regulated gene expression (Sun, 2008). Under shade, the stability of DELLA is reduced due to increased GA levels and leads to promotion of cell elongation (Djakovic-Petrovic et al., 2007; Sun, 2008). Like GAs, brassinosteroid antagonizes light signals and may act in concert with GA and auxin to promote shade avoidance responses (Depuydt and Hardtke, 2011). Light signals perceived by phytochromes are likely integrated into GA signalling through the binding of DELLA to PIF3 and PIF4 (de Lucas et al., 2008; Feng et al., 2008). Given the positive regulatory role of PIF3 and PIF4 in shade and negative regulatory role of DELLA, downregulation of DELLA or overexpression of PIF3 or PIF4 leading to degradation of DELLA may enhance the brassinosteroid and GA-responsive cell elongation.

Ultimately, photosynthesis is the driver of biomass accumulation in plants and may provide one of the best targets for improving yields (Zhu et al., 2010). The most promising biofuel grasses, such as Miscanthus and switchgrass, are C4 plants which have greater photosynthetic conversion rates due to their capacity to concentrate CO2 and minimize photorespiration (Zhu et al., 2008). Surprisingly, few experiments have been conducted to manipulate photosynthetic efficiencies in grasses. The challenge in engineering photosynthetic efficiencies is that, until recently efficient transformation of switchgrass has not been possible and Miscanthus giganteus remains refractory to transgenic manipulation. Another challenge in engineering photosynthesis is that assessments of performance must be conducted at the canopy level rather than when plants are grown in isolation. In closed canopy, the light distribution is extremely unequal, with lower leaves receiving much less light than leaves in the upper canopy, which may be light saturated for photosynthesis. In fact, under full sunlight, available solar energy rapidly exceeds the capacity of the photosynthetic apparatus and up to 80% of the absorbed energy must dissipate or it risks causing damage to the photosystems (Melis, 2009). Decreasing the chlorophyll content in the leaves of the upper canopy, which are exposed to higher light levels, will enable more light to filter to lower leaves in the canopy with less photodamage and photoinhibition in the upper canopy (Ort et al., 2011). Higher chlorophyll levels in the leaves of the lower canopy will contribute to better photosynthetic rates as more light is able to filter to the lower canopy. Differential accumulation of chlorophylls will maximize overall photosynthetic rates through the allotment of available light between the upper and lower canopies. Thus, canopy level-specific engineering of chlorophyll levels may be a mechanism to mitigate the light-saturation of photosynthesis and maximizes carbon assimilation at all levels within the canopy.
In hybrids of Arabidopsis accessions, increases in vegetative biomass have been observed as a result of more chloroplasts and chlorophyll per cell (Fujimoto et al., 2012). Thus, increasing the plastid number per cell is also a potential avenue for photosynthetic manipulation and several genes affecting plastid division are known (Vitha et al., 2001). Redesigning photosynthetic machinery to have more chloroplasts with elevated chlorophyll amounts and light harvesting complexes in the leaves of the lower canopy will contribute to higher photosynthetic capacity per unit of leaf area. Increasing biomass per plant combined with dense planting will significantly increase harvestable biomass from a unit of land area.

Grass architecture can also be targeted for increased photosynthesis. For example, leaf angle and leaf area distribution have been subjected to intense genetic selection to achieve erect canopies and optimal distribution in maize, rice and wheat (Byrt, 2011). The growth of leaf blades is suppressed under low R:FR conditions (McLaren and Smith, 1978) and such reductions in leaf blade area cause photosynthetic rates to decrease. Total leaf blade area that is able to capture solar radiation can be increased by manipulating leaf orientation. In dense canopies, leaves orient themselves in a more erect position (Ballare and Scopel, 1997; Djakovic-Petrovic et al., 2007). Erect leaves are a necessary adaption to obtain high leaf area index (Sinclair and Sheehy, 1999) and have been a target for breeding high-yielding rice (Sakamoto and Matsuoka, 2004). By reducing the levels of brassinosteroids or the number of brassinosteroid receptors, a more erect leaf phenotype can be accomplished (Sakamoto et al., 2006). For example, the d61 mutant carries a lesion in the rice homologue of the BRASSINOSTEROID INSENSITIVE1 (BRI1) brassinosteroid receptor (Li et al., 2002; Nam and Li, 2002). Rice OsBRI1 mutants (Yamamuro et al., 2000) display an erect leaf phenotype. In Arabidopsis, mutations in BRII improve photosynthesis and leaf growth/vascularization and thus lead to increases in whole-plant carbon assimilation and growth (Oh et al., 2011). Targets that can be manipulated to obtain more erect leaves under shade include transcription factors that regulate brassinosteroid responses by interacting with PIFs. BRASSINAZOLE-RESISTANT 1 (BZR1) transcription factor interacts with PIF4 to control the expression of common target genes involved in cell elongation in response to multiple environmental stimuli (Oh et al., 2012). DELLA negatively regulate brassinosteroid signalling by binding BZR1 and blocking its binding to brassinosteroid target genes (Bai et al., 2012). Target genes of BZR1–PIF4 with specific roles under shade will be attractive candidates to alter grass architecture for biomass gains through better leaf orientation. Under dense planting, a more erect leaf phenotype in the lower canopy or in the lower part of the plant may be able to enhance photosynthesis by efficiently capturing sunlight (Sakamoto et al., 2006).

Improvement of biomass properties

Lignocellulose from bioenergy feedstocks is a sustainable source of fermentable sugars for biofuel production. The composition of lignocellulose determines the physical barriers to hydrolytic enzyme digestion and the efficiency of converting biomass to biofuels, which ultimately determines the efficiency and cost of biofuel production. Cell-wall recalcitrance is the largest contributor to the high expense of lignocellulose conversion to biofuels (Simmons et al., 2008); thus, modifying cell-wall properties is a promising strategy to readily convert lignocellulose to the desired plant-based fuels. The process of converting biomass to biofuels is hampered by the presence of lignin in cell walls, which limits not only the degradability but also the digestibility of feedstocks by making polysaccharides unavailable to enzymic digestion. Therefore, altering the lignin profile has been a target of genetic manipulation to reduce the cost of bioenergy production.

Secondary cell walls in fibres and tracheary elements constitute the most abundant biomass produced by plants (Zhong et al., 2006). Thus, modifying the quantity and quality of secondary walls will have a significant impact on plant biomass production. The composition of cell walls is of particular interest in high-density planting of bioenergy grasses since SAR is characterized by the promotion of stem elongation and likely accompanies alterations in cell-wall structure. In Arabidopsis, SAR promotes the expression of genes related to cell walls, cell elongation, cell division, and auxin (Devlin et al., 2003). Microarray analyses of stem transcriptome responses to low R:FR in tomato confirmed that shade induces the genes involved in cell-wall carbohydrate metabolism, but not lignin content (Cagnola et al., 2012). Additionally, several xyloglucan endotransglycosylase-hydrolase (XTH) genes display increased expression in the petioles but not in the blade (Kozuka et al., 2010). Cell-wall extensibility by XTH genes is regulated by phytochromes in several species (Sasidharan et al., 2008, 2010; Sasidharan and Pierik, 2010). Among the cell-wall-associated XTH genes, XYLEM GLUCAN ENDOTRANSGLYCOSYLASE 7 (XTR7) and XYLOGLUCAN ENDOTRANSGLUCOSYLASE HYDROLASE 15 (XTH15) are directly controlled by PIFs through binding to the promoters of these genes (de Lucas et al., 2008; Hornitschek et al., 2012; Leivar et al., 2012b). Controlled overexpression of such cell-wall-loosening enzymes during later developmental stages may result in less recalcitrant cell walls for easier digestibility. Moreover, proteins involved in cell-wall deconstruction (e.g. expansins; Cosgrove, 2005), could also prove good targets for manipulation. To maintain fitness and structural support upon modifications in cell-wall composition, desirable genetic manipulations can be targeted to specific tissues, cell types, or developmental stages (Byrt, 2011). Elucidation of the transcriptional networks that modulate the expression of the key activators and/or repressors underlying the expression of secondary wall biosynthetic genes will provide valuable tools to modify plant biomass quality and production.

The need for a new model system

Significant gains in productivity are likely to be realized through breeding and engineering feedstock plants to
perform under high-density planting. The maize and sorghum genomes provide an opportunity to discover genes that regulate biomass accumulation and improve biomass quality through comparative genomics with feedstocks such as *Miscanthus* and switchgrass that share common ancestry and synteny. However, due to longer generation times and complex genetics, translational research and confirmation of gene function by forward and reverse genetics is challenging in the most promising feedstock species. A new C₄ model species that is closely related to emerging second generation bioenergy grasses will facilitate forward and reverse genetics to optimize biomass quality and quantity.

As a model genetic system, *Setaria viridis* offers a number of favourable attributes including a short generation time of 6–8 weeks, rich genetic diversity, simple diploid genetics, and a sequenced genome. Moreover, *Setaria* species possess many attributes that would accelerate the gene discovery and analysis of regulatory networks of SAR. During domestication of *Setaria italica*, SAR has been selected against for dense planting, but its wild progenitor, *S. viridis*, displays robust responses to shade (Fig. 1). A recently published genome sequence of *S. italica* (Bennetzen et al., 2012) will aid in understanding how genetic and genomic information related to SAR is conserved in the panicoids. The discovery of novel genes responsible for regulation SAR in *S. viridis* and misexpression or up/downregulation of candidate genes will assist the finely tuned genetic manipulation of the physiological and developmental plasticity of SAR for increased planting densities (Doust et al., 2009; Brutnell et al., 2010).

**Summary**

The most promising sources of sustainable lignocellulosic feedstocks are C₄ grasses that include species such as sugarcane, sorghum, maize, *Miscanthus*, and switchgrass. One of the pressing challenges in meeting this goal is to develop germplasm and agronomic practices to optimize the production of the feedstock with as few land, water, and nutrient inputs as possible. Perennial bioenergy feedstocks, such as *Miscanthus* and switchgrass, offer the possibility of reducing the competition between food and fuel, if marginal lands can be used to produce the feedstock with little or no inputs. Unfortunately, both *Miscanthus* and switchgrass are recalcitrant to genetic analysis as they are polyploids with longer generation times, larger genomes, and challenging genetics (e.g. *M. giganteus* is a sterile triploid). To accelerate the process of discovery and germplasm enhancement of these grasses, *S. viridis* will be an advantageous model genetic system to understand and ultimately manipulate carbon assimilation pathways. Furthermore, second-generation bioenergy grasses have not been subjected to selection...
Fig. 2. Schematic representation of a grass feedstock displaying the plasticity of shade avoidance response. (A and B) Phenotype of a shade-responsive grass planted at low density (A) and at high density (B): at low density, the grass produces many tillers and is relatively short in stature; at high planting density, the grass displays characteristic shade-induced morphological changes (i.e. elongated stems, thinner leaves, fewer tillers, and less chlorophyll). (C and D) Phenotype of an engineered feedstock with attenuated shade avoidance response planted at low density (C) and at high density (D): at low density, plants tiller to a limited extent but continue to produce larger plants with more biomass than a conventional feedstock; at high density, leaves remain thick and large and elongated stems thicken resulting in increased biomass and reduced lodging. Engineered plants also display a gradient in chlorophyll accumulation with less chlorophyll in leaves of the upper canopy to allow greater light penetration into the lower canopy, where higher chlorophyll levels will more efficiently harvest light energy.
against negative outcomes of SAR under dense planting. Having close phylogenetic relationship with emerging bioenergy grasses and responsiveness to shade, S. viridis makes an ideal model system to dissect the genetic pathway of SAR. A comprehensive analysis of the genetic pathway will give translational insight into the candidate genes that can be engineered to obtain desirable plant growth, resource partitioning, and architecture under high-density planting, as illustrated in Fig. 2.

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