



Comparison of the growth and biomass production of *Miscanthus sinensis*, *Miscanthus floridulus* and *Saccharum arundinaceum*

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Abstract

Miscanthus and *Saccharum* are considered excellent candidates for bioenergy feedstock production. A field experiment was conducted in Zhejiang province of China to characterize the phenotypic differences in three species, two of *Miscanthus* (*M. sinensis* and *M. floridulus*) and one of *Saccharum* (*S. arundinaceum*), each with two accessions collected from China. Agronomical traits, including plant height, culm number, tuft diameter and culm diameter, were monitored monthly for the first 3 years of growth. For each year of trial, flowering time was observed and biomass yield was harvested. *M. floridulus* produced a superior biomass yield with increasing plant age associated with higher yields (4.18, 24.16 and 29.01 t dry matter/ha in November of years one to three, respectively). Higher culm diameter, plant height and tuft diameter values were observed for *M. floridulus* when compared to the other species. Biomass yield was positively correlated to tuft diameter, culm diameter, culm number and negatively to flowering time, but it showed no correlation with plant height. Tuft diameter and culm diameter could be suitable indicators in the selection of accessions for crop yield at the yield-building phase. Studies of the primary colonizers of *Miscanthus* and *Saccharum* in their original location may be of interest from the perspective of bioenergy germplasm resource collection.

Additional key words: agronomic traits; perennial grasses; correlation analysis.

Abbreviations used: DM (dry matter); DW (dry weight); FW (fresh weight); GDD (growing degree-days).

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Introduction

The recent interest in the potential use of plant-based biofuels to ensure energy security, and to reduce carbon dioxide emission and environmental degradation has progressed to international political agendas (McKendry, 2002; Ibeto *et al.*, 2011). Several perennial grasses, such as *Miscanthus* and *Saccharum*, are promising candidate biomass plants (Clifton-Brown *et al.*, 2008; Saballos, 2008). Both genera are indigenous to tropical and subtropical zones, utilize the C4 pathway of carbon fixation, show good stress resistance and produce high

biomass yields (Heaton *et al.*, 2008). *Saccharum* and closely related taxa (namely *Erianthus*, *Sclerostachya*, *Narenga* and *Miscanthus*) are generally designated as 'Saccharum complex' (Mukherjee, 1957). DNA sequence variations have been analyzed to construct the phylogeny of *Miscanthus s.l.* and *Saccharum s.l.*, which indicates that the two genera are closely related but have different basic chromosome numbers (Hodkinson *et al.*, 2002a).

Miscanthus exhibits high water use efficiency, low energy consumption and produces a large biomass yield (Cosentino *et al.*, 2007; Clifton-Brown *et al.*, 2008).

Generally, *Miscanthus* stands take at least 3-5 growing seasons to become fully established and to reach optimal production. Clifton-Brown & Lewandowski (2002) found in a multisite trial throughout Europe that yields were always lower than 10 t dry matter (DM)/ha in the first two established years. Field trials in Europe and America confirmed the high potential biomass yield of *M. × giganteus*, indicating that it is capable of producing a yield of more than 20 t DM/ha/yr on arable lands (Price *et al.*, 2004). However, this highly productive crop is sterile and reproduces vegetatively from micro-propagated plantlets or rhizomes in spring (Greef & Deuter, 1993). *M. × giganteus* seems to be sensitive to drought and low temperatures, which restricts its industrial production in many regions (Heaton *et al.*, 2010; Anderson *et al.*, 2011). Owing to the narrow genetic pool, establishment costs and sensitivity to cold temperature of *M. × giganteus*, the development of other species must be considered.

The above-ground biomass yield of *M. sinensis* varies by location and year. Clifton-Brown *et al.* (2001) studied the performance of 15 genotypes of *Miscanthus* grown in Europe during their first three years of growth. Wild *M. sinensis* (nonhybrid) genotypes performed optimistic in northerly sites. Yields of these genotypes ranged from 7.1 t DM/ha in Denmark to 14.3 t DM/ha in Portugal. In Honshu Island of Japan above-ground biomass of *M. sinensis* has been reported to range from 1.8 to 13.0 t DM/ha (Stewart *et al.*, 2009). *M. floridulus*, a dominant grass endemic to the coastal regions in Southeast Asia, has been intensely studied in Taiwan (Chou *et al.*, 1991; Hodkinson *et al.*, 2002b; Zhang *et al.*, 2009; Huang *et al.*, 2011) where its growth has been found to be quite poor and it could barely survive winter temperatures below ~ 6°C (Chou *et al.*, 1991). Huang *et al.* (2011) monitored the growth of Taiwanese native *M. floridulus* individuals from different latitudes in their first two years following planting (2009 and 2010) and showed that these strains had vigorous growth at the Chia-Yi Agricultural Experimental Station, reaching 2.5 m after 7 months of cultivation and producing ~ 100 culms per plant. The highest-yielding individual was collected from an altitude of 1000 m, with an autumn yield of 38.0 t DM/ha in 2009. However, the yields in 2010 were lower than those in 2009 because of the excessive rainfall.

Like *Miscanthus*, *Saccharum* is a C4 grass with a tall culm, thick and long internodes, and narrow leaves (Chen & Renvoize, 2006; Saballos, 2008). *S. arundinaceum* has high potential as a germplasm source for sugarcane with useful agronomic traits such as high fiber content, ratooning ability, excellent vigor, and tolerance to biotic and abiotic stresses (Berding & Roach, 1987; Roach & Daniels, 1987). Few studies

have been conducted on the biomass yield and growth performance of *S. arundinaceum*, even in its original location, *i.e.*, China. Among those, comparing different harvest frequencies and clones, Mislevy *et al.* (1997) in the USA found that *S. arundinaceum* produced 4-year average biomass yields ranging from 5.2 to 51.5 t DM/ha/yr. Deren *et al.* (1991) reported second year yields of 4.6-23.3 t DM/ha/yr, depending on clones. Investigations of the biomass yield and agronomic performance of *Miscanthus* and *Saccharum* have mostly been carried out in Europe and the USA but similar efforts are lacking in China. Heaton *et al.* (2010) reported that many primary colonizers had high productivity, which turned out to be a feature that was selected during evolution. Most species of *Miscanthus* and *Saccharum* are endemic to eastern or southeastern Asian countries such as China, Japan and the Philippines (Chen & Renvoize, 2006; Clifton-Brown *et al.*, 2008). To allow biomass species to play a transitional role in long-term bioenergy development in China, their potential needs to be evaluated. To achieve this objective, we compared the agronomic traits and biomass production of two species of *Miscanthus* and one of *Saccharum*.

Material and methods

Plant material and experimental design

Six accessions collected from different climatic regions and elevations of China (Table 1) were transplanted in the field: two of *M. floridulus* (flo1 and flo9), two of *M. sinensis* (sin19 and sin20) and two of *S. arundinaceum* (aru22 and aru30).

The field experiment was conducted from 2009 to 2011 at the experimental farm of Zhejiang A & F University, Hangzhou, Zhejiang province, China (30.23 N, 119.72E). The soil at the experimental site was sand clay loam with a pH of 5.9 and available N, P, and K content of 104.2, 4.1, and 82.6 mg/kg, respectively.

Seven replicates of each accession were planted by hand in March 2009, equidistant in one plot (one plot=21 m²), in rows 50 cm apart, with a planting density of 2 plants/m², resulting in 42 plants/plot. Each plot was replicated four times. The plots were separated by a distance of 1 m in a randomized design. Plants were watered immediately after planting and mechanical weed control was carried out once during the early-season growth period from March to May in 2009. No fertilization or irrigation was applied during the experiment.

Monthly rainfall and air temperature were obtained from the meteorological station nearest to the field

Table 1. Geographical co-ordinates of the *Miscanthus* and *Saccharum* accessions sampled

Accessions	Species	Latitude	Longitude	Site
flo1	<i>M. floridulus</i>	30.07	119.95	Changshan, Zhejiang province
flo9	<i>M. floridulus</i>	29.00	119.10	Longyou, Zhejiang province
sin19	<i>M. sinensis</i>	29.32	121.30	Zhoushan, Zhejiang province
sin20	<i>M. sinensis</i>	34.17	108.57	Xian, Shanxi province
aru22	<i>S. arundinaceum</i>	27.48	120.38	Ruian, Zhejiang province
aru30	<i>S. arundinaceum</i>	28.27	119.54	Qingyuan, Zhejiang province

(Fig. 1). The growing season air temperatures in 2009, 2010 and 2011 were similar, with the highest temperatures (32–35°C) recorded in July and the lowest temperatures (~0°C) recorded in January. The total rainfall varied along the three years. The second year, 2010, was relatively wet (1545 mm) compared to 2009 (1451 mm) and 2011 (1387 mm).

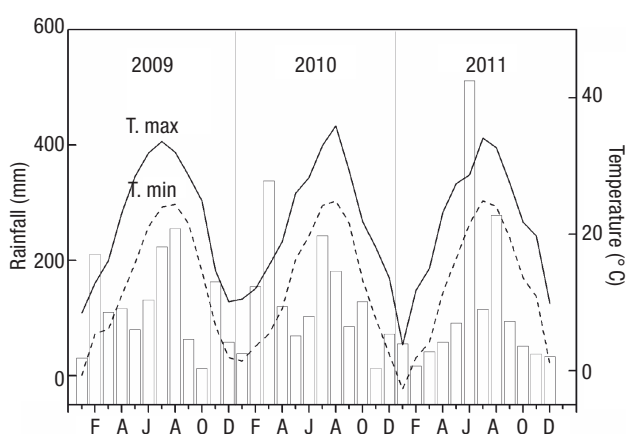


Figure 1. Monthly average minimum and maximum temperatures and precipitation (bars) during the first three years of the Zhejiang A & F University trial.

Agronomic performance measurements

Four growth traits of transplanted plants (plant height, culm number, tuft diameter and culm diameter) were recorded monthly to investigate their dynamics from April to November in 2010 and 2011. Plant height was recorded as the distance between the soil surface and the top of leaves from the tallest culms. The culm number was restricted to those of more than 10 cm. The tuft diameter was measured at ground level. The underground rhizomes of the plants expanded horizontally in multiple directions and formed different size patches of shoots that emerged during the growth season. Therefore, the tuft diameter was recorded on the mean of three distances between two longest outer shoots of the clump that extend outward plagiotropi-

cally. The culm diameter was determined from caliper measurement at ~5 cm aboveground of four randomly chosen culms per plant. All measurements were made on 4 plants per plot. Flowering date was defined as the date when at least one culm had one panicle 1 cm long.

The agronomic traits of each accession were evaluated for response to growing degree-days (GDD), which is a heuristic tool used to predict plant development stages. The plant development stage was defined by the date of the last frost in the spring to the date of harvest (McMaster & Wilhelm, 1997). GDD was determined using the following formula:

$$GDD = \sum_{s2}^{s1} (T_{Max} + T_{Min}) / 2 - T_{base}$$

where T_{Max} and T_{Min} are the daily maximum and minimum air temperatures, respectively; T_{base} is the base temperature taken to be 10°C; and $s1$ and $s2$ are the dates of the emergence in April and of harvest in November, respectively.

Biomass yields measurements

For aboveground biomass estimation, the whole plants were cut by hand in November at ~5 cm from the ground, and they were dried at 80°C until a constant weight was achieved. The aboveground biomass yield was assessed annually as tons of DM per hectare. Fresh weight (FW) and dry weight (DW) were calculated and water concentration was determined as:

$$\text{Water concentration (\%)} = 100 \times (FW - DW) / FW.$$

Statistical analysis

Comparisons of mean values of growth traits were made using ANOVA on four replicates per accession and per plot. The significance of variables and least-squared means were determined using the F test ($p < 0.01$). For multiple comparisons between means,

Student-Newman-Keuls *a*, *b*, *c* tests were used ($p < 0.01$); additionally, the biomass yield response to each variable was also analyzed to understand the underlying basis of the interactions. All the data were processed and analyzed using SPSS version 18.0 (SPSS Inc., Chicago, IL, USA).

Results

Biomass yields and production characters in the first three years of growth

Figure 2 shows the measurements from 2009 to 2011 of the four agronomic traits of *Miscanthus* and *Saccharum* species. *Miscanthus* and *Saccharum* had similar growth patterns of plant height. Most individual accessions were taller in 2010 and 2011 than in 2009, but there was no significant difference between the overall means for 2010 and 2011. Only the plant height of accession 'flo1' increased significantly, from 156 cm to 269 cm, from 2009 to 2011.

The culm diameter of most accessions reached their maximum values in 2010 (Fig. 2). *M. floridulus* had the greatest culm diameter throughout the growth period, over 1.1 cm at the end of the growing season in 2010, while the culm diameters of *M. sinensis* and *S. arundinaceum* were only 0.66 and 1.0 cm, respectively. The new shoot spread was smaller in 2011 than in 2010.

Although culm numbers showed the same pattern in five of the six tested accessions, *i.e.* increasing progressively over the years, different rates of increase among the species could be observed (Fig. 2). For example, culm numbers increased slowly in *S. arundinaceum* ('aru22' and 'aru30') in comparison with the other species, *M. floridulus* and *M. sinensis*. The culm numbers of 'sin20' and 'flo9' increased significantly in each of the three years, while those of 'sin19' and 'flo1' did not increase significantly from 2010 to 2011. The mean value of culm number of *S. arundinaceum* accessions was lower (24/plant) than those of *M. floridulus* and *M. sinensis* (82 and 78/plant, respectively) in 2010. The same situation was also observed in 2011.

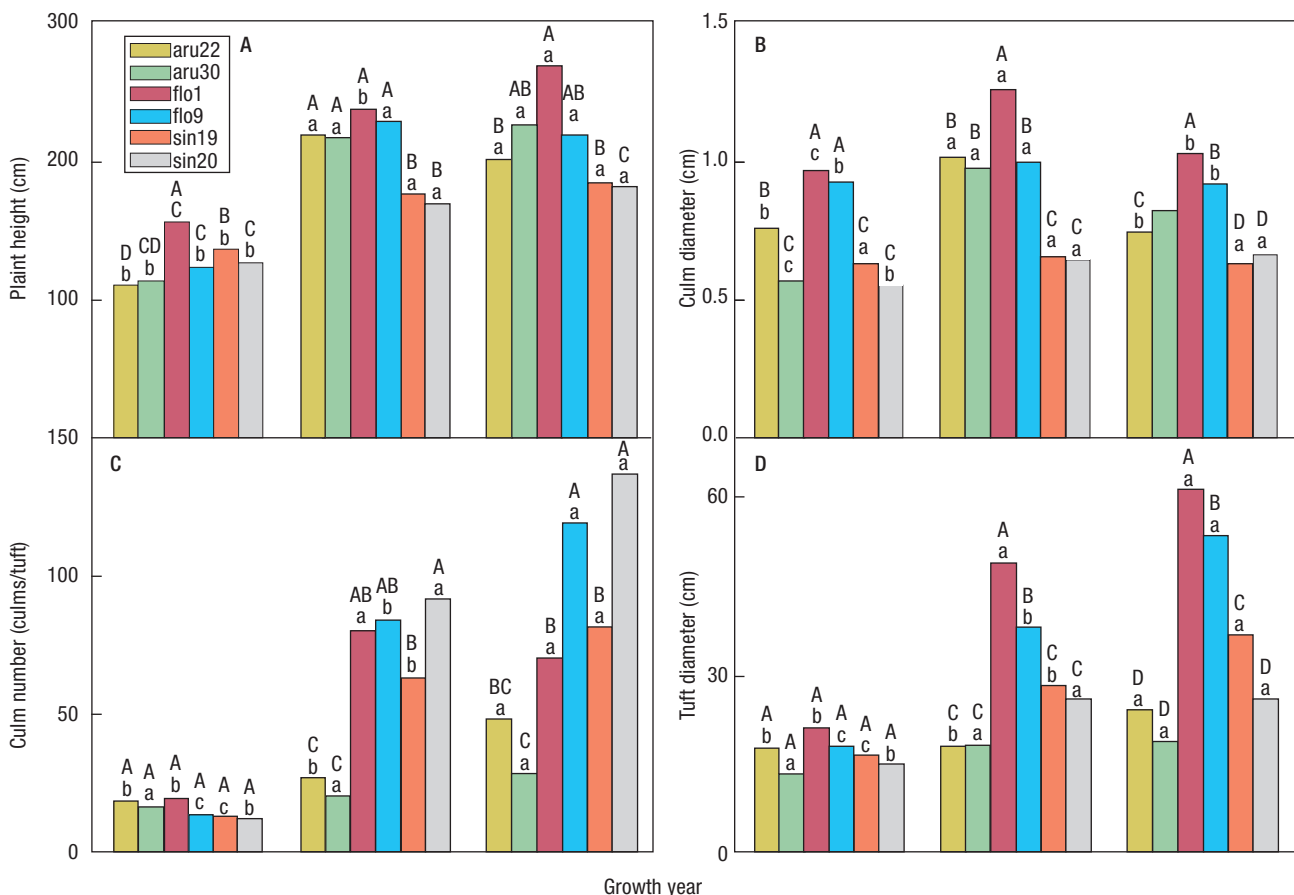


Figure 2. Plant height, culm diameter, culm number and tuft diameter of two accessions of *S. arundinaceum*, *M. floridulus* and *M. sinensis* in the first three years of growth (2009-2011). Lowercase letters indicate significant differences between years for the same accession and trait. Uppercase letters indicate significant differences between accessions in the same year.

The tuft size was mainly controlled by the number of culms. The tuft diameter grew with increasing culm number. However, the tuft diameters in 2011 were significantly different among species. *M. floridulus* ('flo1' and 'flo9') exhibited a significant increase in tuft diameter over time because their rhizomes expanded laterally and formed larger patches than the rhizomes of the other species (Fig. 2). *S. arundinaceum* exhibited an almost stable tuft diameter, with the least new culms. The mean tuft diameters in 2010 were 43 cm for *M. floridulus*, 27 cm for *M. sinensis* and 18 cm for *S. arundinaceum*. In the third year (2011), the mean tuft diameter of *S. arundinaceum* accessions was also lower (21cm) than that of *M. floridulus* and *M. sinensis* (58 cm and 32 cm, respectively) in 2011.

Figure 3 shows the DM aboveground yield of the three species for the three years. The yields of all three species increased from 1st to 2nd year, but the rates of increase differed greatly. The mean DM yields for the three species were very poor in the first year (4.18 t/ha for *M. floridulus*, 0.92 t/ha for *S. arundinaceum*, and 0.86 t/ha for *M. sinensis*). *Miscanthus floridulus*, especially 'flo1', had superior aboveground DM yield when compared to the other species in the latter two years. Its yield increased quickly and was several times greater than that of *S. arundinaceum* or *M. sinensis*. The mean DM yield of *M. floridulus* increased to 24.16 t/ha in 2010 and 29.01 t/ha in 2011, while *S. arundinaceum* had DM yields of 3.81 t/ha in 2010 and 3.50 t/ha in 2011, and *M. sinensis* had 2.35 t/ha in 2010 and 4.32 t/ha in 2011. The water concentration of *Miscanthus* and *Saccharum* in our study ranged from 42% to 58%; however, no significant differences were observed between species and years after planting (data not shown).

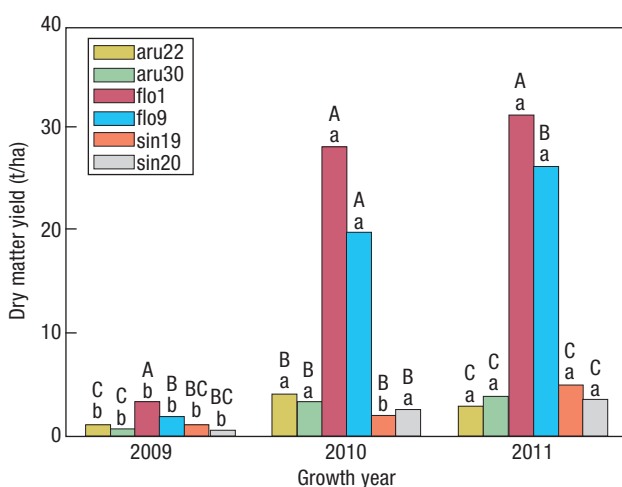


Figure 3. Dry biomass yields of *S. arundinaceum*, *M. floridulus* and *M. sinensis* (two accessions each) in the three years of growth. Lowercase letters indicate significant differences between years for the same accession. Uppercase letters indicate significant differences between accessions in the same year.

Mixed mode analysis using ANOVA showed that most of the agronomic traits and biomass yields were significantly different ($p < 0.01$) among species, years and accessions (Table 2). Variable interaction effects were observed among year-accession, year-species, and year-accession-species. The largest effects on biomass yield were attributed to species, year and then the interaction between year and species. Plant height was significantly affected by the year after planting, species, accession and their interaction. Culm diameter was mainly affected by species. Culm number was attributed to the year of growing, and tuft diameter variation was due to species. The results suggest that the species and the year after planting were the most important factors in determining agronomic traits and DM yield.

Plant development

To understand the dynamics of the tested agronomic traits development, we observed the traits in the three species every month from 2009 to 2011. The same development patterns were found in all the agronomic traits along the three years. Therefore, we analyzed the dynamics of the agronomic traits development only in 2010.

Figure 4 shows that the height of each individual plant generally increased with increasing GDD, creating a logistic curve from the vegetative phase to the flowering phase. The plant height of *M. floridulus* increased rapidly between GDD=0°C and GDD=800°C, which was ascribed to internode elongation. The equilibrium value of plant height was observed at GDD ~ 1500°C in July, when *M. floridulus* flowered. Flowering occurred in the beginning of September for *M. sinensis* and in the middle of October for *S. arundinaceum*. *M. sinensis* had a vegetative growth period of 6 months and achieved its stable plant height at a GDD=2000°C in August. *S. arundinaceum* continued to increase in height even in November.

The culm diameters of *S. arundinaceum* and *M. floridulus* increased over time, but that of *M. sinensis* did not (Fig. 4). *S. arundinaceum* showed rapid increase of culm diameter with increased GDD. The species *M. floridulus* had a slow increase rate of culm diameter but produced the thickest culms in its final developmental stage. The mean culm diameter of *M. sinensis* was maintained at 0.62 cm throughout the plant life cycle in 2010.

New shoots of *M. floridulus* and *M. sinensis* sprouted with increasing GDD with an appreciable amount of shoots occurred from June to September (Fig. 4). *M. sinensis* exhibited the same growth dynamics as *M. floridulus*. *Saccharum arundinaceum* produced few shoots from its rhizomes.

Table 2. Analysis of variance associated with the plant height, culm diameter, culm number, tuft diameter and dry mass yield of *S. arundinaceum*, *M. floridulus* and *M. sinensis* measured in two accessions over the three years of growth

Source	Dependent variable	Degrees of freedom	F statistic	Probability of >F
Year	Plant height	2	100.6	<0.01
	Culm diameter	2	82.7	<0.01
	Tuft diameter	2	135.3	<0.01
	Culm number	2	181.8	<0.01
	Yield	2	751.9	<0.01
Accession	Plant height	1	28.0	<0.01
	Culm diameter	1	29.9	<0.01
	Tuft diameter	1	27.6	<0.01
	Culm number	1	15.5	<0.01
	Yield	1	9.5	<0.01
Species	Plant height	2	52.6	<0.01
	Culm diameter	2	355.5	<0.01
	Tuft diameter	2	172.2	<0.01
	Culm number	2	79.9	<0.01
	Yield	2	1539.1	<0.01
Year × Accession	Plant height	2	5.8	0.348
	Culm diameter	2	1.1	<0.01
	Tuft diameter	2	2.4	0.105
	Culm number	2	9.3	<0.01
	Yield	2	6.9	<0.01
Year × Species	Plant height	4	22.0	<0.01
	Culm diameter	4	21.7	<0.01
	Tuft diameter	4	30.9	<0.01
	Culm number	4	26.0	<0.01
	Yield	4	401.6	<0.01
Accession × Species	Plant height	2	3.2	0.028
	Culm diameter	2	3.9	0.054
	Tuft diameter	2	1.5	0.232
	Culm number	2	14.3	<0.01
	Yield	2	0.6	0.535
Year × Accession × Species	Plant height	4	22.2	<0.01
	Culm diameter	4	9.3	<0.01
	Tuft diameter	4	1.8	0.148
	Culm number	4	6.3	<0.01
	Yield	4	6.5	<0.01

Table 3. Correlations between the dry mass yield and agronomic traits of *S. arundinaceum*, *M. floridulus* and *M. sinensis* (two accessions each) in the three years of growth

	Plant height	Culm diameter	Culm number	Tuft diameter	Flowering time	Yield1	Yield2
Yield1	0.01	0.89*	0.89*	0.79	-0.809		
Yield2	0.69	0.59	0.29	0.87*	-0.904*	0.94**	
Yield3	0.63	0.52	0.34	0.97**	-0.951**	0.90*	0.99**

Yield1, Yield2, Yield3 mean biomass yield for the first, second and third year, respectively. *, **: correlation is significant at the 0.05 and 0.01 levels, respectively.

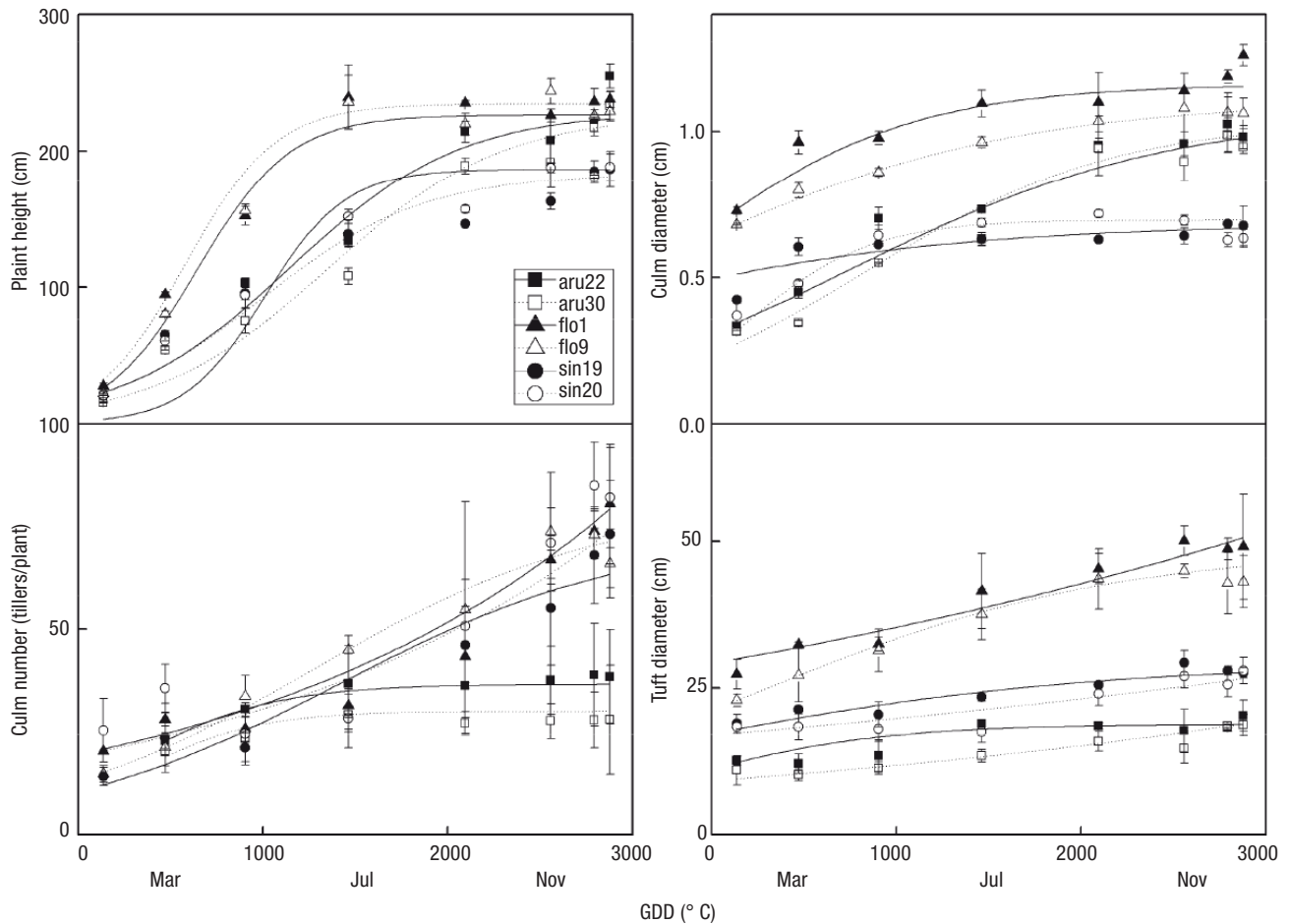


Figure 4. Plant height, culm diameter, culm number and tuft diameter of *S. arundinaceum*, *M. floridulus* and *M. sinensis* (two accessions each) as a function of growing degree-days (GDD) accumulated in 2010. The vertical bars indicate \pm SD.

The tuft diameter of *M. floridulus* increased clearly during the growing season, with new culms produced around the outer clump (Fig. 4). The tuft diameter of *M. sinensis* did not increase considerably during the year, and new shoots were established around the center of the patch. No change in the tuft diameter of *S. arundinaceum* was observed because of its concentrated patch and few emerged shoots.

Correlation between biomass yield and agronomic traits

The accumulation of biomass yield was significantly affected by the agronomical characters measured. Therefore, the correlations between yield and agronomic traits for the first three years of growth were calculated (Table 3). During the first year, the biomass yield was significantly and positively correlated with the culm diameter and culm number. The biomass yield in the second year was correlated to the tuft diameter

and to the first biomass yield ($R^2 = 0.87$ and 0.94 , respectively). A strong correlation ($R^2 = 0.97$) was found between the biomass yield and tuft diameter when yields of crops appeared to have peaked in the third year. There were also strong correlations between the biomass yields in the second and third year ($R^2=0.99$) and between the first and third year ($R^2=0.90$). Biomass yield was also strongly correlated to both culm and tuft diameter. Negative correlations were found between biomass and days to flowering for 2010 and 2011, indicating earlier flowered species tended to achieve higher biomass yield.

Discussion

M. floridulus could be used as candidate biomass crop in China

Although there have been several reports on the superiority of *Miscanthus* \times *giganteus* over *M. sinensis*

in Europe and USA (Clifton-Brown *et al.*, 2001; Anderson *et al.*, 2011), Zub *et al.* (2011) reported about a clone of *M. floridulus* that produced a similar or even greater biomass yield than *M. × giganteus*. Taiwanese native *M. floridulus* accessions collected from different altitudes produced yields of more than 20 t DM/ha/yr after transplantation in the field (Huang *et al.*, 2011). In the present work, we have also found that *M. floridulus* produced an almost three times greater biomass yield than the others in the second year and third year of growth (Fig. 3). During the first three years of growth, *M. sinensis* and *S. arundinaceum* in our study produced less than 5 t DM/ha/yr biomass. These two species were thin-stalked compared with *M. floridulus*, and they were surrounded by weeds during the growing seasons. However, Yan *et al.* (2012) found that the biomass yield of *M. sinensis* could reach ~ 10 t DM/ha/yr in northern China.

Our results showed that *M. floridulus* grew better than *M. sinensis* and *S. arundinaceum*. Sun *et al.* (2010) described the phenological character of *M. floridulus* and showed that this species flowered and produced fruits from May to November. In contrast, the heading and flowering of *M. sinensis* and *S. arundinaceum* occurred in late summer (Saballos, 2008). Wild *M. floridulus* can grow up to 400-600 cm (Sun *et al.*, 2010), in contrast to wild *M. sinensis*, which ranged in Japan 100-200 cm (Stewart *et al.*, 2009). *M. sinensis*, when grown at five different locations in Europe, reached 100-230 cm in height (Clifton-Brown *et al.*, 2001). In our study, *M. sinensis* grew to 180 cm (Fig. 2). Sun *et al.* (2010) reported that both *M. floridulus* and *M. sinensis* are widely distributed in nature, with the culm diameters of wild plants ranging 6-15 mm and 3-10 mm, respectively. In our work, at the end of the growing season, *M. floridulus* and *S. arundinaceum* achieved greater culm diameters than *M. sinensis* (Fig. 2), and the culm number of *M. floridulus* at the final stage of the second year was 80 culms/plant, lower than the number reported by Huang *et al.* (2011) for a crop grown in Taiwan at a density of 1 plant/m², which produced 85-112 culms/plant. Interestingly, the culm number of *M. sinensis* in our study was similar to that of the cultivated *M. sinensis* in Europe (Clifton-Brown *et al.*, 2001). The tuft diameter was enlarged by the production of the new shoots. *M. floridulus* had a larger tuft diameter than *M. sinensis* and *S. arundinaceum* (Fig. 2). This observation might be explained by individual culms competition for nutrients and light in the tuft, which would maintain a suitable density of new shoots. Jezowski (2008) observed that the tuft diameter of *M. sinensis* hybrids could reach ~ 30 cm in the third year, which is consistent with our results.

Species and crop age effects explain the variability of agronomic traits

The observed agronomic traits were influenced by growth year, species and year × species interaction (Table 2). The variation in plant height was clearly explained by the stand age and the species. The *M. floridulus* accessions were taller than those of the other species (Fig. 2). Maughan *et al.* (2012) reported that *M. × giganteus* plant height increased throughout the growing season in the first three years and exhibited similar growth patterns among the different environments in which it was tested. Analysis of variance showed that plant height between different species differed significantly within and between European countries (Clifton-Brown *et al.*, 2001). There was also significant within-species variation in plant height for *M. sinensis* grown in different sites in China (Yan *et al.*, 2012). The species accounted for a large portion of the total variability in culm diameter, making a four-fold greater contribution than growth year. Both *M. floridulus* and *M. sinensis* are widely distributed in nature. At the end of the growing season, *M. floridulus* and *S. arundinaceum* achieved greater culm diameters than *M. sinensis* did (Fig. 2). Tuft diameter was increased by new tiller spreading over the years. Due to the different growth and development patterns of emergence, tiller number was explained more by the growth year, while tuft diameter was more influenced by the species (Fig. 2). According to Stewart *et al.* (2009), clump growth via the generation of new outer shoots appears to cause competition for light, soil nutrients and water. *M. floridulus* expanded horizontally in all directions to form large patches of tillers that competed successfully with weed grasses.

Potential biomass yield could be predicted by agronomic traits and previous yields

Many agronomic traits influence the biomass yield. Clifton-Brown *et al.* (2001) reported an early-flowering *Miscanthus* genotype produced a lower biomass yield than a late-flowering genotype. However, Clifton-Brown & Lewandowski (2002) found that the late-flowering *M. sacchariflorus* had a lower biomass yield than *M. × giganteus*. At the inter-genotypic level, there was a strong positive relationship between biomass yield and plant height (Clifton-Brown *et al.*, 2001; Zub *et al.*, 2011). Yan *et al.* (2012) also found that the biomass yield of *M. sinensis* was positively correlated with plant height and tuft diameter in the second year. Our results showed that, of the species tested, *M. floridulus* had the greatest biomass yield in the second and third years,

even though it flowered earlier (Fig. 3). Furthermore, we observed no significant correlation between plant height and biomass yield during the first three years.

Jezowski (2008) reported that culm number determined the biomass in the first and third years of growth; in the second year, tuft diameter was most highly correlated with biomass yield. Zub *et al.* (2011) also described a strong positive relationship between culm number and aboveground biomass yield. Our results indicated that culm diameter and tiller number were the two main variables that determined the biomass yield in the first year, while tuft diameter was the main index during the second and third year (Table 3). Zub *et al.* (2011) found that the year-to-year relationship between the biomass yields was high regardless of the harvest dates. Clifton-Brown *et al.* (2001) found that the third year biomass yield was correlated to second-year biomass yield ($R^2=0.81$). We also found high and significant correlations of 0.9 between the first- and third-year yields and of 0.99 between the second- and third-year yields. Therefore, the biomass yields in the first and second years of growth could be good indicators of future biomass yield.

In summary, *Miscanthus* and *Saccharum*, perennial grasses endemic to eastern China and recognized as biomass crops for many years, were successfully cultivated over first three years on a sandy clay loam in this region. *M. floridulus* had DM yield above 20 t/ha during the second year, greater culm and tuft diameters, and taller plant height than the other species. Therefore, *M. floridulus* appears to be a promising option for breeding efforts regarding biomass production in regions with a short growing season. Our results indicate that effective selection of appropriate species for breeding purposes could begin with selection for culm diameter and tuft diameter characters. The first-year biomass yield could be considered as a good predictor of the yields in subsequent years. This information can help shorten the time required for selecting high-yield species.

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