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Biology, pretreatment and genetic development of *Miscanthus sinensis*, a biomass crop with great potential in China

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ABSTRACT

Climate changes and energy crisis accelerate the exploration on traditional energy production and new alternative energy sources. Biofuels are important because they could replace petroleum fuels. With high yield production, wide distribution and dramatically high tolerance to biotic and abiotic stress, *Miscanthus sinensis* was considered as one of the most potential energy plants in China. As a relatively new biomass plant, its background knowledge in physiological, pretreatment and genetic investigation is scarce. This review aimed at physiological and characteristics description, as well as pretreatment for ethanol production and genetic improvement of *M. sinensis*. The achievements of genomic and epigenomic research programs in other plants are of great help to better understand the nature of this promising biomass crop species in China. Meanwhile further researches on deconstruction enzymes, genetics and breeding would accelerate the application of *M. sinensis*, with higher yield, better quality, and wider adaptation.

INTRODUCTION

The energy crisis that was caused by climate changes and energy conservation accelerates the exploration in traditional energy production and new renewable energy sources ^[1]. In 2007, China's net import of oil has reached to 186 million tons, accounting for 49.6% of its total oil demand ^[2]. Biofuel is a renewable energy source produced from natural plant materials, such as bioethanol from *Miscanthus*, grain or sugarcane and biodiesel from vegetable oils or animal fats ^[3].

Based on production technologies, biofuels were categorized into to four generations: first (those produced primarily from food crops such as grains, sugar beets and oil seeds), second (those produced from the non-food biomasses including cereal straw, bagasse, forest residues, and purpose-grown energy crops such as vegetative grasses and short-rotation forests), third and fourth generations ^[4]. The second and third generation biofuels are also called advanced biofuels, which are mainly made from non-food crops and algae ^[5]. However, considering the high value of food crops and potential stress of lands, first generation biofuels appear unsustainable ^[4]. Before 2004, corn and wheat are used as the main feedstock to produce ethanol in China ^[2]. Because of the limited grain feedstock, urban air pollution, carbon sequestration, and expansion on food security, the second-generation biofuels have been considered as one of the most potential biofuels in China in the future ^[6].

Bioethanol and biodiesel are two global transportation fuel derived from biomass. The cost of feedstock, conversion process, scale of production and region mainly affected biofuel production costs. For biofuels, the cost of feedstock is a major component of overall costs. Considering biofuel price exclusive of taxes, bioethanol from lignocellulose will be estimated as most promising alternative compared with oil [7]. To satisfy current and future demands, ethanol production from lignocellulosic biomass fermentation is a viable option that does not compete with the food supply. Due to its low cost and high availability, lignocellulosic biomass provides a significant proportion of raw materials for bioethanol production in the long term [6]. In China, industrial biomass can be grown from types of plants, including *Miscanthus*, sweet sorghum, cassava, and sugarcane [8]. About 6.67 million ha reclaimable lands could be used as energy crops lands in China [9]. Currently studied potential biomass species could survive in these extreme lands including sweet sorghum (planted in North of China) and cassava (planted in South of China). In China, *M. sinensis* is widely distributed from Hainan to Heilongjiang, covering latitudes between 18°39'N and 45°22'N, and within an altitude ranging from 3 m to 2109 m. A variety of wild *Miscanthus* species in China have contributed fundamental information for further breeding and genetic improvements.

As one of the top nine potential bio-energy grasses, *Miscanthus* has shown its potential for future renewable second-generation bio-energy [10]. The data from field trials on *Miscanthus* has shown high energy production from many locations in Europe [11,12]. There are three *Miscanthus* species with a high potential of biomass production, *Miscanthus* × *giganteus* (*M* × *giganteus*), *M. sinensis* and *Miscanthus sacchariflorus* (*M. sacchariflorus*). *M* × *giganteus* has been extensively studied and popularly used for biomass production. However, *M* × *giganteus*, widely used in Europe and North America, was derived from a single clone of the spontaneous triploid hybrid between diploid *M. sinensis* and allotetraploid *M. sacchariflorus* [13]. Thus the genetic basis of *M* × *giganteus* is very narrow, which is difficult to be broadened due to its triploid sterility. Genetic improvement of *M* × *giganteus* greatly depends on both *M. sinensis* and *M. sacchariflorus* [13]. *M. sinensis* is a perennial C₄ photosynthetic and rhizomatous grass, with high yield potential, high water use efficiency, low need of fertilizer, and high resistance to extreme environments [14]. Because of its high productivity compared to other C₄ crops within growing season, *M. sinensis* is regarded as a promising crop for feedstock of biomass and alternative bio-fuel in China [15].

Though *Miscanthus* has been advocated as potential second-generation biofuel feedstock by Chinese government, limited information is available on genetics and conversion process of *Miscanthus*. This review would like to focus on the available *M. sinensis* studies for a better understanding of the nature of this promising biomass crop species in China.

MORPHOLOGY AND CHARACTERISTICS OF *M. Sinensis*

Morphology of *M. Sinensis*

Underground stems extend short and thick rhizomes (5-10 cm), and sprout new roots on active nodes to form new tillers (Figure 1c) [16]. An aboveground stem is rigid, cylindrical, unbranched, and mostly hollow (Figure 1d) [17]. In our field trial (conducted in Wuhan, China), *M. sinensis* could reach up to 3.35 m in height (Figure 1a and 1b), higher than that in Japan and Europe, which are 2 m and 2.3 m, respectively [18-21]. Individual leaf blades of *M. sinensis* are 100 cm long on average and range from 2 mm to 20 mm in width [22]. Ligules arising from sheath are 1-2 mm (Figure 1d) [23,24]. In total, about 12-14 leaves per culm grew from the ground until the flag leaf appeared near the panicle. The leaf area index of *M. sinensis* contains a high value, and reaches to a peak of 3.9 ± 0.6 [25,26]. Each tiller precisely produces only one panicle at the top of the stem above the flag leaf. *M. sinensis* genotypes planted in Wuhan start heading at the 120th to 250th day after sprouting in March, while flowering date is mostly from late autumn to early winter over a long time. An individual flower contains 2 stigma and 5 anthers. Each flower contains an awn arising at the upper lemma of 6-12 mm (Figure 1e and 1f). Mature seeds at harvest time are still flanked by lemma and palea adhering to the caryopsis, which contain seedling embryo, and the caryopsis is about 4 mm in length (Figure 1h).

Characteristics of *M. Sinensis* as a Potential Biomass Crop

High biomass yield

M. sinensis is regarded as one of the potential bio-fuel crops, which may be partly due to its relatively high dry-matter yield compared to other plants and primarily benefits from high efficiency of C₄ photosynthesis [25,26]. The mean weight of underground dry-matter is 12.9 t ha⁻¹ in Kawatabi, Japan [27]. According to our field trials, the aboveground biomass yield in China is estimated to range from 0.1 to 38.5 t ha⁻¹. Under field conditions without irrigation and fertilization, the aboveground biomass from Guangdong province of China yielded up to 38.5 t ha⁻¹, which was dramatically higher than that of 22.4 t ha⁻¹ planted in Europe and 12.7 t ha⁻¹ planted in the Mount Aso region of Japan [18,28]. Hence, with high dry-matter yield, *M. sinensis* could play a better role as a

potential bio-fuel crop in China locally.

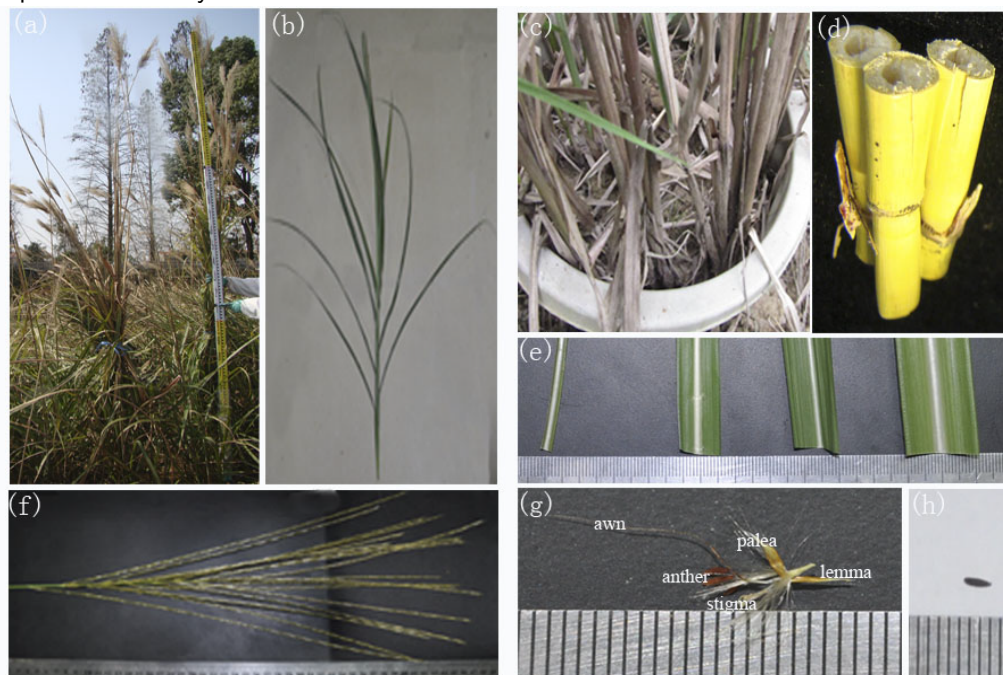


Figure.1: *M. sinensis* morphology description.(a) *M. sinensis* plant in the field in September; (b) vegetative stem of *M. sinensis*; (c) groups of tillers at base of *M. sinensis* plants; (d) rigid, cylindrical, empty stem of *M. sinensis*; (e) leaf of *M. sinensis*, in an average of 15 mm of width (each mark on the graduated scale is 1 mm); (f) seed head (panicle) of *M. sinensis* (each mark on the graduated scale is 1 mm); (g) perfect flower of *M. sinensis*, showing stigma and anthers and the enclosing lemma and palea bracts (each mark on the graduated scale is 1 mm); (h) mature seed of *M. sinensis* at harvest time (each mark on the graduated scale is 1 mm)

M. sinensis plays an important role in breeding new *Miscanthus* cultivars with high quality and performance. As a perennial grass, the yield of *M. sinensis* increased year by year in the early stage of *Miscanthus* field establishment. In the first year, the average biomass yield across Sweden, Denmark, England, and Germany was 0.4 t ha^{-1} , while the third year was 10.9 t ha^{-1} , three times more than that in the second year [29]. Regression analyses of field trials indicated that latitude, height and stem number were the significant factors determining biomass production, while heading date and elevation were also considered to be the important factors affecting yield structure.

High resistance/tolerance to extreme environments

M. sinensis possesses high tolerance to extreme environments in natural or anthropogenic systems including volcanic and coastal areas in acid-sulfate soil with poor nutrient availability, especially a phosphorus (P) deficiency [22,30]. Yoshii clarified the first invader plants in the volcano and reported that the volcanic regions' restoration plants usually included *M. sinensis*, where a high-content of toxic gases and sulfate was always found around acid soil craters without other necessary nutrients for herbaceous investigation. Compared to other *Miscanthus* species, *M. sinensis* could possess a high tolerance to extreme environments, which shows a high potential in breeding new hybrids of good tolerance to abiotic stress. As significant changes of PPDK and Rubisco proteins in C_4 pathway could contribute to the cold-tolerance, *M. sinensis* increases rubisco content to get used to low growth temperatures [31,32]. And within two years of clearing open areas of a cold temperate region in Japan, *M. sinensis* could survive to sprout [33]. Farrell et al. [34] demonstrated that when exposed to frost temperatures of -2°C , -4°C , -6°C , -8°C , and -10°C for 3 hours, only *M. sinensis* hybrid Sin-H9 could tolerate frost with lethal temperatures of 50% death (LT50) at -9.3°C , while other investigated genotypes were all killed. Upon investigating the LT50 value in five *Miscanthus* species, Clifton-Brown found that *M. sinensis* had a much lower LT50 of -6.5°C than that of *M. x giganteus* and *M. sacchariflorus* at -3.4°C [35-36].

M. sinensis also shows good performance when exposed to physiological pressure. In artificially simulated high-salt and drought conditions, *M. sinensis* could still survive with the NaCl concentration of 18 g/L and PEG concentration of 25% (W/W) [37]. Clifton-Brown et al. examined the response of water stress by regulating the amounts of water supply. They found that *M. sinensis* had a strong tolerance to drought and low demands for water and nutrients, as it has a flexible water-saving strategy to maintain green leaf area, by reducing direct response of leaf to drying soil at water-deficient period [38,39]. *M. sinensis*' strong resistance to drought, cold, and poor planting environments on marginal lands makes it a good choice for domestication [40]. In the future, efforts need to be made to identify potential linked markers for morphology, and physiology such as tolerance in nature, which would accelerate the researches on screening, selecting, and breeding in *M. sinensis*. The rhizomes are tuft-forming with high shoot densities, which contribute significantly to prevent water and soil erosion [18]. *M. sinensis* rhizomes branched 3 times per year on average, and more than half of the new tillers would successfully develop aboveground shoots during the same year [28].

INDUSTRIAL PRETREATMENTS FOR ETHANOL PRODUCTION

M. sinensis has high content of holocellulose (72.5 ODW, short for Over-Dried Weight) and α -cellulose (42.2 ODW) content, low ash (0.7 ODW) content and low Klason lignin (19.9 ODW) content [41,42]. Dry stem biomass contained 85-89% of cell wall of *M. sinensis* tested, while cellulose content in cell wall was 32-55% across samples with significant variations [43]. Lignin confers integrity and structural rigidity on the plant cell and shows the most significant resistance against pretreatment to cellulosic ethanol [44]. The lignin content across all *M. sinensis* samples tested was 22-24%. However, lignin content in the *M. × giganteus* was up to 28% [44]. With relatively low lignin content within limited undigested molecular mixture, *M. sinensis* needs lower energy input for cellulose degradation to ethanol, which provides benefits in pretreatment. Sugar content of *M. sinensis* was more than 62%, which mainly consisted of pentose (22.4%) and hexose (40.2%) [45]. Total content of sugar is similar to other major sources of lignocellulosic biomass, such as wheat (69%), corn stover (65%), and rice (63%), indicating its big potential for biomass crops [46-48].

Before cellulose and hemicellulose are converted to ethanol, they require chemical and physical pretreatments followed by enzymatic breakdown of the molecules [49]. A number of alternative systems of pulp production have been investigated and proposed by the scientific community [50]. Compared with sugary and starchy as raw material for bioethanol production, more complex pretreatment and hydrolysis technology are demanded for lignocellulose-based biomass. In fact, lignocellulose pretreatment is the main economic costs in conversion of lignocellulose to ethanol [51]. Once the raw material has been pretreated, lignocellulose must be hydrolyzed to liberate fermentable sugars.

The goal of pretreatment is to break down the lignin structure and disrupt the crystalline structure of cellulose to make it accessible to hydrolysis for conversion to fuels [51,52]. Pretreatment technologies of biomass conversion to lignocellulose include three major processes: chemical, biological, and physical [53]. Since different lignocellulosic materials have different physical and chemical properties, suitable pretreatment technologies should be adopted for each raw material. The pretreatment of *M. sinensis* is currently consisted of chemical, physical-chemical, and biological pretreatments.

Chemical pretreatments

Acid pretreatment could solubilize the hemicellulosic fraction of *M. sinensis* and make the cellulose in plant cells more accessible to enzymes [52]. Aboveground *M. sinensis* stems could be subjected to acid delignification at atmospheric pressure. Under condition of pulping for 75 min with liquor containing 87.8% of formic acid and 0.09 % of HCl, pulp yield relative to dry *M. sinensis* was 46%, while RKL was 3% [54]. Compared to high chemical concentration of 1% H₂SO₄, 0.25% H₂SO₄ showed a better calibration correlation, which makes a larger variation in sugar yields [55].

An alkali-based treatment is another useful process to study ethanol production on *M. sinensis*. Iglesias and colleagues studied the delignification of *M. sinensis* by alkaline treatment with NaOH solution. In the optimal condition of dissolved lignin/weight losses at 0.4, pulp yield about 60% of the lignin [56]. In one step by alkaline pulping, preliminary results have shown that *M. sinensis* can be efficiently delignified to low level (kappa numbers around 25), with 56% pulp yield [57]. Optimized NaOH pretreatment with *Thermotogaelfi* enzyme hydrolysis resulted in 77% delignification, a cellulose yield of more than 95% and 44% hydrolysis of hemicellulose [58].

Besides these methods, other pretreatment are explored to enhance the digestibility of cellulose of *M. sinensis*. Results of thermal, oxidative, lime, liquid hot water, and a combination of ammonia and carbon dioxide pretreatments show significant potential for the digestibility [50]. *M. × giganteus*, catalytic steam gasification provided good production with about 1.7 m³ kg⁻¹daf of gas, containing 50% of H₂ [59]. Murnen and colleagues [60] optimized ammonia fiber expansion pretreatments and enzymatic hydrolysis of *M. × giganteus* to fermentable sugars, which achieved approximately 96% glucan and 81% xylan conversions. Indeed, all the progress in *M. × giganteus* treatment suggests that further researches should be done to enable *M. sinensis* pretreatments for higher pulp production.

Physical - chemical pretreatments

Microwave-based pretreatment can be considered as a physical-chemical process since both thermal and non-thermal affects are often involved. Microwave-assisted ammonium hydroxide followed by phosphoric acid treatments were used to release monomeric sugars from *M. sinensis*. The two-stage pretreatment, treatment with NH₄OH at 120 °C for 15 min followed by treatment with H₃PO₄ at 140 °C for 30 min, impressively provided total monomeric sugar yields of 71.6 g/100 g dried biomass [61].

Hot water does not require addition of chemicals, lessen the need for expensive reactors, avoid catalyst recycle, and overcome neutralization costs. A hot water pretreatment of *M. sinensis* samples was demonstrated at a lower temperature at 121 °C for 20 min in an autoclave. It showed larger variation in sugar yields than chemical pretreatments [55].

Biological pretreatments

Biological pretreatments always employ microbial and enzymatic pretreatments [51,62]. As the unique microorganism that is able to remove lignin from lignocellulosic plant litter, fungi are always explored to upgrade lignocellulosic materials for feed and

paper applications. Till then, many fungi colonies have been selected for further bioconversion studies. As a dominant component of microfungus assemblages on *M. sinensis* leaves in the field, *N. sphaerica* had been evaluated for bioconversion of *M. sinensis* [63].

By collecting fungi associated with decaying *Miscanthus* plants in nature, about 950 fungal colonies were randomly selected out of 4,560 micro-well colonies. The genus of *Sordariomycetes* and *Dothideomycetes* were indicated as the most promising to be used as bioconversion species [64]. An evaluation of biological pretreatment of litter-decomposing fungi to remove lignin from leaves of *M. sinensis* was performed under pure culture conditions; 12 different fungi species could cause the mass loss of leaves ranged from 4.7% to 43.1% of the origin leaf mass [63].

Results from recent studies have shown that lignin degradation by white-rot fungi mainly depends on the features of lignin degrading enzymes such as peroxidases and laccases [65]. Though several white-rot fungi species have been examined on different lignocellulosic biomass showing high delignification efficiency, *Phanerochaete chrysosporium*, *Trametes versicolor* have been examined most potential within *Miscanthus* [65]. *Trametes versicolor* could cause 43.1% and 49.5% mass loss of lignin and total carbohydrates, respectively [63].

In general, biological pretreatment offers advantages such as no chemicals requirement, low cost, and low energy input [66]. However, biological methods develop low hydrolysis rate compared to other technologies [67]. To improve the hydrolysis rate of biological pretreatment for *M. sinensis*, studies to divide more basidiomycetes fungi colonies with ability to delignify *M. sinensis* efficiently should be evaluated.

GENETIC IMPROVEMENT

Phylogenetic and genetic composition of *M. sinensis*

Taxonomically, *Miscanthus* belongs to Saccharineae, Andropogoneae, and Panicoideae in the family of Poaceae and could be classified into four groups of Triarrhena, Eu *Miscanthus*, Karyasua, and Diandra [68]. *M. sinensis* is in PACCAD clade and belongs to Eu *Miscanthus* [69,70]. It is classified to a core group with *M. floridulus*, *M. sacchariflorus*, *M. sinensis subsp. condensatus*, *M. oligostachyus*, and *M. transmorrisonensis* [71].

M. sinensis is a diploid (tetraploid sometimes in nature) and has a basal set of 19 chromosomes and genome size of 5.5 pg [70-73]. Considering the origin of *M. × giganteus*, it has been suggested as the result of hybridization between *M. sinensis* and *M. sacchariflorus* [13,59,74]. Rayburn analyzed the three species by cytometry and stomatal cell and hypothesized that *M. sinensis* provided one genome for *M. × giganteus*, while *M. sacchariflorus* contributed two genomes [72,73]. However, the hybrid origin is still confusing, more molecular and genetic research should be explored to dissect their genome evolution. Although *M. × giganteus* produces a high yield, as a kind of sterile grass, it strongly depends on *M. sinensis* for genetic research to provide high quality and performance. Hence, *M. sinensis* germplasm is an important resource to broaden genetic basis for successful breeding research [93,94].

Conventional breeding

Genetic improvement of *M. sinensis* primarily focused on biomass yield and environmental stress tolerance. Conventional plant breeding technology across different populations results in open hybrid varieties and leads to more changes in gene expression, which may also affect agricultural production. Preliminary studies on *M. sinensis* of tetraploid forms in field experiments have carried out rich lucrative genotypes with greater dry biomass weight, higher cellulose content, and higher resistance to extreme environments in comparison to the controls [21,22]. For example, inbred generation of *M. sinensis* and *M. lutarioriparius* could successfully produce several hybrids with greater cellulose content and lower lignin content, which is of tremendous impact for bio-fuel production. Conventional breeding researches on *M. sinensis* have produced hybrids of high performance. In China, *Miscanthus*' interspecific crosses were performed. They constructed 24 interspecific combinations, 2 backcross populations, 3 F1 sib-mating populations, and 1 recurrent crossing population. The elite F1 hybrid lines captured a higher cellulose and lower lignin content, which are 48.93% and 12.30%, respectively [75]. In Europe, several hybrids were made from two *M. sinensis* populations with a wider temperature ranges tolerance. For example, sin-H6 and sin-H9 showed higher tolerance to suboptimal temperatures and late frost events [59]. Moreover, sin-H9 had higher thermal response rates with a T_b of 6.0 °C and frost tolerance with a lethal temperature of -9.3 °C lower than *M. × giganteus* [34].

Advanced breeding

As one of the new leading candidates to meet biomass demand for future power generation and bio-fuels production, *M. sinensis* needs further genetic improvement using both conventional breeding and modern biotechnical approaches. Advanced breeding reinforces the genetic bottlenecks.

Molecular markers are useful for genetic fingerprinting to identify *M. sinensis* of genetic variations for the breeding program [76,94,95]. A preliminary *M. sinensis* map has already been constructed based on RFLP and SSR markers [77-78]. So far 26 ISSR polymorphic markers, 998 AFLP markers and 14 SSR markers have been identified [59,79,80]. Developing new associated markers

from related crops, such as corn, sorghum, and sugarcane with a rich genomic database, may accelerate breeding procedures. Based on 57 SSR markers derived from *Brachypodium distachyon*, Zhao et al. clustered 21 genotypes of *M. sinensis* into three distinct groups^[81]. Molecular markers provide efficient introgression traits within linked markers^[59]. This procedure will be particularly important in *M. sinensis* as many of the desired traits are expressed only after 2-3 years^[82]. Once the robust associations between these molecular markers and phenotypes have been established, it will be convenient to select desired offspring from a cross in further research.

QTL strategy could be used to investigate the relationship between quantitative traits and loci based on linkage maps, which were usually constructed by molecular markers such as RAPD, SNP, and SSR markers. QTL maps for plant height stem diameter, yield, and combustion quality parameters have been identified in a single *M. sinensis* cross^[82-85, 87]. Though there is a lack of sufficient sequence data for *M. sinensis*, QTL analysis may be an alternative tool to sequence target regions of candidate genes with known functions^[87,88]. Considering the preliminary researches on *M. sinensis*, it could be extended to additional populations, environments and traits. QTLs mapped in other grasses can inform a candidate locus in *M. sinensis*. For example, testing the genetic origins of loci controlling shattering among other model organisms, functions as an abscission layer in millet, seed dispersal in sorghum and maize, and seed shedding in rice could improve development of identification of markers associated with related traits within *M. sinensis*^[89].

Future strategy

Screening *M. sinensis* genotypes in raw data of field trials could optimize biomass yield and quality. As the biomass yield and quality of a particular genotype of one year growth does not always correlated to that measured in the following year, so the identification of the most suitable genotypes requires at least two years. Characteristics such as heading date/flowering time and senescence rate are critical for reproductive success and for optimizing yield and quality. It is essential to determine genotype growth characteristics by measuring plant height shoot density, stem diameter, flowering time, etc. Expensive and intensive observations in agronomy should be conducted to investigate screening for new valuable, novel genotypes with high yield, good quality, and wide adaptation.

The available crop genome sequences can dramatically advance both the theory and application of *M. sinensis* breeding. Whole genomes sequenced in several crops including rice, maize, and sorghum have been characterized. The sequences have opened the door for large-scale transcriptomics and proteomics studies, which has proved to be useful in *Miscanthus* breeding studies^[87,90,91]. The sequence data can be used to study the origin of genes and gene families, track rates of sequence divergence over time, and provide hints about how genes evolve and generate products with novel biological properties^[92]. However, *Miscanthus* genome sequencing is still in its infancy due to its large and highly heterozygous genome. Nevertheless, physical mapping and sequencing of the *M. sinensis* genome have been conducted by several research institutions. A high resolution genetic mapping by genotyping-by-sequencing (GBS) was constructed recently. This genetic map enables informed deployment of the wealth of existing genomic resources of other species to the improvement of *Miscanthus* as a high biomass energy crop^[86]. All these efforts develop the needed background and tools for sequencing the diploid *Miscanthus* genomes and provide excellent tools for unfolding *Miscanthus* evolutionary studies and for optimizing breeding practices. This is a long-term, milestone-based strategy that delivers products and tools while working towards crop improvement, both qualitatively and quantitatively.

CONCLUSIONS

M. sinensis is one of the leading bio-fuel crops with growing importance in the world. Studies on agronomy and taxonomy have shed light on its potential usage as a high-yield biomass. As the progenitor of grass biofuel, *M. sinensis* is central to *Miscanthus* breeding improvement, and contributes two genomes to *M. x giganteus*. The studies of *M. sinensis* in China are in the early stages and mainly focus on field trials for several years, accumulating knowledge of its basic biology. Also, germplasm collections are essential to conserve biodiversity in accelerating breeding process, without strong genetic diversity.

As the main economic costs in conversion of *M. sinensis* to ethanol, more low cost and efficient pretreatment methods should be improved in the future, especially the biological conversion processes. Enzymes that deconstructed cell wall efficiently would contribute better pretreatment property of *M. sinensis*.

As a *Miscanthus* progenitor, *M. sinensis* should be subjected to in-depth studies to evaluate the structural, functional, and regulatory polymorphisms adapting it to environmental stress. More efforts should be made to select new *M. sinensis* hybrids, which are resistant to leaf senescence and drought, with no significant root biomass reduction, from the cross pollination of *M. sinensis* genotypes^[38]. It might significantly accelerate research of both speciation and adaptation processes to stressful and changing environments, once associations between genomic and epigenomic diversities are established. Transgenic technology on *M. sinensis* could target on facilitating biomass dry-matter yield and enabling colonization of extreme environments. Higher yield must be assumed priority, minimizing labor input and land needs. Advanced technologies could focus on important adaptive genes and enable their active introgression for genetic reinforcement to cultivate *M. sinensis* with good quality and quantity.

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