

RESEARCH ARTICLE

The influence of morphological and geographical traits on yield of *Miscanthus sinensis* during the first two years

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ABSTRACT

Miscanthus is a C₄ perennial energy crop distributed in various regions of China. *Miscanthus sinensis* has a high potential for biomass production, great adaptability to marginal land and tolerance to stress environmental. It's very important for the production and development of biofuel to review both the morphological and geographical traits on yield. However, the relevance of biomass yield and the geographical position of habitat are still not well known. In the present study, investigations of heading date, plant height, stem number and dry biomass per plant were carried out to calculate the influence of the morphological traits on yield. The investigation was based on 327 *Miscanthus sinensis* accessions collected from 21 provinces from all over China. The analysis was carried out on the basis of the two-year field experiment. The data for the second year was used to perform the analysis of variance and regression. Statistical data analysis was also performed on the variation of the biomass and morphological traits over successive period of study. The results showed that the growth included plant height and stem number in the following year was much better than the first year, particularly the biomass. Regression analysis revealed that latitude, plant height, and stem number were the key morphological traits that influence the biomass production. Also, heading date and elevation were found to be highly correlated to yield structure. These results demonstrated that the growth and yield were strongly influenced by geographical location, and apart from morphological traits, geographical traits were important factors that affect biomass.

Keywords: Biomass; Geographical traits; Heading date; *Miscanthus sinensis*; Morphological traits

INTRODUCTION

M. sinensis, one kind of *Miscanthus*, originated in the east of Asia and widely distributed all over China. *M. sinensis* can grow beside river bank, agricultural land and even in the hilly areas from lower to the higher elevations (Chou, 2009). In the past decades, the reduction of non-regenerated energy and the increase in energy demand have attracted much interest in the research of an alternative energy. Besides, bioenergy study is the most prevalent topic and attracts wide attention. High-yield perennial grasses are considered as one of the most promising species for commercial bio-energy productions, due to low energy inputs and high outputs (Pyter et al., 2010). At present, production of biomass is significant breeding objective in bioenergy crops such as miscanthus (Heaton et al., 2010; Weijde et al., 2013). Field management practices like fertilization and harvest time can be very promising to improve the biomass quality

(Iqbal and Lewandowski, 2014; Lewandowski et al., 2003; Lewandowski and Heinz, 2003; Lewandowski and Kicherer, 1997). *M. sinensis* is a promising fiber yielding plants which having high potential in temperate climates for sustainable biomass production (Heaton et al., 2010). It is a perennial energy crop by high annual biomass production and high resource-use efficiency (Heaton et al., 2004; Heaton et al., 2008; Lewandowski et al., 2003; Long et al., 2001; Weijde et al., 2013). *Miscanthus* has a high yielding potential up to 40 tones ha⁻¹ year⁻¹, with low input lignocellulosic feedstock. In biomass use yield for a plethora of applications, it shows growing interest, particularly for the production of bioenergy and biofuels (Brosses et al., 2012). Heaton et al. (2004) reported that switchgrass production approximately 12 tons ha⁻¹ lower or half that of *Miscanthus* production observed in Europe. *M. sinensis* is high yield and, relative ease of husbandry using conventional equipment, therefore, it is a target species to renewable energy producers

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(Atkinson, 2009). *M. sinensis* takes in atmospheric CO₂ and assimilating it as plant tissue, and shares with its microbial partners in the soil (Clifton-Brown et al., 2007), or on the soil surface as leaf litter or other dead plant matter, which making it a plants carbon sequester (Heaton et al., 2009). Clifton-Brown et al. (2007) report a sequestration of 0.6 tones *C ha*⁻¹ year⁻¹. *M. sinensis* is also a crop with low nitrogen requirement showing a very impact on biomass production from fertilization (Himken et al., 1997). It also played a major role in reducing nitrogen from surface runoff (Heaton et al., 2008). Its broad range of variation of habitat indicates its adaptability on different kinds of extreme environment. It is one of the parents of *Miscanthus × giganteus* having higher yields than switchgrass (Heaton et al., 2008) a perennial grass as received for its potential as a model bioenergy crop. *M. sinensis* stores approximately 30% of its total dry matter in root and rhizome (Bullard et al., 1995). According to Clifton-Brown et al. (2004), annual yields of *M. sinensis* across Europe depends on types of soil and climatic condition. Its production can range from 10 to 40 tones *ha*⁻¹ year⁻¹. Clifton-Brown et al. (2001) indicated that an average yield of *M. sinensis* could reach 13.4 tones *ha*⁻¹ year⁻¹. According to Clifton-Brown et al. (2001) and Lewandowski et al. (2003), the highest yields without irrigation were observed in autumn (17.9 tones *ha*⁻¹) and winter (13 tones *ha*⁻¹) for *M. sinensis* hybrids and 14.3 and 8.9 tones *ha*⁻¹ for *M. sinensis* (non-hybrid) in the autumn and winter harvests respectively. Work done by Jeżowski (2008) in Poland revealed that winter yields during the third year reached 20.5, 14.9 and 9.8 tones *ha*⁻¹ for a hybrid between *M. sinensis* (2x) and *M. sacchariflorus* (4x), a hybrid between *M. sinensis* (2x) and *M. sacchariflorus* (2x) and a hybrid between two *M. sinensis* (2x) respectively. It is possible that *M. sinensis* can provide a genetic base for breeding because of the wide geographical distribution and genetic diversity and other characteristics. Several studies on traits, biomass yield, crop age, plant height, and tiller have been conducted worldwide (Huang et al., 2011; Jeżowski, 2008; Jeżowski et al., 2011; Zub et al., 2011). Although there have been numerous studies related in the literature, the relevance of biomass yield and the geographical position of habitat is still not well known. The aim of this study is to determine the variation of morphological and geographical traits during the first two years and find out the connections between the traits measured and dry biomass to confirm the key traits of biomass production.

MATERIALS AND METHODS

Plant materials

A total of 327 *M. sinensis* genotypes were collected from 21 provinces in China (Table 1). The geographical locations of these provinces ranged from 18°39.872N - 45°22.671N

Table 1: The information on collecting areas

Collection regions	Collection provinces	Sample number
North of China	Hebei	2
North of China	Shanxi	2
Northeast of China	Liaoning	1
Northwest of China	Gansu	10
Northwest of China	Shaanxi	21
Central of China	Hubei	43
Central of China	Henan	33
Central of China	Hunan	19
East of China	Jiangsu	7
East of China	Shandong	6
East of China	Jiangxi	21
East of China	Anhui	8
East of China	Zhejiang	12
East of China	Fujian	23
Southwest of China	Yunnan	17
Southwest of China	Guizhou	27
Southwest of China	Sichuan	20
Southwest of China	Chongqing	3
South of China	Guangxi	19
South of China	Guangdong	31
South of China	Hainan	3

and 100°10.027E - 127°34.816E covering north, northeast, south, southeast, southwest and central of China. All the resource samples were conserved in Wuhan Botanical Garden.

Field trials

The field experiments were conducted from 2015 to 2016 in a random designed plot of 23m×28m, with proper irrigation and timely weed control in Wuhan Botanical Garden at Chinese Academy of Sciences. Fertilizer was not used during the observation period. The materials were planted during spring period in 2015 and harvested in winter of 2015 and 2016. Analysis of variance of yield structure traits was conducted on plant height (the distance from surface of the soil to the apex of spikelet), tillering (the number of shoots 20 cm above the ground per plant) and ripening time following the description by Jeżowski et al. (2011). Maturity was defined by the heading date. The data was defined the days between March 1st the date of planting and the beginning of flowering. Heading date (the days between March 1st and the beginning of heading) and flowering date (the days between March 1st and the date that more than 50% headed) were added to the survey data the second year. The yield was defined in the form of plant biomass. Measurements were taken after harvest of each year. Biomass (plant weight of dry yield) was determined by the average weight of each plant.

Statistical analyses

The data from planting until peak yield in the second year of cultivation were taken for analysis. In order to compare

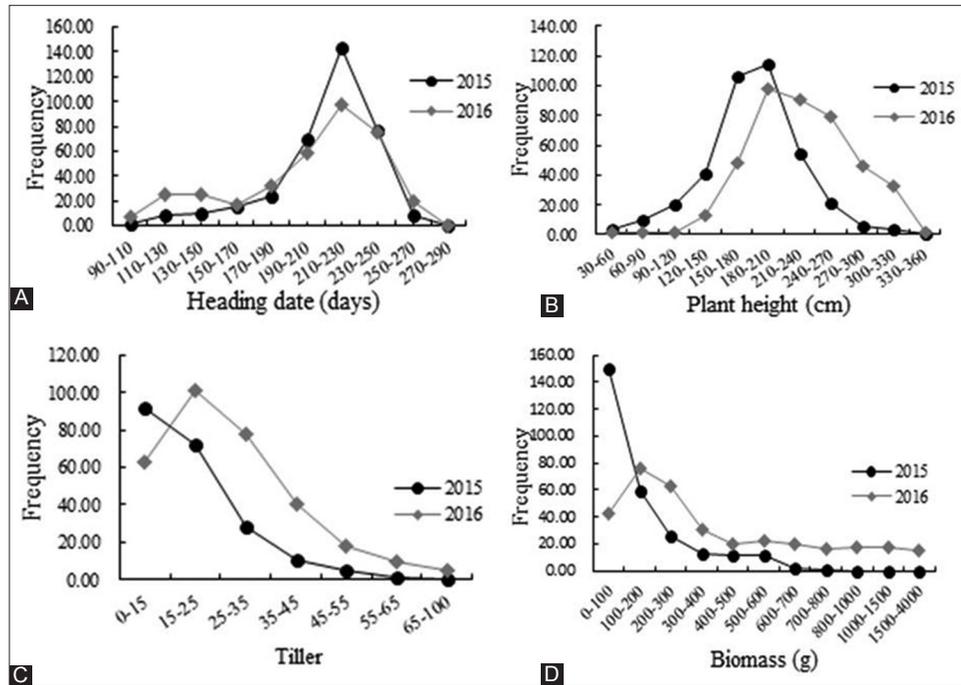


Fig 1. The distribution of frequency of heading date (A), plant height (B), tiller(C), biomass (D) in the two years.

the samples from each years of the study, the results of the measurements were used to conduct a variance of analysis and two-factor analysis of variance. In the least significance difference (LSD) test was conducted at the 0.05 level of significance. Moreover, hypotheses of the geographical position of habitat were tested concerning the differences in the morphological traits obtained between the first and second years. The correlation between the traits affecting production and geographical factors were explored by excel. The correlations between the traits measured in this study were also analyzed. Multiple regression analysis were carried out using SPSS 13.0 (SPSS Inc., USA) to confirm the key traits that influence the biomass production (Soleymanifard et al., 2012).

RESULTS

Agronomic traits

The date on vegetative growth phase of *M. sinensis* collected started from June and lasted to November which is considered to be early winter in Wuhan. Most samples headed from about 150 days to 260 days after germination in the two years. Heading peak was observed on day 240 after germination (Fig. 1A). Samples collected from Liaoning, Shandong, Gansu, Shanxi, Henan and Hubei provinces headed earliest while samples from Guangdong, Hainan, Yunnan, and Fujian provinces entered the mating season at the latest. Fig. 1B presents the analysis of plant height, the result showed that plant height mainly concentrated in the range from 19 to 27.5 cm. Most samples were more

Table 2: Traits comparison between the first and second year

Contrast	Heading date	Plant height	Tiller	Biomass
Y2-Y1	64.428**	5.478*	8.321**	102.758**

Y1- The first year of the experiment

Y2- The second year of the experiment

*Significant at the 0.05 level, **Significant at the 0.01 level

than 15 cm and up to 33.5 cm maximum for the second year. Higher samples were from Sichuan (Southwest of china), Guangdong (South of China), Guangxi (South of China), and Fujian (East of China). Generally, samples were taller than the first year, with 35 cm equally, even up to 2m partly. At the same time, the number of tillers per plant of each genotype showed a significant difference during the successive year of survey (Fig. 1C). The minimum of tillers per plant was only 10, but the maximum was up to 95. The result proved the strong variation of tiller ability. Interestingly, tiller has the same situation as plant height that the genotypes with more tillers concentrated in the same province. The biomass ranged from 200 gram to more than 800 gram in the first year but increased many folds the next year (Fig. 1D). Outstanding performance on plant height, tiller and biomass were reported from the samples collected from Sichuan, Guangdong, Guangxi and Fujian provinces. The samples collected from Guangdong were even up to 4000 gram in the second year. In order to understand how the variations of traits affect biomass over successive years of cultivation, it is reasonable to analyze the differences in these traits between the two experimental years. Generally, heading date, plant height, tiller, and biomass all showed significant differences (Table 2). The decrease of heading

date for the second year indicated that samples headed earlier in the second year probably due to the adaptive process to the environment change. But the same trend in the two years suggested that the length of the growth period was considered to be decided by genetic evolution year in year out. The plant height of the second year was significantly higher than the first year. The large increase in biomass in the second year was probably due to the augment of tiller and plant height (Figure 2).

Correlation analysis of the morphological traits

The correlation analysis was performed and the results are presented on Table 3. The results showed no correlation between plant height and heading date, full heading date, flowering date. Vegetative traits and plant height have a highly significant influence on leaf weight, stem weight, and biomass. The results of the main effect were positive. At the same time, it was noticed that there was a highly significant correlation between the heading date and the ratio of leaf and stem weight. The strong correlation between stem number and plant height suggested that higher altitude was accompanied by more vigorous tillers. Moreover, the ratio

of leaf and stem weight showed significantly positive effect on biomass. It indicated that the heading date could affect the composition of biomass. The late heading will lead to a higher ratio, more leaves and less stem, and finally higher biomass further. The stable interaction between biomass and the ratio of leaf and stem weight suggested that the composition of biomass could affect yield. It could be inferred that heading date, plant height and stem number which are supposed to be the key factors affecting biomass.

Correlation between morphological and geographical traits

From the correlation analysis of geographical parameter and individual traits, heading date and ratio of leaf/stem were strongly negatively correlated with both longitude and latitude (Table4). Latitude had a strong negative influence on all individual traits. With exception of leaf/stem, all traits were negatively correlated with elevation. These results proved that the growth and yield were strongly influenced by geographical location. It inferred that populations at lower latitude and elevation would enter the reproductive stage later and had a longer vegetative

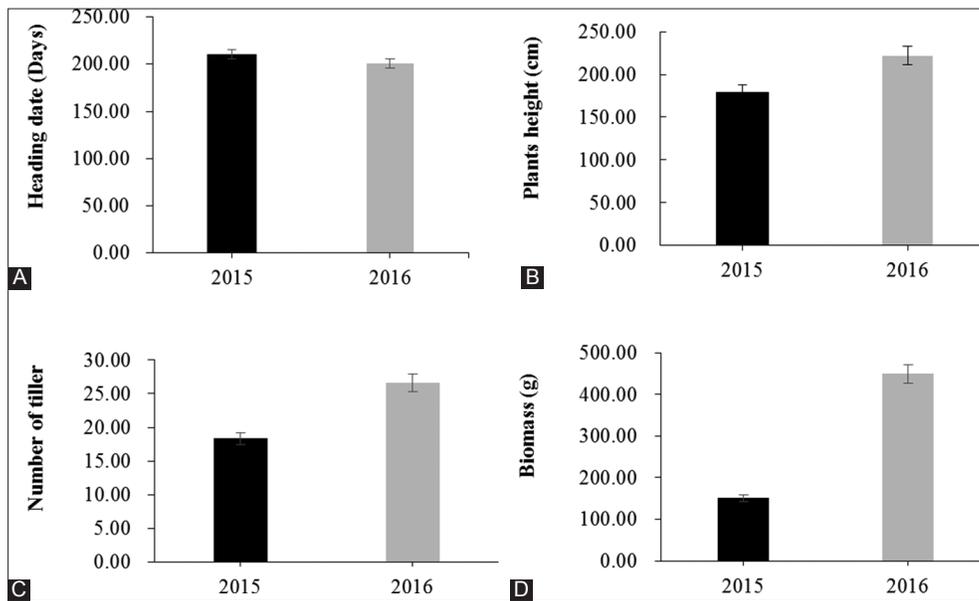


Fig 2. Bar graph showing (A) Heading date, (B) Plant height, (C) Tiller and (D) Biomass of successive year of survey.

Table 3: Correlation analysis of morphological traits and yield

	Heading date	Full heading date	Flowering date	Plant height	Stem number	Leaf weight	Stem weight	Ratio of leaf/stem
Full heading	0.979**							
Flowering date	0.991**	0.975**						
Plant height	0.094	0.094	0.099					
Stem number	0.103	0.106	0.122*	0.200**				
Leaf weight	0.511**	0.512**	0.505**	0.480**	0.514**			
Stem weight	0.382**	0.325**	0.323**	0.628**	0.582**	0.841**		
Ratio of leaf/stem	0.369**	0.361**	0.353**	-0.228**	-0.072	0.314**	-0.198**	
Biomass	0.462**	0.461**	0.456**	0.552**	0.558**	0.977**	0.931**	0.138*

*Correlation is significant at the 0.05 level, **Correlation is significant at the 0.01 level

Table 4: Correlation analysis of geographical and morphological traits

	North latitude	East longitude	Elevation (m)
Heading date	-0.898**	-0.109*	-0.186**
Full heading	-0.902**	-0.105	-0.165**
Flowering date	-0.904**	-0.083	-0.192**
Plant height	-0.126*	0.033	-0.244**
Stem number	-0.124*	-0.023	-0.078
Leaf weight	-0.536**	-0.015	-0.251**
Stem weight	-0.363**	0.061	-0.309**
Ratio of leaf/stem	-0.357**	-0.188**	0.127*
Biomass	-0.491**	0.008	-0.276**

*Correlation is significant at the 0.05 level, **Correlation is significant at the 0.01 level

Table 5: Multivariate analysis of variance of morphological traits on biomass

Source of variation	DF	R ²	F	P
Heading date	109	0.413	1.330	0.041*
Plant height	124	0.542	1.783	0.000**
Stem number	59	0.543	5.055	0.000**
Heading date×plant height	75	0.993	1.745	0.249
Heading date×stem number	124	0.986	2.395	0.016*
Plant height×stem number	121	0.993	1.159	0.475

R²: coefficient of determination; DF: degree of freedom; F: F value; P: probability; * significant at 0.05 **, significant at 0.01

growth. Similarly, the plants with higher plant height and biomass most likely appeared in the lower latitude and elevation areas. The inference may due to the higher growth temperature in those areas. Moreover, we could get both materials propagated early in the high latitude and elevation areas and materials with great biomass in the low latitude and elevation areas. Both latitude and elevation were important factors of growth. The investigation conducted in the successive years of vegetation confirmed that the materials collected from southern China grew higher and lush, but late heading. Differently, the materials from northern areas enter into the reproductive period earlier.

Key traits for biomass production

In order to understand the key traits that influence biomass production, further analysis of all production traits was conducted. The result of multivariate analysis of variance in table 5 demonstrates the strong impact of biomass which verified the inference that heading date, plant height and stem number supposed to be the key traits for biomass. There is no significant effect of the interaction between the heading date and plant height on the biomass. The interaction of plant height and stem number presented no apparent value of the main effect either. On the contrary, the interaction of heading date and stem number showed a significant effect of biomass.

Based on the analysis above, all traits measured that may affect the biomass were treated simultaneously in order to obtain the regression equation on biomass.

Regression equation was conducted with biomass(Y) as the dependent variable and heading date(X₁), full heading date(X₂), flowering date(X₃), latitude(X₄), longitude(X₅), elevation(X₆), plant height(X₇) and stem number(X₈) as the independent variables. The correlation coefficient R was 0.743, and the coefficient of determination R² was 0.553. The analysis of variance showed F was 121.130, P<0.01 which meant the equation was effective. The equation $Y=244.056-35.922X_4+3.521X_7+14.111X_8$ indicated that there was a linear relationship between biomass and latitude, plant height and stem number. When the plant height and stem numbers were fixed, biomass increased 279.978 as latitude reduced one degree. When the latitude and stem number were fixed, biomass increased 247.577 as plant height increased one unit. Similarly, when the latitude and stem number were fixed, biomass increased 258.167 as stem number increased by one.

DISCUSSIONS

The temperature difference for a year is very big between North and South of China, for the lowest temperature is -22 °C and the highest temperature is 44 °C. The influence of geographical from different temperature showed that the samples headed earlier mostly came from the north and northeast areas of China probably due to the low temperature and windy. On the contrary, the samples that came from the south of China headed at the latest likely because of perennial high temperature and sufficient sunlight. The North China area has short sunshine time and fast cooling, so the plants there reproduce earlier and are shorter hence the problem of plant breaking as a result of strong wind is mitigated. The environmental conditions in the southern parts of China make the plants have a long growth and enter into reproductive period very late. The samples which had better plant height, tiller and biomass were from Sichuan, Guangdong, Guangxi and Fujian provinces which indicated that the materials which came from these areas had the most potential. In addition, this situation suggested the correlation between plant height, tiller, and biomass. Important character especially biomass showed great changes between the first and second years of cultivation. The result showed that most samples grew better and provided a higher yield in the second year of cultivation. This is inconsistent with the findings of Clifton-Brown et al. (2001) and Jeżowski et al. (2011). The increase of plant height along with the increase of tiller may be the main reason for the significantly increase of biomass yield. Plant height increases the efficiency in the use of light. The ratio of leaf/stem had a significant positive effect on biomass, so it could be speculated that samples with more leaves could harvest more presumably due to the more leaves and the stronger photosynthesis.

Considering the factors related to biomass, the crop age turns out to be one important factor. The biomass increase accompanied by the growth. Our results concur to that of Zub et al. (2011) and Jeżowski et al. (2011) that there is a significant augment between the two consecutive years of cultivation which indicated the connection between yield and crop age. Moreover, an increase in yield from the first year of cultivation to second was reported by Clifton-Brown et al. (2001). Lewandowski et al. (2003) further proved that peak yields can be attained in two years. Our results further revealed that plant height and tiller were key factors for biomass which agrees with the findings of Jeżowski (2008) and Huang et al. (2011). In their study, Jeżowski (2008) and Huang et al. (2011) noted that biomass of *Miscanthus* species had a significantly positive correlation to plant height and tiller number. According to Huang et al. (2011), elevation is an important variable that can influence the biomass of *Miscanthus* species. Our study reveals that apart from morphological traits, geographical traits were also important factors that affect biomass. This could be explained by the low temperature in high altitude area and also temperatures variations with latitude changes.

CONCLUSION

As one of the most potential energy crop, *Miscanthus sinensis* has attracted much attention worldwide. *M. sinensis* is widely distributed in China. The 327 samples collected from various parts of China basically representative populations of Chinese. There are lots of diversities of each individual trait. The wealth of resources provides a guarantee to develop new varieties. The observed morphological traits variation in the two years indicates that *M. sinensis* grow rapidly from the second year. Although the growth was very rapid within the two years, a question still remains about the year where the peak is reached. This, therefore, creates a gap for future research. In our study, we concluded that latitude, plant height, and stem number are the key factors that directly influenced the biomass. In addition, heading date and elevation also played key role in the resulting biomass.

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Authors' Contributions

Nuananong Purente, Qiang Li, Jing Zhang; Designed and performed experiments, analysed data and co-wrote the paper. Junhua Peng; Performed transporter experiments. Lanming Cui; Co-analysed data. Miao He.; Supervised the research.

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