A DISSERTATION FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

Genetic Diversity and Biomass Yield Potential of Korean *Miscanthus*

BY

SOO-HYUN LIM

AUGUST, 2015

MAJOR IN CROP SCIENCE AND BIOTECHNOLOGY

DEPARTMENT OF PLANT SCIENCE

THE GRADUATE SCHOOL OF SEOUL NATIONAL UNIVERSITY

Genetic Diversity and Biomass Yield Potential of Korean *Miscanthus*

UNDER THE DIRECTION OF PROF. DO-SOON KIM
SUBMITTED TO THE FACULTY OF THE GRADUATE SCHOOL
OF SEOUL NATIONAL UNIVERSITY

BY
SOO-HYUN LIM

MAJOR IN CROP SCIENCE AND BIOTECHNOLOGY
DEPARTMENT OF PLANT SCIENCE
APRIL, 2015

APPROVED AS A QUALIFIED DISSERTATION OF SOO-HYUN LIM
FOR THE DEGREE OF DOCTOR OF PHILOSOPY
BY THE COMMITTEE MEMBERS
JUNE, 2015

CHAIRMAN ______________________________
Byun-Woo Lee, Ph. D.

VICE-CHAIRMAN ______________________________
Do-Soon Kim, Ph. D.

MEMBER ______________________________
Hee-Jong Koh, Ph. D.

MEMBER ______________________________
DoKyoung Lee, Ph. D.

MEMBER ______________________________
Jong-Woong Ahn, Ph. D.

Genetic Diversity and Biomass Yield Potential of Korean *Miscanthus*

Soo-Hyun Lim
Crop Science and Biotechnology
Department of Plant Science
The Graduate School
Seoul National University

GENERAL ABSTRACT

Miscanthus is a potential bioenergy crop due to its C_4 photosynthesis, perennial and rhizomatous growth, rapid growth and high biomass productivity, and broad environmental adaptability. Korea is known as a core region that has diverse *Miscanthus* genetic resources. Particularly *M. sinensis* and *M. sacchariflorus*, which are considered to be two most important parental candidates for developing high yield cultivars, are native to Korea. However, no study has been reported to evaluate genetic diversity and biomass yield potential of Korean *Miscanthus*. Therefore, this study was conducted to investigate genetic diversity by assessing morphological, phenological and agronomic traits of Korean *Miscanthus* and to evaluate biomass yield potential by determining the relationships between

phenological, canopy structural and agronomic traits with biomass yield in a field located in Suwon and Yeoju, Korea for 5 years from 2010 to 2014.

To investigate genetic diversity by morphological traits, 280 *Miscanthus* accessions that consist of 184 *M. sinensis* and 93 *M. sacchariflorus* collected from Korea and other East Asian countries including China, Japan, and Russia were planted in the *Miscanthus* germplasm field located in Suwon, Korea in 2010. Three reference accessions, one *M.* × *giganteus*, one *M. floridulus*, and one *M. lutarioriparius*, were also planted. Twenty morphological traits in leaf, stem, and inflorescence were assessed and then used for phylogenetic analysis. Among these traits, the presence of awn in spikelet, stem growth habit, new autumn shoot emergence, and the ratio of callus hair to spikelet were the key traits to distinguish between *M. sinensis* and *M. sacchariflorus*, while the other traits were associated with genetic diversity among accessions within the same species or the same cluster. Both *M. sinensis* and *M. sacchariflorus* were individually clustered into five groups with *M. floridulus* belonging to *M. sinensis* IIa group, while *M. lutarioriparius* and *M.* × *giganteus* into *M. sacchariflorus* V group. Principal coordination analysis (PCoA) of morphological traits found 24 intermediate accessions, seven *M. sinensis* and 17 *M. sacchariflorus*, positioned between *M. sinensis* and *M. sacchariflorus*. Chromosome counting revealed that five accessions, one *M. sinensis* and four *M. sacchariflorus*, were triploid, suggesting natural hybrids between *M. sinensis* and *M. sacchariflorus*.

Traits associated with biomass yield such as phenological and agronomic traits were assessed in the four-year field trial in Suwon, Korea from 2010. Correlation analyses among phenological and agronomic traits, biomass yield and geographic latitude of collection site showed that heading date, leaf growth traits and stem growth traits were closely related with biomass yield. Latitude and heading date exhibited a significant negative correlation, and heading date showed a significant positive correlation with biomass yield. The presence of significant relationships between latitude and agronomic traits suggests that the accessions collected from different geographical latitudes can provide more genetically diverse materials for breeding. The growth of top three leaves (flag, the second and the third leaf) showed positive correlation with other agronomic traits such as stem diameter and stem dry weight, and then biomass yield. Agronomic traits assessed in the second year after planting also had a strong correlation with biomass yield assessed in the fourth year after planting. In particular, the leaf area, stem diameter and stem dry weight of the second year were significantly related with the fourth year biomass yield. It suggested that the earlier agronomic traits assessed in the s year can be used for screening *Miscanthus* genetic resources and lines with high biomass yield potential. Despite of general relationships between phenological and agronomic traits and biomass yield, poor relationships were often observed between traits and latitude collection sites. These diverse patterns of relationship with phenological and agronomic traits imply that the tested *Miscanthus* accessions possess high genetic diversity in their biomass yield potential.

For better understanding of biomass formation in *Miscanthus* and establishing an ideo-type with high biomass yield for future breeding, canopy structures of 17 *Miscanthus* accessions, 8 *M. sinensis*, 6 *M. sacchariflorus*, and 3 triploid *Miscanthus*, were assessed in the field located in Yeoju, Korea, for 3 years from 2012 to 2014. Canopy diameter, canopy height, canopy area and canopy volume of each plant were measured together with no. of stem and stem dry weight per plant every year, and then stem no. per canopy area, stem dry weight per canopy area, and stem dry weight per canopy volume were calculated. Annual canopy development rates in all the canopy-associated parameters were finally estimated by linear regression analyses. Canopy area and volume of *M. sinensis* were relatively slowly developed due to its rhizome growth habit as compared to *M. sacchariflorus* and triploid *Miscanthus*, which show rapid outwards rhizome growth. Regarding biomass accumulation per a unit area and volume of canopy, *M. sinensis* showed greater biomass density due to its compact growth habit than *M. sacchariflorus*, while triploid *Miscanthus* showed intermediate biomass accumulation. These results suggest that canopy developmental traits are dependent on *Miscanthus* species thus it can be used for discriminating *Miscanthus* species. Correlation analyses of canopy traits with agronomic traits and biomass yields revealed that canopy diameter, canopy height, canopy area and canopy volume are significantly related with biomass yield, but the extent of significance of these relationships depends on *Miscanthus* species. In *M. sinensis*, canopy volume followed by canopy diameter and canopy area were the most determinant for biomass yield, while canopy height followed by canopy diameter and canopy volume in *M. sacchariflorus* and canopy diameter followed by canopy area and canopy volume in triploid *Miscanthus* were the most important determinant. These results suggest that for biomass production, outwards expansion of canopy structure is important for *M. sinensis* and triploid *Miscanthus*, while vertical expansion of canopy structure is important for *M. sacchariflorus*. Therefore, canopy parameters provided a clue of desirable canopy structure of *Miscanthus* for future breeding toward greater biomass yield potential of *Miscanthus* cultivar.

Key words: Agronomic trait, biomass yield, breeding, canopy structure, genetic diversity, *Miscanthus*, morphological trait

Student number: 2008-21273

Genetic Diversity and Biomass Yield Potential of Korean *Miscanthus*

CONTENTS

GENERAL ABSTRACTS ………………………………………………………. i

CONTENTS ……..……………..………...……………….………… vi

LIST OF TABLES ………………………………………...….…………… x

LIST OF FIGURES ………………………………….....…….……….... xii

LIST OF APPENDICES ………………………………………………. xv

ABBREVIATIONS ………………………………………….……….. xvii

GENERAL INTODUCTION …………………………...….…………..... 1

LITERATURE REVIEW …………………………………….……………. 4

REFERENCES ……………………………………........….….…….. 12

CHAPTER I. Genetic diversity of Korean *Miscanthus* based on morphological trait analysis and identifying intermediate *Miscanthus*

ABSTRACT …………………………………….……………………. 17

INTRODUCTION …………………….……….....…………………… 19

MATERIALS AND METHODS ………….….……...………………... 21

Collection of *Miscanthus* germplasm …….….………...……………… 21

Morphological traits of *Miscanthus* accessions …….…...……………... 22

Phylogenetic analysis 23
RESULTS 24
Genetic Diversity of morphological traits in *Miscanthus* accessions 24
Phylogenetic relationship of *Miscanthus* accessions........................ 30
Intermediate type of *Miscanthus* accessions 36
DISCUSSION 42
Key traits to classify *Miscanthus* species 42
Intermediate type of *Miscanthus* accessions 43
REFERENCES 45

CHAPTER II. Relationships between phenological traits and agronomic traits revealed key traits determining *Miscanthus* biomass yield

ABSTRACT 50
INTRODUCTION 52
MATERIALS AND METHODS 54
Field experiment 54
Phenological traits and agronomic traits of *Miscanthus* accessions 54
Statistical analysis 56
RESULTS 57
Phenological traits of *Miscanthus* accessions 57
Growth developments of *Miscanthus* accessions 58
Biomass yield of *Miscanthus* accessions 60

Relationship among the phenological traits, agronomic traits and biomass yield 64

DISCUSSION 75

Key traits determining *Miscanthus* biomass yield in early stage of *Miscanthus* planting 75

Relationship among the phenological traits, agronomic traits and biomass yield 76

REFERENCES 78

CHAPTER III. Relationship between agronomic traits and canopy structure in determining *Miscanthus* biomass yield

ABSTRACT 82

INTRODUCTION 84

MATERIALS AND METHODS 86

Assessment of canopy structure development of *M.* × *giganteus* 86

Assessment of canopy structure of *Miscanthus* species 90

Distribution of stem number and stem dry weight 91

Statistical analysis 91

RESULTS 92

Canopy structure development of *M.* × *giganteus* and its association with biomass 92

Comparison of canopy structure between the *Miscanthus* species …....... 98

Relationship among the agronomic trait, canopy structure development and biomass yield ……………………….…………………….................. 110

DISCUSSION ………………………………………………….……… 116

Relationship between species and canopy structure development ..…... 116

Relationship among the agronomic trait, canopy structure development and biomass yield ………………………..……………………........... 117

REFERENCES …………………………………………………......….. 119

APPENDIX ………………………………………………………….. 123

ABSTRACT IN KOREAN …………………………………...…….. 132

LIST OF TABLES

Table 1. *Miscanthus* species and their major characteristics (Renvoize, 1992; Royal Botanic Garden Kew web; Chung and Kim, 2012) ……... 8

Table 1-1. Summary of morphological traits of *Miscanthus* species collected from East Asia ……………………………………………….... 28

Table 1-2. Summary of main morphological traits among the *M. sinensis*, *M. sacchariflorus*, *M.* × *giganteus*, and intermediate type ………... 38

Table 1-3. Summary of morphological traits of *Miscanthus* showing intermediate characteristics. M-045, M-160 and M-168 is reference accessions of *M. sinensis* and *M. sacchariflorus* (4n and 2n), respectively …………………………………………………. 41

Table 2-1. Range of variation assessed in the 2nd year phenological traits and agronomic traits of *Miscanthus* accessions ……………………. 61

Table 2-2. Range of variation assessed in the 4th year phenological traits and agronomic traits of *Miscanthus* accessions …………………….. 62

Table 2-3. Summary of correlation analyses among the 2nd year agronomic traits, the 4th year biomass yield and latitude of collection site of *M. sinensis and M. sacchariflorus* accessions ………….……… 65

Table 3-1. Summary of canopy structure parameters of *M.* × *giganteus.* The values in the parentheses are standard errors (*df* = 6) ………….. 94

Table 3-2. Summary of stem dry weight and number of stem distribution parameters following Gaussian distribution curve in each planting year …………………………………………………………….. 97

Table 3-3. Summary of number of stems distribution parameters following Gaussian distribution curve in each year after planting ………. 102

Table 3-4. Summary of stem dry weight distribution parameters following Gaussian distribution curve in each year after planting ………. 103

Table 3-5. Summary of canopy structure parameters of *Miscanthus* species ……………………………………………………………... 105

Table 3-6. Summary of canopy structure related with biomass yield of *Miscanthus* species …………………………………………… 107

LIST OF FIGURES

Figure 1-1. Collection sites of *Miscanthus* accessions in Korea, Japan, China and Russia …………………….……………………………… 22

Figure 1-2. Phylogenetic tree for 277 *Miscanthus* accessions with 3 outgroup *Miscanthus* species based on 20 morphological trait analyses ………………………………………………………….... 32

Figure 1-3. Phylogenetic tree for 184 *M. sinensis* with *M. floridulus* based on 20 morphological trait analyses ……………………………… 33

Figure 1-4. Phylogenetic tree for 93 *M. sacchariflorus* with *M.* × *giganteus* and *M. lutarioriparius* based on 20 morphological trait analyses ………………………………………………………….... 35

Figure 1-5. Principal coordinate analysis of all *Miscanthus* accessions. Yellow dots in the blue circle indicate *M. sinensis*, green dots in the green circle located *M. sacchariflorus*, and yellow and green dots in the red circle showing intermediate types …………….. 37

Figure 2-1. Box plot indicating median (line), interquartile range (boxes), and 5% to 95% percentile (whiskers) for each phenology and agronomic traits assessed in the 4th year *Miscanthus* after planting ……………………………………………………… 63

Figure 2-2. Relationship between main agronomic traits assessed in the 4th year after planting of pooled *Miscanthus* …………………….. 66

Figure 2-3. Relationship between main agronomic traits assessed in the 4th year after planting of *M. sinensis* ……………………………... 68

Figure 2-4. Relationship between main agronomic traits assessed in the 4th year after planting of *M. sacchariflorus* ……………………… 70

Figure 2-5. Correlation between main agronomic traits assessed in three years after planting and the 4th year biomass yield. *M. sinensis* (A, B and C), *M. sacchariflorus* (D, E and F), and Pooled (G, H and I). *, ** and *** indicate statistical significance at the 0.05, 0.01 and 0.001 levels, respectively …………………………………..... 72

Figure 2-6. Associations among the phenological trait (heading date), main agronomic traits assessed in the 2nd year *Miscanthus* after planting, and the 4th year biomass yield. *M. sinensis* (A), *M. sacchariflorus* (B), and Pooled (C). ** and *** indicates statistical significance at the 0.01 and 0.001 level, respectively ………... 74

Figure 3-1. Canopy area and canopy volume of *M.* × *giganteus* assessed in the 1st (A), the 2nd (B), the 3rd (C), and the 4th year (D) after planting. The parameter *r* and *h* are maximum radius (1/2 × canopy diameter) and maximum stem height (canopy height), respectively …... 88

Figure 3-2. One example of stem distribution of *M.* × *giganteus* assessed in the 1st year (◆), the 2nd year (△), the 3rd year (■) and the 4th year (○) after planting ………………………………………... 89

Figure 3-3. Simulated distribution of number of stem (A) and stem dry weight (B) per unit distance from the center of *M.* × *giganteus* for 4 years since the first planting in 2011. The simulated values were calculated using the Gaussian distribution curve (equation 3.4) and the parameter estimates (Table 3-1) ……………………… 96

Figure 3-4. Distribution of number of stem (*M. sinensis* (A), *M. sacchariflorus* (B) and hybrid *Miscanthus* (C)) and stem dry weight (*M. sinensis* (D), *M. sacchariflorus* (E) and hybrid *Miscanthus* (F)) per unit distance from the center of *Miscanthus* accessions in the 3rd year after planting …………………….. 101

Figure 3-5. Relationship between canopy structure parameters and biomass yield of *Miscanthus* species ……………………………….... 109

Figure 3-6. Association among the 3rd year agronomic traits, canopy structure parameters and biomass yield of *M. sinensis* ($2n = 2x = 38$). *, ** and *** indicate statistical significance at the 0.05, 0.01 and 0.001 levels, respectively ……………………………….. 111

Figure 3-7. Association among the 3rd year agronomic traits, canopy structure parameters and biomass yield of *M. sacchariflorus* ($2n = 4x = 76$). *, ** and *** indicate statistical significance at the 0.05, 0.01 and 0.001 levels, respectively ……………………….…... 113

Figure 3-8. Association among the 3rd year agronomic traits, canopy structure parameters and biomass yield of hybrid *Miscanthus* ($2n = 3x = 57$). *, ** and *** indicate statistical significance at the 0.05, 0.01 and 0.001 levels, respectively …………………...…….. 115

LIST OF APPENDICES

APPENDIX 1. Summary of morphological trait in *Miscanthus* accessions ……………………………………………………. 123

APPENDIX 2-1. Range of variation assessed in the 1st year phenological traits and agronomic traits of *Miscanthus* accessions .. 125

APPENDIX 2-2. Range of variation assessed in the 3rd year phenological traits and agronomic traits of *Miscanthus* accessions .. 126

APPENDIX 2-3. Summary of correlation analyses among the 1st year agronomic traits, the 4th year biomass yield and latitude of collection site of *M. sinensis and M. sacchariflorus* accessions ……………………………………...… 127

APPENDIX 2-4. Summary of correlation analyses among the 3rd year agronomic traits, the 4th year biomass yield and latitude of collection site of *M. sinensis and M. sacchariflorus* accessions ……………... 128

APPENDIX 2-5. Summary of correlation analyses among the 4th year agronomic traits, the 4th year biomass yield and collection site latitude of *M. sinensis and M. sacchariflorus* accessions ………………………………………….. 129

APPENDIX 2-6. Relationship between main agronomic traits assessed in the 1st year after *Miscanthus* planting ………….......... 130

APPENDIX 2-7. Relationship between main agronomic traits assessed in the 2nd year after *Miscanthus* planting …………….......... 130

APPENDIX 2-8. Relationship between main agronomic traits assessed in the 3rd year after *Miscanthus* planting …………….......... 131

ABBREVIATIONS

2D PCoA	2-Dimentional Principal Coordinate Analysis
ANOVA	Analysis of Variance
CA	Canopy Area
CV	Canopy Volume
DM	Dry Matter
DW	Dry Weight
LAI	Leaf Area Index
LSD	Least significant difference
MTA	Mean Tip Angle or The leaf inclination angle
UPGMA	Unweighted Pair Group Method with Arithmetic average

GENERAL INTRODUCTION

Miscanthus is a C_4 perennial rhizomatous plant and shows high biomass yield potential and broad environmental adaptability from tropical to temperate climates. Since the 1980s, *Miscanthus* has been studied as a bioenergy crop in several European countries including Denmark, Germany, and UK, and cultivated for commercial biomass production.

It is *Miscanthus* × *giganteus* that is commercially cultivated in Europe. *Miscanthus* × *giganteus* is a triploid hybrid ($2n = 3x = 57$) resulted from a natural hybridization between *Miscanthus sinensis* ($2n = 2x = 38$) and *Miscanthus sacchariflorus* ($2n = 4x = 76$) (Greef *et al.*, 1997; Hodkinson *et al.*, 2002b and c; Rayburn *et al.*, 2009). *Miscanthus* × *giganteus* is a promising bioenergy crop due to cold tolerance, C_4 photosynthesis, high water-use efficiency, low input of fertilizer, and high biomass yields in a variety of environments (Clifton-Brown *et al.*, 2001; Clifton-Brown *et al.*, 2002; Clifton-Brown *et al.*, 2007; Lewandowski *et al*, 2003; Naidu *et al.*, 2003). Due to its high biomass yield and broad environmental adaptation, it is now commercially cultivated as a bioenergy crop in Europe and intensively studied in USA (Heaton *et al.*, 2008).

Due to its triploid characters, *M.* × *giganteus* is sterile and cannot produce viable seed for propagation (Linde-Laursen, 1993). Thus, *M.* × *giganteus* can be propagated only through rhizomes or cultured tissues. This nature supports that *M.* × *giganteus* has low invasive risk. However, it limits breeding a new cultivar from this sterile *M.* × *giganteus* as this cannot be crossed with other *Miscanthus* to introduce a new trait. Furthermore, *Miscanthus* commercially being cultivated is from a single clone of *M.* × *giganteus* and thus has no genetic diversity. This suggests high risk of diseases and pest outbreaks in the near future (Clifton-Brown *et al.*, 2001; Prasifka *et al.*, 2009). Increasing genetic diversity by introducing new *Miscanthus* cultivars is urgently required to solve such risks. To breed a new cultivar, it is required to understand genetic diversity of *Miscanthus* species based on morphological and phenotypic trait, and various traits associated with biomass yield, stress tolerance and so on by long-term phenotyping of *Miscanthus* germplasm collected from various geographic localities. There is little research about genetic diversity based on diverse traits such as morphological traits, phenological traits, and agronomical trait related with biomass yield of *Miscanthus* species.

Miscanthus requires at least three years for assessing its biomass yield potential, which is a challenging problem for *Miscanthus* breeding and production researches. If we can estimate biomass yield potential earlier than 3 years after planting, our efforts for such researches will be significantly reduced and time for breeding a new cultivar will be shorten. *Miscanthus* shows different growth habit in each species, thus it determined different canopy developments and affected to establish potential biomass yield in each *Miscanthus* species. Canopy developments can distinguish *Miscanthus* species and predict potential biomass yield by canopy structure parameters, these parameters can be using in breeding new *Miscanthus* cultivar as a target traits for improving potential biomass yield.

Despite the fact that *Miscanthus* is native species in East Asia where diverse *Miscanthus* germplasms can be easily collected, not many efforts have been made to evaluate genetic diversity in morphologic and agronomic traits including biomass yield potential of *Miscanthus* collected in this region based on long-term phenotyping study.

Therefore, this study was conducted with the following main objectives.

Chapter I

1) to investigate genetic diversity of *Miscanthus* accessions collected in Korea and neighboring countries

Chapter II

1) to assess the phenological and agronomic traits of *Miscanthus* accessions in the long-term field study

2) to investigate the relationships among the phenological traits, agronomic traits, and biomass yield of *Miscanthus* accessions

Chapter III

1) to characterize the canopy structure development of *Miscanthus* species

2) to determine the relationship between canopy structure and *Miscanthus* biomass yield

LITERATURE REVIEW

1. Bioenergy crop

Climate change and increasing demand of energy resources required new fuels replaced fossil fuels (Oliver *et al.*, 2009). Bioenergy could reduce emission of carbon dioxide because of it's produced from organic matters. Fossil fuels should be replaced to bioenergy fuels (Yuan *et al.*, 2008). After harvest, bioenergy crop converted to bio-oil, such as alcohol, bioethanol, and bio-butanol to replaced fossil fuels. Oil plant such as oilseed rape, sun flower, and peanut produce biodiesels for fuels. Starch-based plant such as potato, soy bean, sweat potato, cassava, corn, wheat, barley, rice, and sorghum produce alcohol and bioethanol from starch (Yuan *et al.*, 2008). Sugar-based plant such as sugar beet and sugar cane produce bioethanol from sugar. To produce amount of biofuels, bioenergy crop should be produce high biomass yield and tolerance to abiotic stress or poor environment. Thus, Woody crops such as willow and poplar are widely utilized, as well as perennial grasses such as *Miscanthus* and common reed cultivated as a cellulose-based bioenergy crop (Fischer *et al.*, 2005). Additionally, genetic modification plant also researched to improve biomass yield, enhanced to abiotic stress, low input to manage bioenergy crops (Kirakosyan and Kaufman, 2009).

However, oil plant, starch-based plant, and sugar-based plant were used for food or feedstock, thus these plants caused competitions with human or livestock. Therefore, bioenergy crop is a grown for bioenergy feedstock rather than food supply.

1.1. The first generation bioenergy crop

The first generation bioenergy can be produced from chemical components of agricultural crops such as oil, starch, and sugar, which can be extracted and converted to bio chemicals, replace the fossil fuel (De Oliveira *et al.*, 2005). The first generation bioenergy crop composed of oil-based crop, starch-based crop, and sugar-based crop. Oil palm, oilseed rape, sun flower, and peanut were included oil-based bioenergy crop. Potato, sweet potato, cassava, corn, wheat, barley, rice, and sorghum were included starch-based bioenergy crop. Sugar beet and sugar cane included sugar-based bioenergy crop (De Vries *et al.*, 2010). However, these bioenergy crops would result in severe competition between food supplies and energy, which is probably not sustainable in the long term given that the net energy and carbon dioxide balance is not favorable (De Oliveira *et al.*, 2005; Farrell *et al.*, 2006).

1.2. The second generation bioenergy crop

The second generation bioenergy crops have recently gained attention as a result of the limitations of the first generation bioenergy crops, such as competition between food supplies and energy (Yuan *et al.*, 2008). The second generation bioenergy crop produces bioethanol and bio-butanol from lignocellulose biomass. They produce ethanol and butanol from lignocellulose through fermentation of biomass. Additionally, they directly used as a fuels in place of fossils, because of high converting costs and rate to produce bioethanol and butanol. The second generation bioenergy crops derived from lignocellulose plant materials such as a *Miscanthus*, common reed, willow, and poplar are considered as more sustainable energy than the first generation bioenergy. The second generation bioenergy crop had no competition with food supply or feedstock. Furthermore, these crops had tolerance to abiotic stress and high biomass yield potential.

2. *Miscanthus*

2.1. Taxonomy

The genus *Miscanthus* distributed from the tropics and subtropics, and different species are widely distributed climatic regions in East Asia (Greef and Deuter 1993). *Miscanthus* belongs to the subtribe *Saccharineae* of the tribe *Andropogoneae*, which is in the family *Graminae*.

Miscanthus genus broadly classified into 15 species by Renvoize and Gilbert (1992) (Table 1), although some other taxonomist reported more species (Chou 2009; Lee 1995). South East Asia, the pacific Islands, and tropical Africa also distributed other *Miscanthus* species (Chen and Renvoize, 2006).

However, within the *Miscanthus* genus, there are confused in few species (Lewandowski *et al.* 2000; Renvoize and Gilbert 1992).

2.2. Habitat and growth physiology

Species of interest for biomass production are *M. sinensis* (diploid), *M. sacchariflorus* (diploid in Korea and China, tetraploid in Korea and Japan), *M. floridulus* (diploid), *M. lutarioriparius* (diploid), and *M.* × *giganteus* (triploid). These species are native to the East Asian (Korea, Japan, China, and Russia). The other species are known from the Himalayas and southern Africa (Hodkinson *et al.* 2002a) but are of no interest for biomass production because of their genotypic difference with East Asian *Miscanthus* species. Therefore, East Asia is the core area of *Miscanthus* genetic resource for developing *Miscanthus* as a bioenergy crop.

2.3. Genetic information

Miscanthus has a basic chromosome number of 19. The triploid genotype *M.* × *giganteus* ($2n = 3x = 57$) believe that natural hybrid between *M. sinensis* (diploid, $2n = 2x = 38$) and *M. sacchariflorus* (tetraploid, $2n = 4x = 76$) (Greef and Deuter 1993). As an allotriploidy, *M.* × *giganteus* is sterile and impossible maturing fertile seeds (Linde-Laursen 1993).

The collecting *Miscanthus* germplasm and use of diverse characteristic of germplasm are important factors in *Miscanthus* breeding. In East Asia, *Miscanthus* putative species for breeding, such as *M. sinensis* and *M. sacchariflorus* has a significant genetic diversity (Jørgensen and Muhs 2001). However, triploid *M.* × *giganteus* has a very low level of genetic diversity because its genetic and cultivating background (Greef *et al.* 1997; Hodkinson *et al.* 2002b) that they all propagated from the same clone of *M.* × *giganteus*. For this reason, *M. sinensis* and *M. sacchariflorus*, putative parental species could research to breed new Miscanthus cultivar, overcoming the disadvantage of *M.* × *giganteus.*

Table 1. *Miscanthus* species and their major characteristics (Renvoize, 1992; Royal Botanic Garden Kew web; Chung and Kim, 2012).

Miscanthus **spp.**	Chromosome number	Natural habitat or origin	Major characteristics
M. sinensis	$2n = 38$	Asia temperate and tropical	On mountain slopes, highlands, open grassy places and wasteland, caespitose, rhizomes short
M. floridulus	$2n = 38$	Asia temperate and tropical, Pacific	On wasteland, slopes and grassy places, caespitose, culm erect; 1.5 ~ 4.0 m long
M. sacchariflorus	$4n = 76$	Asia temperate	On mountain slopes, roadsides, plains and river banks, culms solitary, rhizomes elongated
M. lutarioriparius	$4n = 76$	South China	Rhizomes short, scaly, culms geniculately ascending
M. × *giganteus*	$3n = 57$	Japan	Hybrid between *M. sinensis* and *M. sacchariflorus*
M. tinctorius	NA*	Japan	Caespitose, rhizomes short, hairy adaxially
M. oligostachyus	NA	Eastern Asia	Culms solitary, rhizomes short, knotty, culms erect
M. transmorrisonensis	NA	Taiwan	Evergreen leaves, closely related to *M. sinensis*
M. fuscus	NA	Asia tropical	Culms erect, reed-like, leaf sheath longer than adjacent culm internode
M. nudipes	$2n = 40$	China, India	Culms erect, culm nodes pubescent, leaf blade margins smooth
M. nepalensis	$2n = 40$	Nepal, Himalaya	Caespitose, rhizomes short, culms erect, ligule a ciliolate membrane
M. violaceus	NA	Africa tropical	Caespitose, culms erect, 2.0 ~ 4.0 m long, leaf blade apex acute
M. junceus	NA	Africa tropical	Caespitose, culms erect, 1.0 ~ 3.0 m long, leaf blade apex acuminate
M.sorghum	NA	South Africa	Caespitose perennial, culms 1.5 ~ 4.0 m high
M. ecklonii	NA	South Africa	Caespitose, rhizomes absent or short, culms erect, 1.0 ~ 4.0 m long

* NA: not available

3. Genetic diversity

The use of germplasm for the development of *Miscanthus* will be an important approach to create genetic diversity in *Miscanthus* breeding program. Selecting useful diversity from the genetic resources available will be an enormous challenge. Understanding of genetic diversity of crop will helpful to understand crop evolutionary relationship among the germplasm, to improve breeding strategies using by genetic diversity information (Bretting and Widrlechner, 1995). However, there is no evaluation on genetic diversity of *Miscanthus* species (Yook *et al.*, 2014). Evaluation based on agronomic traits plays an important role in application of *Miscanthus* breeding.

3.1. Phenotypic diversity (Morphological traits)

Phenotype includes qualitative and quantitative characteristics. Qualitative characteristics, like leaf color, hair color, and flower color etc. are often controlled by single genes and less influenced by environmental factors. In contrast, quantitative characteristics such as stem height, number of stem, biomass yield etc. are controlled by polygenes and strongly influenced by environmental factors.

According to the growing number of phenotypic traits in a comparison of breeding pool, the number of genes involved in the control of phenotypic traits should increase, resulting in improving the utility of phenotypic diversity in predicting genotypic diversity (Cui *et al.*, 2001).

4. Phylogenetic relationship

Phylogenetics is the evolutionary relationship among the related crop species. Phylogenetic analysis is exhibited as branching, treelike diagrams that represent a calculated pedigree of the genetically or inherited relationship among organ, molecule, or both (Baxevanis and Ouellette, 2004).

Member of a group or shared common evolutionary history or more related to each other than another group closely estimated in cladistics. A given group shared common features each other than distant ancestors (Fiona and Detlef, 2004). A phylogenetic tree is a mathematical model of evolution. Tree composed of ingroup and outgroups. They linked with branch, node and root. Phylogenetic analysis estimated difference in DNA-sequence structure, distance matrix between sequence, restriction data, and allele data. Phylogenentics tree represented correlation between allele or distance among the phenotypic matrix (Krane and Raymer, 2003).

5. Plant canopy structure

Plant canopy structure is the spatial concept of plant growth development from the above ground in single plant or plant community (Russell *et al.*, 1990).

Development of stems and branches support consisting of biomass in plant growth. In above ground, leaf and stem characteristics were important factors to develop plant canopy structure, which determine the plant biomass yield. These growth development characteristics had difference in each species or cultivars.

In agricultural production, stem height, proportion of leaf coverage, and stem density are important factors for the assessment of crop establishments (Ehlert *et al*., 2008). Crop biomass yield depends on their morphological and physiological characteristics, such as dry matter accumulation. Based on these parameters, expected crop yields can be appraised and the amount of fertilizers and pesticides for the site-specific crop management can be optimized (Ehlert and Dammer, 2006). Furthermore, in harvesting with machinery, canopy structure determine the condition of harvest processing (Ehlert *et al*., 2008). Biomass yield of crop production, either grazed or mechanically harvested, is determined by crop community size, canopy structure, and developmental status of tiller populations within the crop community. Canopy structure parameters include stem height and stem densities, LAI, the angle of leaf inclination, leaf blade length and width, and internode length. Growth development in crop canopy structure that occurs during plant growth can be important determinants of potential productivity in perennial grasses (Redfearn *et al*., 1997). In this reason, crop breeders considered crop canopy structures such as stem height, number of stem, leaf area index, or tolerance to abiotic stress to increase crop biomass yield.

Reference

Baxevanis AD, Ouellette BF. 2004. *Bioinformatics: a practical guide to the analysis of genes and proteins*: John Wiley & Sons.

Bradshaw JD, Prasifka JR, Steffey KL, Gray ME. 2010. First report of field populations of two potential aphid pests of the bioenergy crop *Miscanthus* × *giganteus*. The Florida Entomologist **93**: 135–137.

Bretting P, Widrlechner MP. 1995. Genetic markers and plant genetic resource management. Plant Breeding Reviews **13**: 11-86.

Chen SL, Renvoize SA. 2006. *Miscanthus* Andersson. In ZY Wu, PH Raven, eds, Flora of China, Vol. 22, Beijing, Science Press, St. Louis, MO, Missouri Botanical Garden Press 581-583.

Chou CH. 2009. *Miscanthus* plants used as an alternative biofuel material: The basic studies on ecology and molecular evolution. Renewable Energy **34**: 1908-1912.

Christian DG, Bullard MJ, Wilkins C. 1997. The agronomy of some herbaceous crops grown for energy in southern England. Aspects of Applied Biology, Biomass and Energy Crops **49**: 41–52.

Christian DG, Haase E. 2001. Agronomy of *Miscanthus*. In: *Miscanthus* for Energy and Fibre (eds Jones MB, Walsh M), pp. 21–45. James & James, London.

Clifton-Brown JC, Breur J, Jones MB. 2007. Carbon mitigation by the energy crop, *Miscanthus*. Global Change Biology **13**: 2296–2307.

Clifton-Brown JC, Lewandowski I, Andersson B, Basch G, Christian DG.

2001. Performance of 15 *Miscanthus* genotypes at five sites in Europe. Agronomy Journal **93**: 1013-1019

Clifton-Brown JC, Lewandowski I, Bangerth F, Jones MB. 2002. Comparative responses to water stress in stay-green, rapid and slow senescing genotypes of the biomass crop, *Miscanthus*. New Phytologist **154**: 335–345.

Cui Z, Carter TE, Burton J W Jr, and Wells R. 2001. Phenotypic diversity of modern Chinese and north American soybean cultivar. Crop Science **41**: 1954-1967.

Daniels J, Roach BT. 1987. A taxonomic listing of *Saccharum* and related genera. Sugar Cane Spring Suppl 16-20.

De Oliveira MED, Vaughan BE, Rykiel EJ. 2005. Ethanol as fuel: energy, carbon dioxide balances, and ecological footprint. BioScience **55**: 593-602.

De Vries SC, van de Ven GWJ, van Ittersum MK, Giller KE. 2010. Resource use efficiency and environmental performance of nine major biofuel crops, processed by first-generation conversion techniques. Biomass and Bioenergy **34**: 588-601.

Ehlert D, Horn HJ, and Adamek R. 2008. Measuring crop biomass density by laser triangulation. Computers and Electronics in Agriculture **61(2)**: 117-125.

Ehlert D, Dammer KH, 2006. Widescale testing of the Crop-meter for site-specific farming. Precision Agriculture, http://dx.doi.org/10.1007/s11119-006-9003-z.

Farrell AE, Plevin RJ, Turner BT, Jones AD, O'hare M, Kammen DM.

2006. Ethanol can contribute to energy and environmental goals. Science **311**: 506-508.

Fiona SLB, Detlef DL. 2004. Bioinformatics: a practical guide to the analysis of genes and proteins: Phylogenetic analysis. John Wiley & Sons

Fischer G, Prieler S, van Velthuizen H. 2005. Biomass potentials of miscanthus, willow and poplar: results and policy implications for Eastern Europe, Northern and Central Asia. Biomass and Bioenergy **28**: 119-132.

Greef JM, Deuter M. 1993. Syntaxonomy of *Miscanthus* × *giganteus* GREEF et DEU. Angewandte Botanik **67**: 87-90.

Greef JM, Deuter M, Jung C, Schondelmaier J. 1997. Genetic diversity of European *Miscanthus* species revealed by AFLP fingerprinting. Genetic Resources and Crop Evolution **44**: 185-195.

Hodkinson TR, Chase MW, Lledo MD, Salamin N, Renvoize SA. 2002a. Phylogenetics of *Miscanthus*, *Saccharum* and related genera (*Saccharinae, Andropogoneae, Poaceae*) based on DNA sequences from ITS nuclear ribosomal DNA and plastid trnL intron and trnL-F intergenic spacers. Journal of Plant Research **115**: 381-392.

Hodkinson TR, Chase MW, Renvoize SA. 2002b. Characterization of a genetic resource collection for *Miscanthus* (*Saccharinae, Andropogonae, Poaceae*) using AFLP and ISSR PCR. Annals of Botany **89**: 627-636.

Jørgensen U, Muhs HJ. 2001. *Miscanthus* breeding and improvement. In MB Jones, M Walsh, eds, *Miscanthus* for Energy and Fibre, James & James Ltd., London, pp. 68-85.

Kirakosyan A, Kaufman PB. 2009. Recent advances in plant biotechnology. Dordrecht, Springer.

Krane DE, Raymer ML. 2003. Fundamental Concepts of Bioinformatics: Pearson Education International.

Lee SC. 1995. Taxonomy of *Miscanthus* (*Poaceae*) in Taiwan. Ph.D. Dissertation, Department of Biology. Taiwan University, Taipei, Taiwan.

Lewandowski I, Clifton-Brown JC, Scurlock JMO, Huisman W. 2000. *Miscanthus*: European experience with a novel energy crop. Biomass and Bioenergy **19**: 209-227.

Lewandowski I, Scurlock JMO, Lindvall E, Christou M. 2003. The development and current status of perennial rhizomatous grasses as energy crops in the US and Europe. Biomass and Bioenergy **25**: 335-361.

Linde-Laursen IB. 1993. Cytogenic analysis of *Miscanthus* '*Giganteus*' and interspecific hybrid. Hereditas **119**: 297-300.

Lo CC, Chen YH. 1989. Breeding *Saccharum-Miscanthus* hybrids for fiber resource. Proceedings of the International Society of Sugar Cane Technologists (ISSCT): 892-893.

Naidu SL, Moose SP, AL-Shoaibi AK, Raines CA, Long SP. 2003. Cold tolerance of C4 photosynthesis in *Miscanthus* × *giganteus*: adaptation in amounts and sequence of C4 photosynthetic enzymes. Plant Physiology **132**: 1688–1697.

Oliver RJ, Finch JW, Taylor G. 2009. Second generation bioenergy crops and climate change: a review of the effects of elevated atmospheric CO2 and drought on water use and the implications for yield. Global Change Biology Bioenergy **1**: 97-114.

Prasifka JR, Bradshaw JD, Meagher RL, Nagoshi RN, Steffey KL, Gray ME. 2009. Development and feeding of fall armyworm on *Miscanthus* × *giganteus* and switchgrass. Journal of Economic Entomology **102**: 2154–2159.

Rayburn AL, Crawford J, Rayburn CM, Juvik JA. 2009. Genome Size of Three *Miscanthus* Species. Plant Molecular Biology Reporter **27**: 184-188.

Redfearn DD, Moore KJ, Vogel KP, Waller SS, and Mitchell RB. 1997. Canopy architecture and morphology of switchgrass populations differing in forage yield. Agronomy Journal **89(2)**: 262-269.

Renvoize S, Gilbert D. 1992. Taxonomy and cultivars. In Rutherford, MC Heath, eds, The Potential of *Miscanthus* as a Fuel Crop, ETSU Report No. **B 1354**, London: DTI, pp. 21-29.

Spencer JL, Raghu S. 2009. Refuge or reservoir? The potential impacts of the biofuel crop *Miscanthus* × *giganteus* on a major pest of maize. PLoS ONE. **4**: e8336.

Yook MJ, Lim SH, Song JS, Kim JW, Zhang CJ, Lee EJ, Ibaragi Y, Lee GJ, Nah GJ, Kim DS. 2014. Assessment of genetic diversity of Korean *Miscanthus* using morphological traits and SSR markers. Biomass and Bioenergy **66**: 81-92.

Yuan JS, Tiller KH, Al-Ahmad H, Stewart NR, Stewart CN. 2008. Plants to power: bioenergy to fuel the future. Trends in Plant Science **13**: 421-429.

CHAPTER I. Genetic diversity of Korean *Miscanthus* based on morphological trait analysis and identifying intermediate *Miscanthus*

ABSTRACT

Miscanthus is a potential bioenergy crop due to its C_4 perennial growth, high biomass yield production. To obtain genetic diversity for future breeding program, we collected 280 accessions of *Miscanthus* germplasm from Korea and other East Asian regions and performed morphology-based phenotyping using twenty morphological traits in leaf, tem, and inflorescence. There are two distinctive species groups, *M. sinensis* and *M. sacchariflorus,* which are considered to be two most important parental candidates for breeding of high yielding cultivars, in morphological trait analyses. Among 20 morphological traits, we found that four major traits, namely the presence of awn in spikelet, rhizome growth habit, new autumn shoot emergence, and the ratio of callus hair to spikelet are the key traits to distinguish between *M. sinensis* and *M. sacchariflorus*. Principal coordination analysis (PCoA) of morphological traits found 24 intermediate accessions, seven *M. sinensis* and 17 *M. sacchariflorus, which were initially classified as M. sinensis* and *M. sacchariflorus*, positioned between *M. sinensis* and *M. sacchariflorus*. Chromosome counting revealed that 5 accessions, 1 *M. sinensis* and 4 *M. sacchariflorus*, are triploid, suggesting natural hybrids between *M. sinensis* and *M. sacchariflorus*. Our results suggested that phylogenetic analyses using morphological traits are effective for the diversity assessment and identification of phylogenic groups of *Miscanthus* species. This phylogenetic group based on morphological traits will provide effective parental selection for future breeding of a highly productive *Miscanthus* cultivar.

Key words: *Miscanthus spp.*, morphological trait, phylogenetic analysis, genetic diversity, breeding

Introduction

Miscanthus has been studied as a potential bioenergy crop in Europe since the 1980s (Lewandowski *et al.*, 2003). Especially, the triploid hybrid *M.* × *giganteus* has been widely cultivated as a dedicated bioenergy feedstock (Hodkinson and Renvoize, 2001). However, mass production of *M.* × *giganteus*, which is a sterile hybrid, requires high costs to propagate through vegetative propagation and tissue culture (Christian *et al.*, 2005). Furthermore, it is difficult to improve *M.* × *giganteus* through breeding due to its triploid nature (Lafferty and Lelley, 1994) as *M.* × *giganteus* is an allotriploid hybrid ($2n = 3x = 57$) between *M. sinensis* ($2n = 2x = 38$) and *M. sacchariflorus* ($2n = 4x = 76$) (Hodkinson *et al.*, 2002a). Therefore, its putative parents, *M. sinensis* and *M. sacchariflorus*, are gaining attention for the future breeding with potential high diversity in two parents.

Thus, the collection and use of diverse *Miscanthus* germplasm is a critical factor in *Miscanthus* breeding. The high levels of genetic diversity have been found among the parental species (*M. sinensis* and *M. sacchariflorus*) (Jørgensen *et al.*, 2001). Recently, for this reason, some research groups in Korea, Japan, and China have also started collecting their own *Miscanthus* germplasm and began breeding a new *Miscanthus* cultivar as a bioenergy crop (Chung and Kim, 2012). The assessment of the genetic diversity in crop species is of interest for the conservation of genetic resource, broadening of the genetic base, and practical applications in breeding programs (Amini *et al.*, 2008). In order to design an appropriate breeding program, it is important to know how much the phenotypic and genetic variation of a trait is heritable (Kearsey and Pooni, 1996).

Andersson was described the taxonomy of *Miscanthus* using morphological traits in 1885 (Scally *et al.*, 2001). Related inflorescence traits, such as the length of inflorescence axis, the length of raceme, arrangement of the spikelet, shape of glume, the presence of glume hair, and the presence of awn in spikelet were classified *Miscanthus* genus (Lee 1964 a, b and c; Clayton and Renvoize, 1986; Hodkinson *et al.*, 1997). Lee (1993) also reported qualitative trait, such as elongated rhizome, awn in spikelet, bud at the node, bunch or scattered type of growth habit, and the presence of leaf hair could distinguish *Miscanthus* and *Triarrhena*. However, these traits were not clear to classify all *Miscanthus* species. Few *Miscanthus* had intermediate type of between *M. sinensis* and *M. sacchariflorus*. Scally *et al*. (2001) also reported morphological classification using the qualitative traits is difficult to apply with another grasses. Only use quantitative traits also difficult to distinguish members of the *Miscanthus* genus (Hodkinson *et al*., 2002b; Renvoiz *et al*., 1997). For this reason, Sun *et al*. (2010) trying to use inflorescence traits and vegetative traits to classify *Miscanthus* genus.

Therefore, this study was conducted to determine its genetic diversity and phylogenetic relationships of *Miscanthus* accessions based on morphological traits. Also, morphological trait analyses were conducted to find the key traits of *Miscanthus* in order to classify its species and to assess the polymorphism of intra- and inter specific variation and phylogenetic relationship within and between species.

Materials & Methods

Collection of *Miscanthus* germplasm

Miscanthus accessions (280 *Miscanthus* accessions composed of 184 *M. sinensis*, 93 *M. sacchariflorus,* 1 *M.* × *giganteus*, 1 *M. floridulus* and 1 *M. lutarioriparius*) were collected in Korea, Japan, China and Russia during 2008-2011 as marked in Figure 1-1. We collected 149 *M. sinensis* and 70 *M. sacchariflorus* from different geographical sites in Korea, which were maintained at the *Miscanthus* germplasm field of Seoul National University, Suwon (N 37° 16' 12.1", E 126° 59' 27.5"), Korea. Nitrogen fertilizer was applied at 60 kg N ha^{-1} $year^{-1}$ in early June and weed management was conducted manually and using herbicide if necessary. All the accessions were planted at a density of 1 plant m^{-2} with three replications and grown for four years. When we collect a *Miscanthus*, we classified *Miscanthus* species using by shape of rhizome and growth habit of stem. We obtained *Miscanthus* from Japan (12 *M. sinensis* and 4 *M. sacchariflorus*), China (5 *M. sinensis* and 10 *M. sacchariflorus*) and Russia (12 *M. sinensis* and 9 *M. sacchariflorus*). As a outgroup species, *M. floridulus* (collected from China) and *M. lutarioriparius* (collected from China) were also included.

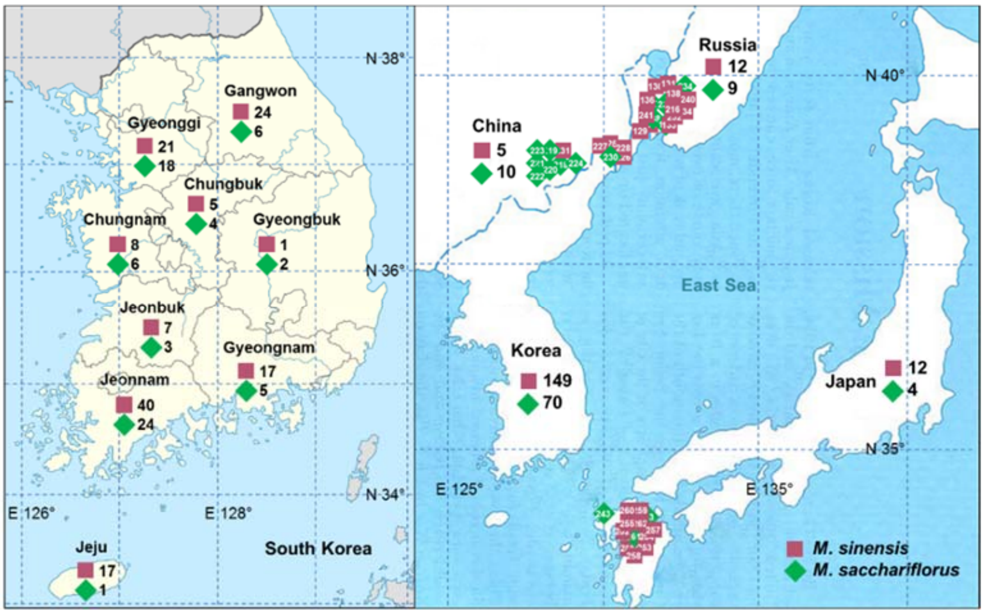


Figure 1-1. Collection sites of *Miscanthus* accessions in Korea, Japan, China and Russia

Morphological traits of *Miscanthus* accessions

We maintained collected *Miscanthus* at the *Miscanthus* germplasm field of Seoul National University, Suwon, Korea (N 37° 16’ 12.1”, E 126° 59’ 27.5”). Twenty morphological traits were evaluated to assess the phenotypic diversity of the *Miscanthus* accessions using phylogenetic analysis. The morphological traits included those associated with the leaf (the shape of leaf cross section, the presence of ligule hair, auricle, leaf hair and collar hair, leaf sheath hair, and the leaf dry weight at harvest), culm (growth habit, the presence of branch, stem diameter, and the presence of new shoot emergence in autumn), floret (the presence of an awn, no. of stigma/anther, and color of stigma/anther), panicle (the shape of panicle, and panicle length), and spikelet (touch of callus hair and ratio between callus hair and spikelet) (Appendix 1).

Phylogenetic analysis

Phylogenetic analyses of morphological traits were performed using the NTSYS-pc program version 2.21 (Rohlf, 2002). Morphological trait data were converted for standardization with the STAND module. The STAND module was used prior to SIMINT to reduce the effects of different scales of data in different morphological traits data. Morphological similarities between accessions were measured using the CORR coefficient based on standard morphological traits data with the SIMINT module. The unweighted pair group arithmetic average (UPGMA) was used to generate clusters of accessions following the SAHN module. The clustering results were used to plot a dendrogram following the TREE module. Principal coordinate analysis (PCoA, = Multidimentional scaling, MDS) based on standard morphological traits data with the SIMINT module using the DIST coefficient were used to visualize phenotypic similarities and relationship among the accessions using NTSYS-pc program version 2.21 (Rohlf, 2002).

Results

Genetic diversity of morphological traits in *Miscanthus* accessions

After all of the accessions were classified based on Renvoize and Gilbert (1992), phenotypic assessment was performed to investigate genetic diversity of *Miscanthus* accessions by observing morphological traits. Morphological traits showed diverse patterns in each organ.

Leaf morphologies were showed diverse characters in all the *Miscanthus* species. The shape of leaf cross section showed four-types as a flat shape, flat and rolled shape, flat and V-shape, and V-shape (Appendix 1). Portion of flat shape, flat and V-shape, flat and rolled shape, and V-shape were 64.3 %, 28.9 %, 4.3 %, and 2.5 %, respectively. *M. sinensis* had diverse shape of leaf cross section. Flat and rolled shape (37 %) and V-shape (3.8 %) showed only in *M. sinensis*. Most of *M. sacchariflorus* had a flat type leaf (86.0 %). All accessions of *Miscanthus* did not have an auricle and had a ligule as a membrane type (Appendix 1 and Table 1-1). Most of accessions had ligule hair (90.4 %), and they differed in amount of hair. Most of accessions (59.3 %) had a short and little amount of ligule hair, and some accessions (31.1 %) had a long and many ligule hair. Leaf hair showed different pattern between *M. sinensis* and *M. sacchariflorus*. 88.2 % of *M. sacchariflorus* had no leaf hair, while 86.4 % of *M. sinensis* had leaf hair. 82.1 % of accessions had no collar hair, and no difference in portions between *M. sinensis* and *M. sacchariflorus*. 72.1 % of the *Miscanthus* accessions did not have leaf sheath hair. Dry weight of leaf at harvest composed of leaf blade dry weight and leaf sheath dry weight of each stem. Dry weight of leaf represented leaf canopy formation. Leaf dry weight of *M. sinensis* ranged from 0.77 to 40.32 g, while this of *M.*

sacchariflorus showed 0.63 to 25.95 g. Most of *M. sacchariflorus* (92.5 %) were inferior to average of leaf dry weight (mean = 10.05 g). It represented that *M. sacchariflorus* leaf was shattered more than *M. sinensis* leaf at harvest.

Stem morphologies were determined canopy structure of *Miscanthus* species. *M. sacchariflorus* showed unique characters in stem organ such as branching node and buds, while *M. sinensis* did not shown such traits. Growth habit divided *Miscanthus* into three types as a bunch type, scattered type, and intermediate type (Appendix 1). Most of *M sinensis* belong to the bunch type of growth habit, and most of *M. sacchariflorus* belong to the scattered type. Interestingly, three *M. sinensis* accessions and eleven *M. sacchariflorus* accessions showed different type of growth habit compared to typical one. They mixed bunch type and scattered type of growth habit like a *M.* × *giganteus* (Appendix 1 and Table 1-1). The presence of stem branching (axillary branches) at the node was clearly showing difference between *M. sinensis* and *M. sacchariflorus*. There was no stem branching in *M. sinensis*, while twelve *M. sacchariflorus* and *M. lutarioriparius* had a stem branching at the node (Appendix 1). Stem diameter of *M. sinensis* (mean = 5.98 mm) was greater than *M. sacchariflorus* (mean = 4.90 mm). Stem diameter of *M. sacchariflorus* ranged from 3.03 to 9.05 mm, while *M. sinensis* varied from 1.41 to 10.06 mm. New shoot emergence in autumn also clearly distinguish between *M. sinensis* and *M. sacchariflorus*. All of *M. sinensis* had a newly emerged shoot in autumn, however *M. sacchariflorus* did not emerge new shoot in autumn (Appendix 1).

Floret is commonly used to identify plant species. It could applicate *Miscanthus* species, *M. sinensis* had an awn in their floret, while *M. sacchariflorus* did not have an awn. However, our observing in this study, two

M. sinensis did not have an awn and eight *M. sacchariflorus* had an awn. Awn length of *M. sinensis* (mean = 8.14 mm) was longer than *M. sacchariflorus* (mean = 4.9 mm). For all of accessions, each floret had two stigmas and three anthers. There were no differences on the number of stigmas and anthers between *M. sinensis* and *M. sacchariflorus*. Color of stigmas and anthers also did not differ between *M. sinensis* and *M. sacchariflorus*. When the first stigma and the first anther were initiated, color was purple and yellow, respectively (Appendix 1 and Table 1-1).

Panicle shape of *Miscanthus* accessions showed four-types, as a diamond shape, flat shape, tip-rolled shape, and sector shape. 73.9 % of all *Miscanthus* accessions showed flat shape, and the other accessions showed equally partitioned in each shape. Panicle length of accessions ranged from 10 cm to 54 cm. There were no trends between *M. sinensis* (mean = 28.9 cm) and *M. sacchariflorus* (mean = 28.3 cm). Ten out of all accessions had long panicles more than 40 cm. These accessions were *M. sacchariflorus* except one *M. sinensis*. *Miscanthus* × *giganteus* and *M. lutarioriparius* also had long panicles over 40 cm.

Many taxonomist use the ratio of callus hair and spikelet to classify *Miscanthus* species. The ratio of callus hair to spikelet was showed different ratios between *Miscanthus* species. The ratios were approximately 1.0 in *M. sinensis* and *M. floridulus* and were close to 2.0 in *M. sacchariflorus*, *M. lutarioriparius* and *M.* × *giganteus*. Interestingly, Twenty-three out of all *M. sacchariflorus* showed 0.9 to 1.4 ratios, while nine *M. sinensis* showed 1.5 to 1.6 ratios. 82.1 % of all accessions had silky callus hair, while the other accessions had rough callus hair. All of *M. sacchariflorus* had silky callus hair, and 30 accessions of *M. sinensis* had a rough callus hair.

Twenty morphological traits were very diverse in all the *Miscanthus* accessions. In these traits, the presence of new autumn shoot, the presence of awn, and the ratio of callus hair to spikelet were clearly distinguished between the species.

Table 1-1. Summary of morphological traits of *Miscanthus* species collected from East Asia

Organs	Traits	Unit	*M. sinensis* (*n* = 184)	*M. sacchariflorus* (*n* = 93)	*M. × giganteus*
Leaf	Leaf cross section shape	Flat (%)	52.7	86.0	100.0
		Flat-V (%)	37.0	14.0	0.0
		V (%)	3.8	0.0	0.0
		Flat-roll (%)	6.5	0.0	0.0
	Ligule hair	x (%)	12.5	3.2	0.0
		+ (%)	46.2	85.0	100.0
		++ (%)	28.8	10.8	0.0
		+++ (%)	12.5	1.1	0.0
	Auricle	○ (%)	0.0	0.0	0.0
	Leaf hair	○ (%)	86.4	11.8	0.0
	Collar hair	○ (%)	16.3	19.4	0.0
	Leaf sheath hair	○ (%)	32.1	19.4	100.0
	Leaf canopy (Leaf dry weight)	g	12.40	4.94	15.95
Culm	Growth habit	Bunch (%)	98.4	0.0	0.0
		Scattered (%)	0.0	85.0	0.0
		Intermediate (%)	1.6	15.1	100.0
	Branch	○ (%)	0.0	12.9	0.0
	New shoot emergence in Autumn	○ (%)	100.0	0.0	0.0
	Stem diameter	mm	5.98	4.90	8.40
Floret	Awn	○ (%)	98.9	8.6	0.0
	Awn length	mm	8.06	0.42	0.00
	No. of stigma	Number	2.0	2.0	2.0

Table 1-1. Continued

Organs	Traits	Unit	*M. sinensis* (n = 184)	*M. sacchariflorus* (n = 93)	*M.* × *giganteus*
Floret	No. of anther	Number	3.0	3.0	3.0
	Stigma color	Color	Purple	Purple	Purple
	Anther color	Color	Yellow	Yellow	Yellow
Panicle	Panicle shape	Diamond (%)	8.2	10.8	0.0
		Flat (%)	74.5	73.1	100.0
		Roll (%)	9.2	5.4	0.0
		Sector (%)	8.2	10.8	0.0
	Panicle length	cm	28.30	28.87	39.25
Spikelet	Ratio between callus hair and spikelet	Ratio	1.13	1.67	1.74
	Touch of callus hair	Silky (%)	72.8	100.0	100.0

Phylogenetic relationship of *Miscanthus* accessions

To investigate genetic diversity of Miscanthus species, we assessed morphological traits collected Miscanthus accessions, and then drawn a phylogenetic tree (Figure 1-2) based on assessed morphological traits (Appendix 1). All of the accessions were classified into two major groups, *M. sinensis* and *M. sacchariflorus*. *Miscanthus floridulus* was classified into *M. sinensis* group, and *M. × giganteus* and *M. lutarioriparius* was classified into *M. sacchariflorus* group (Figure 1-2). *Miscanthus sinensis* was classified into five major groups (Figure 1-3). Two sub-clusters of group I were named as sub-group Ia and Ib, and group II was sub-divided into two sub-groups named IIa and IIb. The other groups were named III through V. *M. sinensis* Group I was composed of 38 accessions, which had leaf sheath hair and silky callus hair. Sub-group Ia includes 16 *M. sinensis* had flat type of leaf cross section. Sub group Ib was composed of 22 *M. sinensis* had the other types of leaf cross section. All accessions collected from southern part of Korea and Japan classified into sub -group Ib except 2 accessions, other. Group II, which was composed of 41 *M. sinensis* and *M. floridulus* did not have leaf sheath hair and had a silky callus hair. Sub group IIa and IIb clustered by presence of leaf hair. Interestingly, a *M. sinensis* accession, M-276, in sub-group IIb, did not have an awn in floret. And other accessions, M-085, M-105 and M-194, were triploid by chromosome counting (data not shown). Group III, which composed of 47 *M. sinensis*, had rough callus hair. There were no collar hairs in group I, II and III, while group IV was composed of 21 accessions had collars hair. Group V was composed of 37 accessions, which had silk callus hair. Interestingly, group V also clustered with intermediate *Miscanthus* accessions, M-021, 062, 072, 110, 116 and 172. M-021 did not have an awn in floret. M-062, 072 and 172 had an intermediate type of growth habit. M-110 and 116 were diploid level of *M. sacchariflorus* (Personal communication with HR Park).

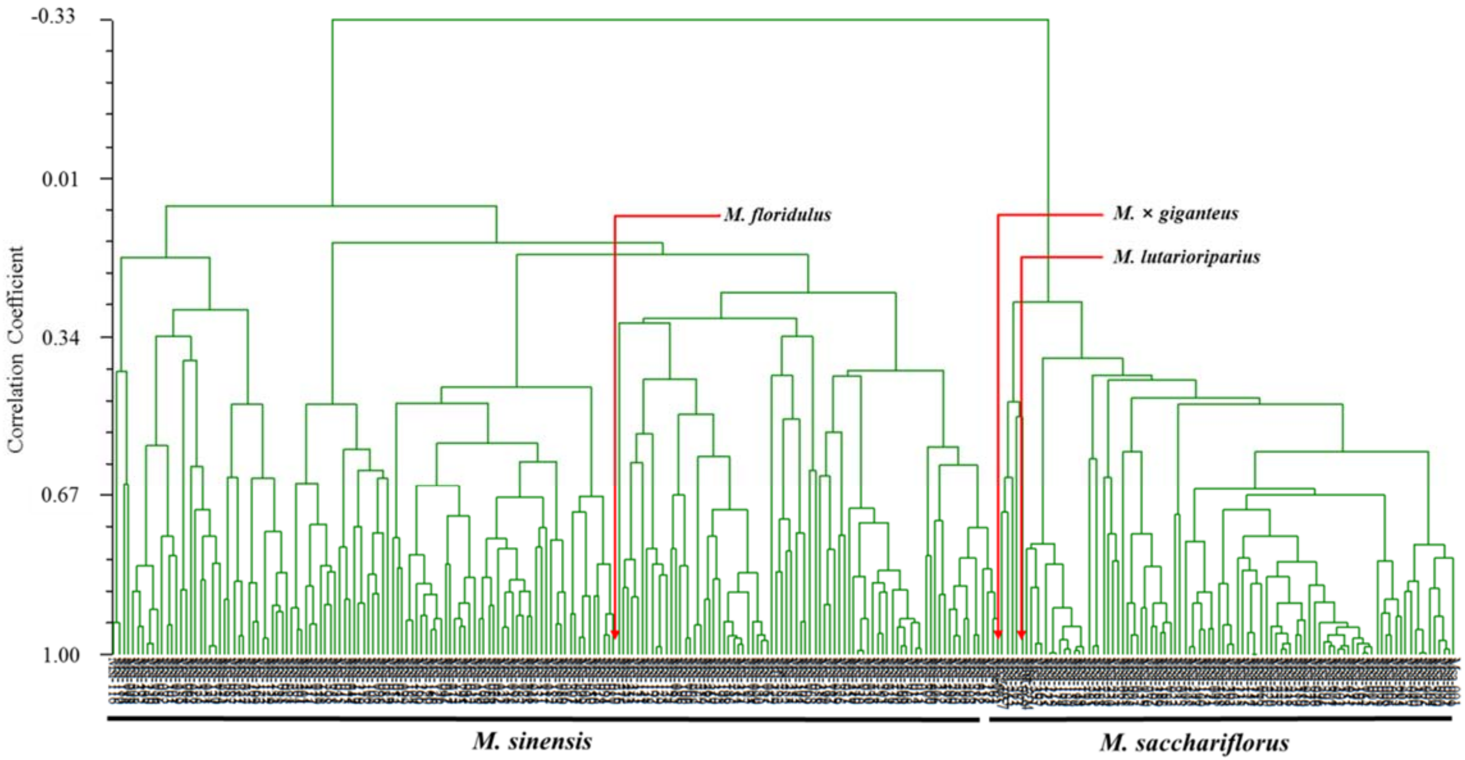


Figure 1-2. Phylogenetic tree for 277 *Miscanthus* accessions with 3 outgroup *Miscanthus* species based on 20 morphological trait analyses.

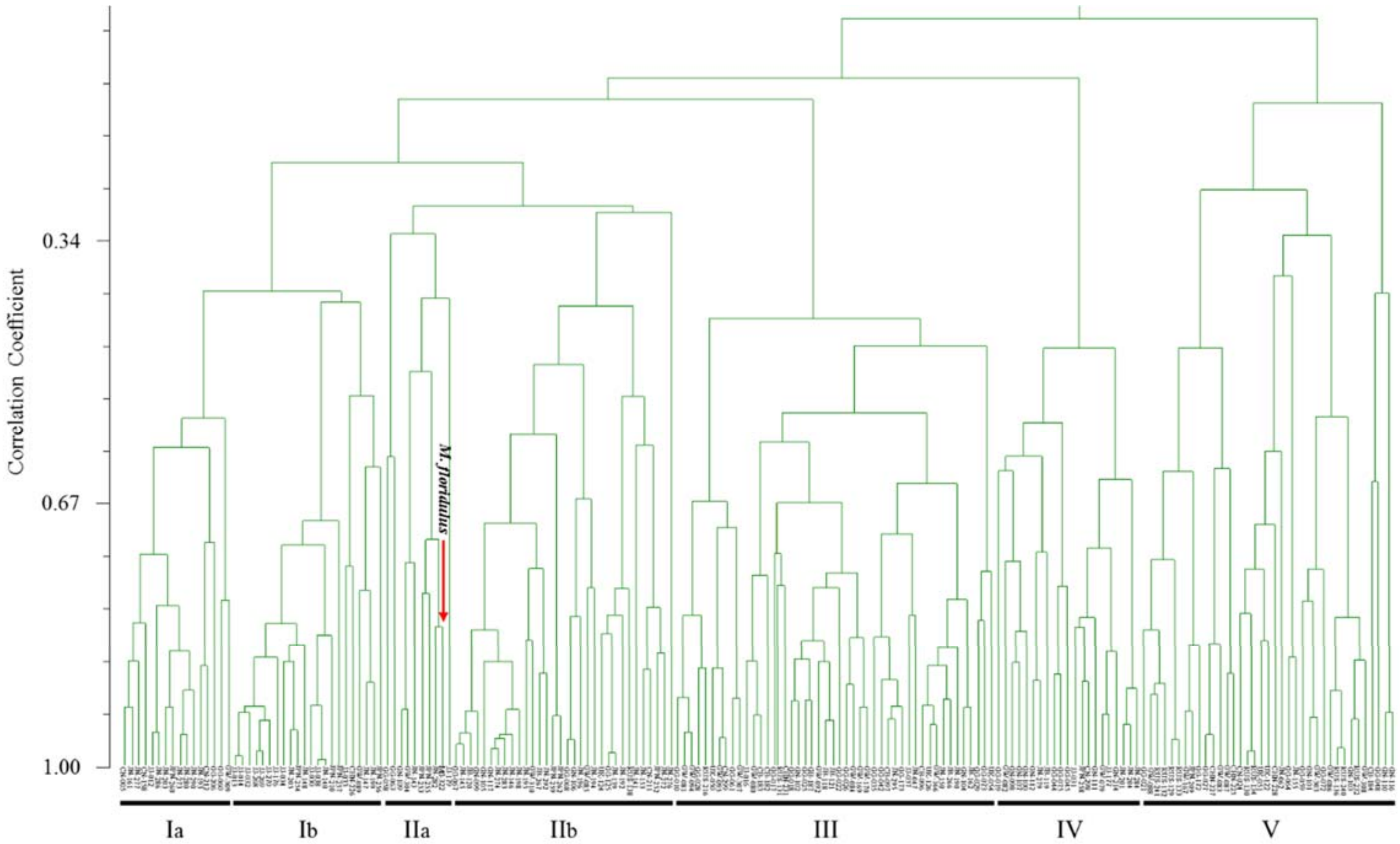


Figure 1-3. Phylogenetic tree for 184 *M. sinensis* with *M. floridulus* based on 20 morphological trait analyses.

M. sacchariflorus accessions were clustered within five groups (Figure 1-4). Two sub cluster of group I were named Ia and Ib. The other groups were named II through V. 57 accessions clustered with group I, and sub group Ia was composed of 11 *M. sacchariflorus*. In sub group Ia, except accession M-001 collected from Gyeonggi province, all accessions were determine tetraploid by flow cytometry. Interestingly, M-001 was triploid, which had an awn in floret and intermediate growth habit. Sub group Ia had collar hair, while sub-group Ib did not have collar hair. In sub group Ib, M-108 and M-152 collected from Gyeongnam province and Jeonnam province, respectively, had an intermediate type of growth habit and are triploid (Personal communication with HR Park). In previous study (reference), we confirmed that M-108 was identical to *M.* × *orgiformis* Honda (Ibaragi *et al.*, 2013). This finding suggests that these two accessions may be the intermediate types of *M. sinensis* and *M. sacchariflorus*, which could be resulted from interspecific outcrossing. Group III, which composed of 18 accessions did have any significant patterns or trends among traits. In group III, some traits showed intermediate type such as growth habit, presence of awn and ratio of callus hair to spikelet. Group IV composed of 12 *M. sacchariflorus* and *M. lutarioriparius*, which had a branch at the node. Group V composed of 3 triploid *Miscanthus* (*M.* × *giganteus* and 2 *M. sacchariflorus*; Personal communication with HR Park) and *M. sacchariflorus*, which had intermediate type of *M. sinensis* and *M. sacchariflorus*.

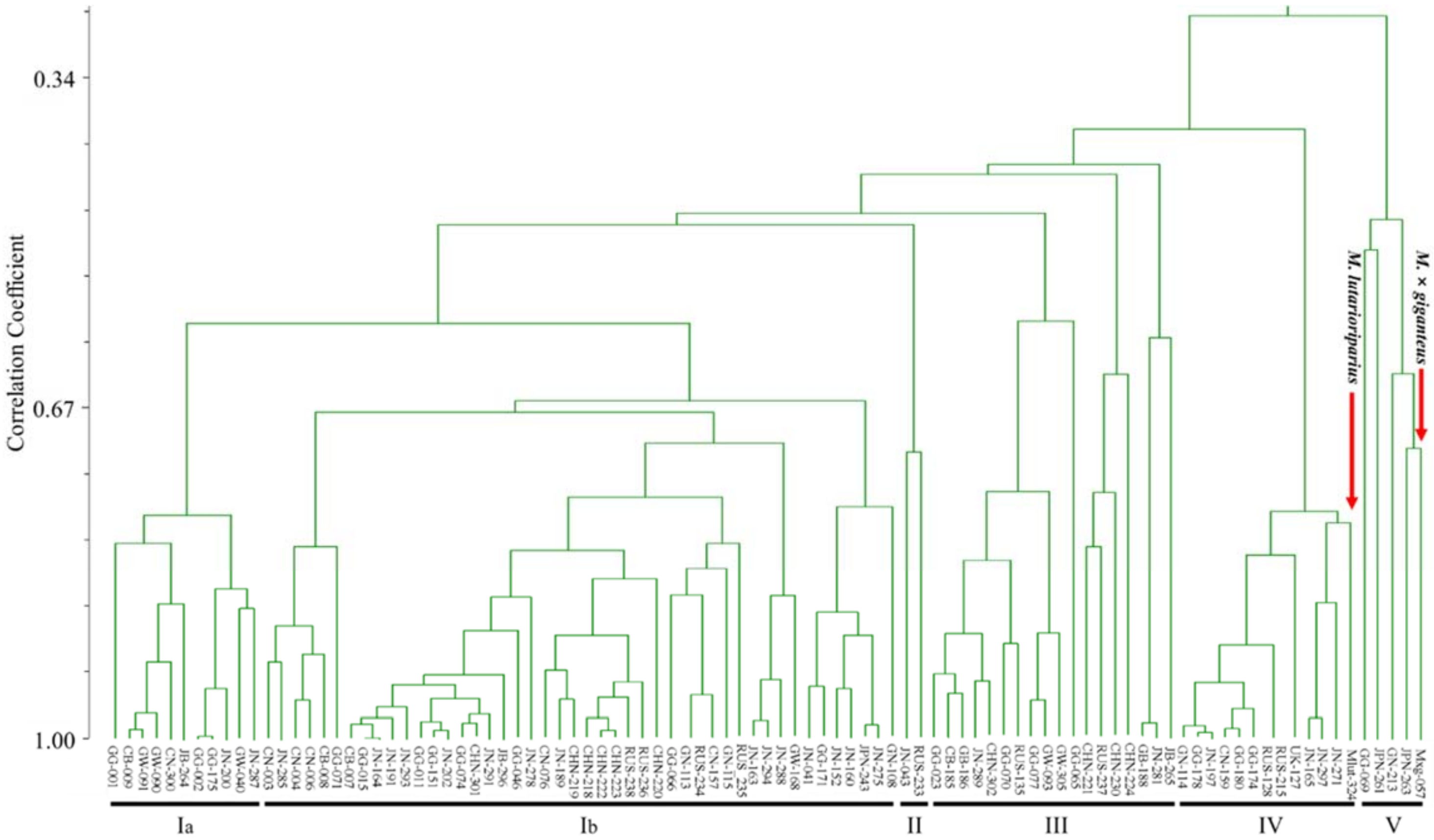


Figure 1-4. Phylogenetic tree for 93 *M. sacchariflorus* with *M.* × *giganteus* and *M. lutarioriparius* based on 20 morphological trait analyses.

Intermediate type of *Miscanthus* accessions

The 2D principal coordinate analysis (PCoA) based on morphological traits was used to assess distance among the 280 *Miscanthus* accessions (Figure 1-5). Interestingly, all of the accessions clustered into 3 groups, as *M. sinensis*, *M. sacchariflorus* and intermediate type of *M. sinensis* and *M. sacchariflorus* (Figure 1-5). *Miscanthus* × *giganteus* and *M.* × *orgiformis* (M-108) grouped within other intermediate types. Table 1-2 showed the summary of morphological traits showing intermediate characteristics. Reference accessions of *M. sinensis* have leaf hair, collar hair and awn. However, M-021 and M-276 did not have awn in floret. The other intermediate accessions did not have leaf hair and collar hair like *M. sacchariflorus*. M-048 and M-276 had a similar ratio of callus hair to spikelet as *M. sacchariflorus*. *Miscanthus sacchariflorus* also showed similar patterns in morphological traits. Intermediate type of *M. sacchariflorus* had diverse intermediate traits than *M. sinensis*. Reference accessions of *M. sacchariflorus* did not have leaf hair, collar hair and awn. However, intermediate *M. sacchariflorus* did not follow the morphological traits of typical *M. sacchariflorus*. M-065, 188, 213, 221, 230, 261, 263, 265 and 281 had leaf hair or collar hair and short ratio of callus hair to spikelet. M-066, 069, 070, 071, 108, 115 and 135 did not have leaf hair and collar hair, but did not have an intermediate type of growth habit.

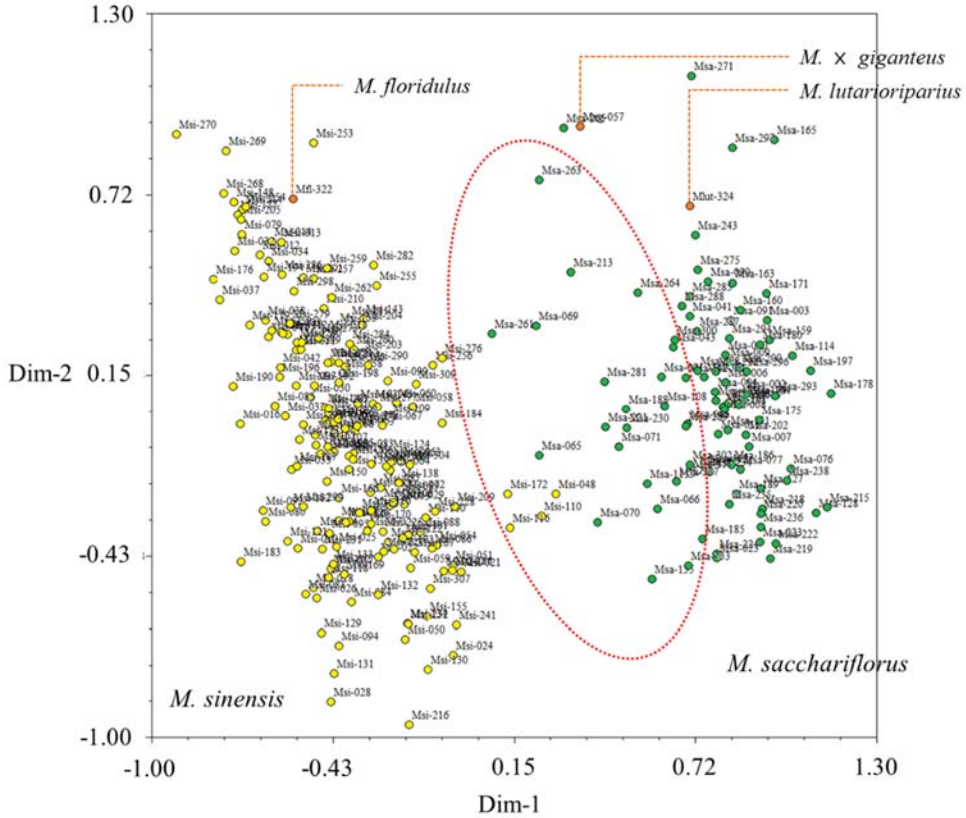


Figure 1-5. Principal coordinate analysis of all *Miscanthus* accessions. Yellow dots in the blue circle indicate *M. sinensis*, green dots in the green circle located *M. sacchariflorus*, and yellow and green dots in the red circle showing intermediate types.

Table 1-2. Summary of main morphological traits among the *M. sinensis*, *M. sacchariflorus*, *M. × giganteus*, and intermediate type.

Traits	*M. sinensis*	*M. sacchariflorus*	*M. × giganteus*	Intermediate type
Growth habit	Bunch 	Scattered 	Intermediate 	Bunch or Scattered or Intermediate 
Leaf/ Collar	Pilose 	Glabrous 	Glabrous 	Pilose or glabrous 
Awn	Awned 	No awn 	No awn 	Awned or No awn 
Rhizome	Sympodial 	Monopodial 	Monopodial 	Sympodial or monopodial or Intermediate 

Table 1-3 summarized main morphological traits of *Miscanthus* spp. Intermediate type of *Miscanthus* had diverse morphological traits, which were very different from those of typical *M. sinensis* and *M. sacchariflorus*. *Miscanthus sinensis* and *M. sacchariflorus* could be distinguished by the presence of autumn new shoot and awn. As summarized in Table 1-1, all the *M. sinensis* accessions produced autumn new shoot, while all the *M. sacchariflorus* did not produce them. Interestingly, some *M. sinensis* have no awn, while some *M. sacchariflorus* have awns. Therefore, we presumed that some accessions might be the result of hybridization between *M. sinensis* and *M. sacchariflorus*. Principal coordinate analysis showed some intermediate types of accessions placed between *M. sinensis* and *M. sacchariflorus* (Figure 1-5). In total, 24 accessions, seven *M. sinensis* and 17 *M. sacchariflorus*, were selected as tentative intermediate types (Table 1-3). In morphological traits, two of the seven *M. sinensis* accessions had no awn and eight of the 17 *M. sacchariflorus* accessions had awns. In stem growth habit, 10 of the 17 *M. sacchariflorus* accessions showed intermediate growth habit unlike a typical *M. sacchariflorus,* which had a scattered growth habit such as M-160 and M-165 (Table 1-3). In the case of callus hair/spikelet ratio, many of selected intermediate accessions showed around 1.4, which was the intermediate value for *M. sinensis* and *M. sacchariflorus*, whose typical ratios were reported to be around one and two, respectively (Liu, 1997; Sun *et al.*, 2010). Flow cytometry revealed six diploid and one triploid *M. sinensis*, and nine diploid, four triploid, and four tetraploid *M. sacchariflorus*. Chromosome counting finally confirmed that one *M. sinensis* and four *M. sacchariflorus* accessions were triploid. Therefore, these five triploids might be hybrids resulted from hybridization between diploid *M. sinensis* and tetraploid *M. sacchariflorus*. Even in the case of diploids, considering their morphological traits, some of them may also be derived from hybridization between diploid *M. sinensis* and diploid *M. sacchariflorus*. This finding suggests that these morphological traits can be indicators to classify an intermediate type of *M. sinensis* and *M. sacchariflorus*, which could have resulted from interspecific outcrossing.

Table 1-3. Summary of morphological traits of *Miscanthus* showing intermediate characteristics. M-045, M-160 and M-168 is reference accessions of *M. sinensis* and *M. sacchariflorus* (4*n* and 2*n*), respectively.

Species	Code	Ploidy level	Leaf			Stem		Spikelet	
			Leaf hair	Collar hair	Leaf sheath hair	Autumn new shoot	Growth habit	Awn	Callus hair/ Spikelet
M. sinensis	M-045 (Reference)	2*n*	○	○	×	○	B	○	1.3
	M-021	2n	○	×	×	○	B	×	1.4
	M-048	2n	×	○	×	○	B	○	1.6
	M-110	2n	×	×	×	○	B	○	1.4
	M-116	2n	×	×	×	○	B	○	1.3
	M-172	2n	×	×	×	○	B	○	1.4
	M-194	3n	○	×	×	○	B	○	1.2
	M-276	2n	○	×	×	○	B	×	1.5
M. sacchariflorus	M-160 (Reference)	4*n*	×	×	×	×	S	×	2.0
	M-168 (Reference)	2*n*	×	×	○	×	S	×	1.6
	M-001	3*n*	×	○	○	×	I	○	1.9
	M-065	2*n*	○	×	×	×	I	○	1.1
	M-066	2*n*	×	×	×	×	S	○	1.1
	M-069	2*n*	×	×	×	×	I	○	1.4
	M-070	2*n*	×	×	×	×	I	○	1.3
	M-071	2*n*	×	×	×	×	I	○	1.4
	M-108	3*n*	×	×	×	×	I	○	2.0
	M-115	2*n*	×	×	×	×	I	×	1.2
	M-135	2*n*	×	×	×	×	I	○	1.4
	M-188	4*n*	○	×	×	×	S	×	1.4
	M-213	3*n*	○	○	○	×	S	×	1.3
	M-221	2*n*	○	○	○	×	S	×	1.3
	M-230	2*n*	○	○	×	×	S	×	1.5
	M-261	3*n*	○	×	×	×	I	×	0.9
	M-263	4*n*	○	×	○	×	S	×	1.6
	M-265	4*n*	○	○	○	×	S	×	1.4
	M-281	4*n*	○	×	×	×	S	×	1.3
M. × giganteus	M-056 (Reference)	3*n*	×	×	○	×	I	×	1.7

Discussion

Key traits to classify *Miscanthus* species

There is no agreement yet on the taxonomical definition of *Miscanthus* species (Clifton-Brown *et al.*, 2008; Sun *et al.*, 2010). Normally, the rhizome, the ratio of the callus hair to spikelet, the presence of an awn, and inflorescences were distinctively different between *M. sinensis* and *M. sacchariflorus* (Renvoize and Gilbert, 1992; Lee, 1964 a, b and c; Lee, 1996). However, among the morphological traits assessed in this study, new shoot emergence in autumn was the most distinct trait, which successfully separate *M. sinensis* and *M. sacchariflorus* accessions, indicating that it can be used to distinguish between two species. All of the *M. sinensis* accessions had autumn new shoots from the soil surface, mainly had an awn in spikelet, and showed a bunch style growth habit, while *M. sacchariflorus* had no awn and no new shoots emerging in autumn and showed a scattered growth habit. These results clearly demonstrated the possibility to distinguish between *M. sinensis* and *M. sacchariflorus* by the selected 20 morphological traits. Similar results were also reported by Chae *et al.* (2014) and Yook *et al.* (2014). They also clearly distinguished *Miscanthus* species using both morphological traits and molecular markers. However, they could not distinguish intermediate type of *M. sinensis* and *M. sacchariflorus*. Sun *et al.* (2010) also classified not only by inflorescences morphological traits but also vegetative morphological traits, which were both qualitative and quantitative traits. However, when only using morphological traits, they failed to clearly distinguish populations based on collection sites in the same species. Interestingly, triploid *Miscanthus* clustered independently with other accessions. Intermediate type of *Miscanthus* also clustered together (Sub group IIb and group V of *M.*

sinensis; Group III and group V of *M. sacchariflorus*). There are clustered intricately by diverse morphological traits in each sub group. Furthermore, morphological traits could not clearly distinguish accessions based on their collection sites. Chae *et al.* (2014) and Yook *et al.* (2014) also found morphological traits that could not clearly distinguish accessions based on their collection sites. These results confirmed that morphological traits have a limitation in detecting variation between accessions. Smith and Smith (1992) reported that these limitations were caused by low polymorphism, low heritability, late expression, and vulnerability to the environmental influences of morphological traits. Thus, clustering only based on morphological traits may not be sufficient to classify by collection sites. Ghafoor *et al.* (2002) also reported the necessity of cluster analyses based on molecular markers to determine the extent of genetic diversity. Clustering using both morphological traits and molecular markers, particularly those interrelated with each other, may provide better insight into the genetic variation and relationship of various *Miscanthus* accessions. Intermediate type of *Miscanthus* also reason why they intricately clustered each accessions. Therefore, we need more study about intermediate type of *Miscanthus* accessions.

Intermediate type of *Miscanthus* accessions

Our results about morphological traits, most of *M. sinensis* and *M. sacchariflorus* accessions were distinguished by the presence of new shoot emergence in autumn. However, some *Miscanthus* accessions clustered independently. In Figure 1-5, some *Miscanthus* accessions were positioned between *M. sinensis* and *M. sacchariflorus*. Morphological traits of these

Miscanthus showed intermediated types (Table 1-2 and 1-3). In previous study, M-108 confirmed to *M.* × *orgiformis* Honda (Ibaragi *et al*., 2013), which had similar rhizome and distribution pattern of hairs on the lower glume, but had an apparently long geniculate awn on the spikelet similar to *M. sinensis*. In the previous study, M-108 confirmed triploid level with M-001, M-085, M-105, M-152, M-194, M-213 and M-261. They grouped independently with typical *Miscanthus* accessions or clustered together with triploid *Miscanthus* and intermediate type of *Miscanthus*. Dwiyanti *et al.* (2013) also conducted morphological traits and genetic analysis of putative triploid *Miscanthus* hybrid collected in Japan. They assessed the presence of awn and leaf sheath hair. Hybrid *Miscanthus* possessed hairs on their sheaths, as did tetraploid *M. sacchariflorus*. All putative hybrids had awns on their florets, as observed in *M. sinensis*. Some of our intermediate type of *M. sacchariflorus* also had a leaf sheath hair and awn in floret. Through chromosome counting, some accessions were confirmed as triploid *Miscanthus*, while the other accessions were confirmed as diploid and tetraploid by flow cytometry. Therefore, we need further study about intermediate type of *Miscanthus* to conduct chromosome counts for confirming ploidy level of intermediate type of *Miscanthus* accessions.

References

Amini F, Saeidi G, Arzani A. 2008. Study of genetic diversity in safflower genotypes using agro-morphological traits and RAPD markers. Euphytica **163**: 21.

Christian DG, Yates NE, Riche AB. 2005. Establishing *Miscanthus sinensis* from seed using conventional sowing methods. Industrial Crops and Products **21**: 109.

Chae WB, Hong SJ, Gifford JM, Rayburn AL, Sacks EJ, Juvik JA. 2014. Plant morphology, genome size, and SSR markers differentiate five taxonomic groups among accessions in the genus *Miscanthus*. Global Change Biology Bioenergy **6(6)**: 646-660.

Chung JH, Kim DS. 2012. *Miscanthus* as a potential bioenergy crop in East Asia. Journal of Crop Science and Biotechnology **15**: 65-77.

Clayton WD, Renvoize SA. 1986. Genera Graminum, grasses of the world. *Kew Bulletin Additional Series XIII*.

Clifton-Brown JC, Chiang YC, Hodkinsn T. 2008. *Miscanthus*: genetic resources and breeding potential to enhance bioenergy production. In: Genetic improvement of bioenergy crops (ed Vermerris W), pp. 273–294. Springer, New York.

Dwiyanti MS, Rudolph A, Swaminathan K, Nishiwaki A, Shimono Y, Kuwabara S, Matuura H, Nadir M, Moose S, Stewart JR, Yamada T. 2013. Genetic Analysis of Putative Triploid *Miscanthus* Hybrids and

Tetraploid *M. sacchariflorus* Collected from Sympatric Populations of Kushima, Japan. Bioenergy Research **6**: 486-493.

Ghafoor A, Ahmad Z, Qureshi A, Bashir M. 2002. Genetic relationship in *Vigna mungo* (L.) Hepper and *V. radiata* (L.) R. Wilczek based on morphological traits and SDS-PAGE. Euphytica **123(3)**: 367-78.

Hodkinson TR, Chase MW, Takahashi C, Leitch IJ, Bennett MD, Renvoize SA. 2002a. The use of DNA sequencing (ITS and trnL-F), AFLP, and fluorescent in situ hybridization to study allopolyploid *Miscanthus* (Poaceae). America Journal of Botany **89**: 279-286.

Hodkinson TR, Chase MW, Renvoize SA. 2002b. Characterization of a genetic resource collection for *Miscanthus* (*Saccharinae Andropogoneae Poaceae*) using AFLP and ISSR PCR. Annals of Botany **89**: 627–636.

Hodkinson TR, Renvoize S. 2001. Nomenclature of *Miscanthus* × *giganteus* (Poaceae). Kew Bulletin **56**: 759-760.

Hodkinson TR, Renvioze SA, Chase MW. 1997. Systematics of *Miscanthus*. In: Biomass and Bioenergy crops (eds Bullard M J *et al.*) Aspects of Biology **49**. Association of Applied Biologist. Warwick, UK. pp 189–197.

Ibaragi Y, Lim SH, Yook MJ, Chang JS, Kim DS. 2013. Taxonomic notes on Korean *Miscanthus* × *orgiformis* Honda (*Poacea*) e a new record from Korea. Journal of Japanese Botany **88(3)**: 184e7.

Jaroslav D, Jan B. 2005. Plant DNA Flow Cytometry and Estimation of Nuclear Genome Size. Annals of Botany **95**: 99–110.

Jørgensen U, Muhs HJ. 2001. *Miscanthus* breeding and improvement. In: Jones MB WM, editor. *Miscanthus* for Energy and Fibre. London, UK: James & James Ltd.; pp. 68-85.

Kearsey MJ, Pooni HS. 1996. The genetical analysis of quantitative traits. London, UK: Chapman and Hall.

Kim S, Rayburn AL, Lee DK. 2010. Genome Size and Chromosome Analyses in Prairie Cordgrass. Crop Science **50**: 2277-2282.

Lafferty J, Lelley T. 1994. Cytogenetic studies of different *Miscanthus* species with potential for agricultural use. Plant Breeding **113**: 246.

Lee YN. 1964a. Taxonomic studies on the genus *Miscanthus*: relationships among the section, subsection and species, part 1. Journal of Japanese Botany **39**: 196–205.

Lee YN. 1964b. Taxonomic studies on the genus *Miscanthus*: relationships among the section, subsection and species, part 2. Journal of Japanese Botany **39**: 257–265.

Lee YN. 1964c. Taxonomic studies on the genus *Miscanthus*: relationships among the section, subsection and species, part 3. Journal of Japanese Botany **39**: 289–298.

Lee YN. 1993. Manual of the Korean grasses. Ewha Wonmans University Press, Seoul, Korea, pp. 49-62.

Lee YN. 1996. Flora of Korea. Seoul, Korea: Kyohaksa; pp. 1032-1034.

Lewandowski I, Scurlock JMO, Lindvall E, Christou M. 2003. The development and current status of perennial rhizomatous grasses as energy crops in the US and Europe. Biomass and Bioenergy **25**: 335-361.

Liu L. 1997. *Miscanthus, Diandranthus, Triarrhena*. In: Chen SL, ed. *Flora reipublicae popularis sinicae*. **10**. Bejing: Science Press 4–26.

Rayburn AL, Auger JA, Benzinger EA, Hepburn AG. 1989. Detection of Intraspecific DNA Content Variation *in Zea mays* L. by Flow Cytometry.

Renvoize SA, Gilbert D. 1992. Taxonomy and cultivars. In Rutherford, MC Heath, eds, The Potential of *Miscanthus* as a Fuel Crop, ETSU Report No. **B 1354**, London: DTI, pp. 21-29.

Renvoize SA, Hodkinson TR, Chase MW. 1997, Miscanthus in Britain: a molecular based review of diversity in the living resources held in the UK and available in Europe. Ministry of Agriculture Fisheries, and Food, Research Development MAFF QA3580.

Rohlf FJ. 2002. NTSYS-pc: numerical taxonomy and multivariate analysis system. 2.21 ed. New York: Applied Biostatistics.

Scally L, Hodkinson TR, Jones MB. 2001. Origins and taxonomy of *Miscanthus*. In: *Miscanthus* for energy and fibre. (eds Jones MB, Walsh M), pp. 1–9. James & James, London, UK.

Smith JSC, Smith OS. 1992. Fingerprinting Crop Varieties. In: Donald LS, editor. Advances in Agronomy: Academic Press, pp. 85-140.

Sun Q, Lin Q, Yi Z, Yang Z, Zhou F. 2010. A taxonomic revision of *Miscanthus* s. l. (*Poaceae*) from China. Botanical Journal of the Linnean Society **164**: 178–220.

Yook MJ, Lim SH, Song JS, Kim JW, Zhang CJ, Lee EJ, Ibaragi Y, Lee GJ, Nah GJ, Kim DS. 2014. Assessment of genetic diversity of Korean *Miscanthus* using morphological traits and SSR markers. Biomass and Bioenergy **66**: 81-92.

CHAPTER II. Relationships between phenological traits and agronomic traits revealed key traits determining *Miscanthus* biomass yield

ABSTRACT

4-years field experiments were conducted to investigate phenotypic traits associated with biomass yield of 281 *Miscanthus sinensis* and *M. sacchariflorus* accessions collected from Korea and its neighboring East Asian countries. Five phenological traits and nineteen agronomic traits associated with biomass yield were assessed to investigate their genetic diversity and relationships with biomass yield and latitudes of collection sites, where *Miscanthus* accessions were collected. Correlation analyses among phenological and agronomic traits, biomass yield and geographic latitude of collection site revealed that heading date, leaf length, leaf area and stem growth traits (stem height, number of stem, stem dry weight, and stem diameter) were closely related with biomass yield. Latitude of collection site exhibited a significant negative correlation with heading date, and heading date showed a significant positive correlation with biomass yield. The presence of significant relationships between latitude and agronomic traits suggests that the accessions collected from different geographical latitudes can provide more genetically diverse materials for breeding. The growth of top three leaves (flag, the second and the third leaf) showed positive correlation with other agronomic traits such as stem height, stem diameter and stem dry weight, and then biomass yield. Agronomic traits assessed in

the second year after planting also showed a strong correlation with biomass yield assessed in the fourth year after planting. In particular, the leaf area (r = 0.435, $p < 0.01$), stem height (r = 0.402, $p < 0.01$), stem diameter (r = 0.423, $p < 0.01$) and stem dry weight (r = 0.554, $p < 0.001$) of the second year were significantly related with the fourth year biomass yield. This finding suggests that the earlier agronomic traits assessed in the second year can be used for screening *Miscanthus* genetic resources and breeding lines with high biomass yield potential. Despite of general relationships between phenological and agronomic traits and biomass yield, poor relationships were often observed between traits and latitude collection sites. These diverse patterns of relationship with phenological and agronomic traits imply that the tested *Miscanthus* accessions possess high genetic diversity in their biomass yield potential.

Key words: *Miscanthus***,** phenological trait, agronomic trait, biomass yield, correlation analysis, genetic diversity

Introduction

Bioenergy production from cellulosic feedstock is a major challenge and requires significant breeding efforts to maximize biomass production (Courtney *et al.*, 2011). The study of genetic diversity in crop species has contributed to the conservation of genetic resources, broadening of the genetic bases, and practical applications in breeding programs (Amini *et al.*, 2008). To design a relevant breeding program, it is important to know how much the phenotypic variation of a trait is heritable and diverse (Kearsey and Pooni, 1996).

Chung and Kim (2012) was summarized that several factors how *Miscanthus* was became an ideal alternative bioenergy source. *Miscanthus* species is exceptional among C_4 species for its biomass productivity in temperate climates (Dohman and Long, 2009). *Miscanthus* × *giganteus* has been commercially cultivated for biomass production due to its high biomass yield and environmental adaptation to cold temperate regions. Many long-term field trials conducted in Europe reported that maximum biomass yield potential of *M.* × *giganteus* was over 20 dry matter (DM) Mg ha^{-1} $year^{-1}$ in central Europe and 30-40 DM Mg ha^{-1} $year^{-1}$ in southern Europe (Danalatos *et al.*, 2007; Schwarz *et al.*, 1994). However, cultivated *Miscanthus* species was limited in *M.* × *giganteus*, and it was from a single clone, it can cause problems in reduce genetic diversity and breeding new *Miscanthus* cultivar. *Miscanthus* × *giganteus* had weakness to water stress, cold tolerance, and high risk of disease and pests (Clifton-Brown *et al.*, 2001; Ings *et al.*, 2013; Prasifka *et al.*, 2009).

Thus, to breed new *Miscanthus* cultivar, based on phenotypic and agronomical traits of parental *Miscanthus* species, such as *M. sinensis* and *M. sacchariflorus* was required to understand their genetic diversity and use materials for breeding new *Miscanthus* cultivar. Furthermore, potential biomass yield was affected by interactions between phenological and agronomical traits of *Miscanthus* accessions. For the high biomass yield, vegetative period should be longer than reproductive growth period. Furthermore, photosynthesis rate, plant growth rate, number of stem and dry weight of stem is important traits to determine their biomass productivity. *Miscanthus* species was took four years after planting to reach stable maximum potential biomass yield. It was limiting factor to reduce breeding period of new *Miscanthus* cultivar. Thus, if *Miscanthus* potential biomass yield can be estimated in earlier stage of planting than the stage when we can estimate potential biomass yield, our efforts for breeding a new *Miscanthus* cultivar can be significantly reduced and the breeding period can also be shorten.

Therefore, this study was conducted to assess phenological and agronomic traits related with biomass yield. For this purpose, we assessed phenological and agronomic traits in the 4-years field trials and conducted correlation analysis between these traits and biomass yield.

Materials & Methods

Field experiment

Miscanthus accessions (281 *Miscanthus* accessions composed of 4 *M.* × *giganteus*, 93 *M. sacchariflorus* and 184 *M. sinensis*) were collected in Korea, Russia, Japan, and China during 2008-2011. We maintained collected *Miscanthus* at the *Miscanthus* germplasm field of Seoul National University, Suwon (N 37° 16’ 12.1”, E 126° 59’ 27.5”), Korea. Nitrogen fertilizer was applied at 60 kg N ha^{-1} $year^{-1}$ in early June and weed management was conducted manually and using herbicide if necessary. All the accessions were planted at a density of 1 plant m^{-2} with three replications and grown for four years.

Phenological traits and agronomic traits of *Miscanthus* accessions

From the first year after planting, we assessed 24 agronomic traits: shoot emergence date, the first leaf/flag leaf emergence date, heading date, flowering date, flag leaf/the second leaf/the third leaf length and width, leaf area, stem height, no. of stem, stem dry weight, leaf blade dry weight, leaf sheath dry weight, total stem dry weight, stem diameter, stem density, and estimated yield were assessed. Shoot emergence date was measured when shoots were emerged from the ground. The first leaf emergence date was assessed when the first leaf was emerged from the shoot. Flag leaf date was measured when flag leaf emerged from the tip of the stem. Heading date was measured when panicle was emerged from the tip of the stem. Flowering date was recorded when stigma was initiated from the floret. Leaf length and width were measured when panicle was fully emerged. The second leaf was selected when the second leaf from the tip of the stem excluded flag leaf. The third leaf was also selected when the third leaf from the tip of the stem excluded flag leaf. Leaf area was calculated as follows (Clifton-Brown *et al.*, 2000),

$$\text{Leaf area } (cm^2) = 0.74 \times leaf\ length \times leaf\ width \qquad (2.1)$$

At maturity in November, stem height, the number of stem and stem diameter were measured prior to harvest. Stem height was assessed from the soil surface to the tip of the panicle. The number of stems was count as the number of productive stems per plant in the space of $0.01m^2$. When the maximum biomass yield assessed we counted a total number of stems per plant. After oven dry of harvested stems at 80 °C for 48 hours, the stem dry weight, leaf blade dry weight, and leaf sheath dry weight were recorded. Stem diameter was determined at the mid-point between the second or the third basal nodes using vernier calipers. Stem density was calculated using stem diameter, stem length, and stem dry weight. We sampled stem at the mid-point between the second or the third basal nodes. After that stem volume calculated using stem diameter and stem length, and then stem dry weight divided by stem volume. Stem density was calculated as follows,

$$\text{Stem density } (g\ cm^{-3}) = \frac{stem\ dry\ weight}{\pi \times \left({stem\ diameter}/{2} \right)^2 \times stem\ length} \qquad (2.2)$$

The maximum biomass yield (the fourth year biomass yield; Mg ha^{-1}) was then estimated based on plant dry weight and the number of plants at a planting space of 1.0 m × 0.75 m, equivalent to 13,300 plants ha^{-1}.

Statistical analysis

Correlation coefficients were calculated among the phenological traits, agronomical traits and the maximum biomass yield of *Miscanthus* accessions. To examine the relationship among the phenological traits, agronomical traits and the maximum biomass yield of *Miscanthus* accessions, linear regression analysis were conducted. All statistical analyses were performed by using JMP 12 software (SAS Institute).

Results

Phenological traits of *Miscanthus* accessions

To understand relationship between the phenological and agronomic traits related biomass yield of *Miscanthus* species through yearly planting, we conducted phenological traits related vegetative stage and reproductive stage of *Miscanthus* such as shoot emergence and heading date, respectively (Table 2-1 and 2-2, Appendix 2-1 and 2-2). Agronomic traits related biomass yield of *Miscanthus* such as leaf area, stem height, number of stems, stem dry weight, stem diameter, stem density, and estimated yield were assessed in each planting year (Table 2-1 and 2-2, Appendix 2-1 and 2-2). It was showed the range of variation among the accessions for phenological traits and agronomic traits associated with biomass yield over four years, continuously. There are very diverse variations observed for all the traits.

In the first year after planting, the earliest shoot emergence initiated on sixty-sixth of Julian date, while the latest shoot emerged on one hundred fifty sixth of Julian date (Appendix 2-1). The range between earliest shoot emergence and latest shoot emergence gradually reduced following the yearly cultivation in each species. The first leaf emergence date also showed similar trend with shoot emergence date. Interestingly, reproductive stage, such as flag leaf initiation, heading date and flowering date showed no difference the range between earliest initiation and latest initiation following the year (data not shown). To produce high biomass yield, length of vegetative growth is important factor. Shoot emergence and heading date were related canopy duration of *Miscanthus*. Canopy duration was determined length of vegetative stage of *Miscanthus*. Thus, *Miscanthus* which had long canopy duration produce high biomass yield. Especially, *Miscanthus* accessions collected from Russia and northern China had an early heading and flowering, while collected from Jeju Island and southern Japan had a late heading and flowering. It means that vegetative stage of *Miscanthus* delayed by late heading and flowering. Delayed vegetative stage can produce more biomass in *Miscanthus* cultivations.

Growth developments of *Miscanthus* accessions

Leaf area of all *Miscanthus* species was gradually increased yearly and each species (Table 2-1 and 2-2, Appendix 2-1 and 2-2). Mean of total leaf area of *M. sinensis*, diploid *M. sacchariflorus* and tetraploid *M. sacchariflorus* were 267.0, 164.0, and 247.7 cm^2 assessed in the fourth year, respectively (Table 2-2). *M.* × *giganteus* had the largest total leaf area among the *Miscanthus* species assessed in the fourth year (mean = 454.3 cm^2) (Table 2-2). Stem height was also increased significantly through the cultivation period (Table 2-1 and 2-2, Appendix 2-1 and 2-2). Mean stem height of tetraploid *M. sacchariflorus* (272.4 cm) was taller than *M. sinensis* (231.7 cm), and diploid *M. sacchariflorus* (216.0 cm) assessed in the fourth year (Table 2-2). *M.* × *giganteus* (380.9 cm) was the tallest species among the *Miscanthus* accessions (Table 2-2). Maximum stem height was no difference between *M. sinensis* and tetraploid *M. sacchariflorus*, while minimum height was showed difference between species. *M. sinensis* showed diverse range of stem height and had a shortest accession. Number of stem in 0.01 m^2 also increased through the cultivation period, however there are no difference between species (Table 2-1 and 2-2, Appendix 2-1 and 2-2). In the fourth year after planting when we assessed total number of stems, there was big difference between the species. *M. sacchariflorus* had a stem almost twice as many as *M. sinensis* (data not shown). Total stem dry weight was consisted of stem dry weight, leaf sheath dry weight, and leaf blade dry weight. Total stem dry weight was also increased during the planting year. Mean dry weight of *M. sinensis* (27.1 g stem^{-1}) was higher than diploid (9.8 g stem^{-1}) and tetraploid (21.5 g stem^{-1}) *M. sacchariflorus* assessed in the fourth year (Table 2-2), especially leaf sheath and leaf blade were twice as heavy as *M. sacchariflorus* (data not shown). Stem dry weight and total stem weight per plant of *M.* × *giganteus* much heavier than other species during the planting year. Mean stem diameter was increased during the planting year, and it also *M.* × *giganteus* thicker species than others (Table 2-1 and 2-2, Appendix 2-1 and 2-2). However, some *M. sinensis* and tetraploid *M. sacchariflorus* had thicker stem diameter than *M.* × *giganteus* assessed in the fourth year (9.4 mm and 10.2 mm, respectively) (Table 2-2). Stem density did not show significantly difference between species and planting year (Table 2-1 and 2-2, Appendix 2-1 and 2-2). Stem density of tetraploid *M. sacchariflorus* showed a little higher than *M. sinensis*. There was no trend during the yearly planting. Interestingly, growth development of tetraploid *M. sacchariflorus* showed good performance than diploid *M. sacchariflorus* in all the traits, while diploid *M. sacchariflorus* was more diverse than tetraploid *M. sacchariflorus* in range of agronomic traits (Figure 2-1).

Biomass yield of *Miscanthus* accessions

In the early stage of planting (the first and the second year), estimated yield of *Miscanthus* was lower than estimated yield of the third and the fourth year, because of its growth development characteristic (Table 2-1 and 2-2, Appendix 2-1 and 2-2). Mean estimated yield of *M. sinensis*, diploid *M. sacchariflorus*, tetraploid *M. sacchariflorus*, and *M.* × *giganteus* in the second year after planting (3.3, 2.4, 3.9, and 4.1 Mg ha^{-1}, respectively) (Table 2-1) increased a little than the first year (1.1, 0.7, 0.7, and 0.8 Mg ha^{-1}, respectively) (Appendix 2-1), did not reach the maximum biomass yield. In the fourth year, estimated biomass yields ranged from 0.3 to 32.9 Mg ha^{-1}, with mean biomass yield of 10.6, 6.6, 11.3 and 18.5 Mg ha^{-1} in *M. sinensis,* diploid and tetraploid *M. sacchariflorus*, and *M.* × *giganteus,* respectively (Table 2-2). Biomass yield in the third and the fourth year were increased dramatically than the second year biomass yield. The fourth year biomass yield also increased than the third year biomass yield, however they did not increased dramatically as the third year biomass yield. Interestingly, maximum biomass yield of *M. sinensis* (32.9 Mg ha^{-1}) and *M. sacchariflorus* (30.6 Mg ha-1) were higher than *M.* × *giganteus* (18.5 Mg ha^{-1}) in the fourth year (Table 2-2). Range of biomass yield also showed similar patterns compared with other traits in each species (Figure 2-1).

Table 2-1. Range of variation assessed in the 2nd year phenological traits and agronomic traits of *Miscanthus* accessions.

Traits	Unit	*M. sinensis* (*n* = 181)				*M. sacchariflorus* (2*n*; *n* = 32)				*M. sacchariflorus* (4*n*; *n* = 57)				*M.* × *giganteus*
		Min	Max	Mean	Median	Min	Max	Mean	Median	Min	Max	Mean	Median	
Shoot Emergence	Julian date	77.0	123.0	92.5	91.0	87.0	99.0	94.8	95.0	90.0	100.0	96.1	96.0	102.0
Heading date	Julian date	179.0	273.0	232.4	236.0	174.0	254.0	209.0	200.0	231.0	268.0	242.7	242.0	251.5
Flag leaf area	cm^2	0.7	56.9	11.7	8.6	1.3	34.6	7.4	3.2	0.8	34.1	9.8	8.4	30.4
2nd leaf area	cm^2	7.8	167.6	62.6	58.7	10.0	113.9	38.2	26.6	24.8	117.5	57.1	54.6	139.7
3rd leaf area	cm^2	9.9	196.0	74.1	71.2	8.7	120.1	42.0	27.8	28.0	146.8	66.9	69.8	161.7
Total leaf area	cm^2	18.4	394.8	148.4	140.0	21.8	260.7	87.6	56.8	56.2	267.6	133.8	135.2	331.8
Stem height	cm	65.0	231.7	163.0	168.3	106.7	238.3	162.6	157.3	76.7	242.7	189.1	198.3	240.4
No. of stem (0.01 m^2)	Number	2.0	20.0	6.3	6.0	4.0	16.0	6.4	4.0	2.0	12.0	6.9	7.0	5.8
Total stem DW	g $stem^{-1}$	0.9	68.3	21.0	18.6	2.4	24.8	7.9	6.3	2.9	61.7	14.5	12.8	45.8
Stem diameter	mm	1.0	7.0	4.2	4.2	2.1	4.8	2.9	2.8	2.5	6.3	3.7	3.6	5.9
Stem density	g cm^{-3}	0.1	0.6	0.4	0.4	0.2	0.6	0.4	0.4	0.3	0.7	0.5	0.5	0.4
Estimated yield	Mg ha^{-1}	0.05	10.3	3.3	3.0	0.4	12.2	2.4	1.5	0.5	7.9	3.9	3.6	4.1

Table 2-2. Range of variation assessed in the 4th year phenological traits and agronomic traits of *Miscanthus* accessions.

Traits	Unit	*M. sinensis* (*n* = 181)				*M. sacchariflorus* (2*n*; *n* = 32)				*M. sacchariflorus* (4*n*; *n* = 57)				*M.* × *giganteus*
		Min	Max	Mean	Median	Min	Max	Mean	Median	Min	Max	Mean	Median	
Shoot Emergence	Julian date	92.0	100.0	96.7	97.0	94.0	98.0	95.8	96.0	94.0	99.0	96.9	97.0	97.3
Heading date	Julian date	170.0	273.0	227.6	233.0	172.0	252.0	202.4	190.5	210.0	266.0	238.4	239.0	235.8
Flag leaf area	cm^2	1.5	98.3	18.5	14.4	2.2	47.1	10.2	6.7	2.6	32.6	14.1	12.9	50.1
2nd leaf area	cm^2	9.6	295.1	120.6	115.9	28.9	142.5	75.2	71.5	71.9	235.8	114.2	107.3	202.6
3rd leaf area	cm^2	11.5	287.4	127.9	122.0	25.6	141.4	78.6	73.0	78.1	232.2	119.4	112.4	201.6
Total leaf area	cm^2	22.6	680.8	267.0	252.3	69.9	293.4	164.0	150.5	157.6	475.2	247.7	239.5	454.3
Stem height	cm	49.0	362.7	231.7	230.3	155.7	279.7	216.0	212.2	162.3	367.7	272.4	272.3	380.9
No. of stem (0.01 m^2)	Number	4.0	36.0	11.7	11.0	7.0	29.0	11.4	7.0	4.0	22.0	12.7	13.0	10.8
Total stem DW	g $stem^{-1}$	0.6	81.5	27.1	23.9	2.5	31.2	9.8	8.1	8.8	64.1	21.5	18.0	76.1
Stem diameter	mm	1.6	9.4	5.8	5.6	3.5	7.4	4.5	4.3	3.9	10.2	5.7	5.6	9.0
Stem density	g cm^{-3}	0.1	0.5	0.3	0.3	0.2	0.5	0.3	0.3	0.3	0.6	0.4	0.4	0.4
Estimated yield	Mg ha^{-1}	0.3	32.9	10.6	10.2	1.0	22.7	6.6	4.0	2.5	30.6	11.3	10.8	18.5

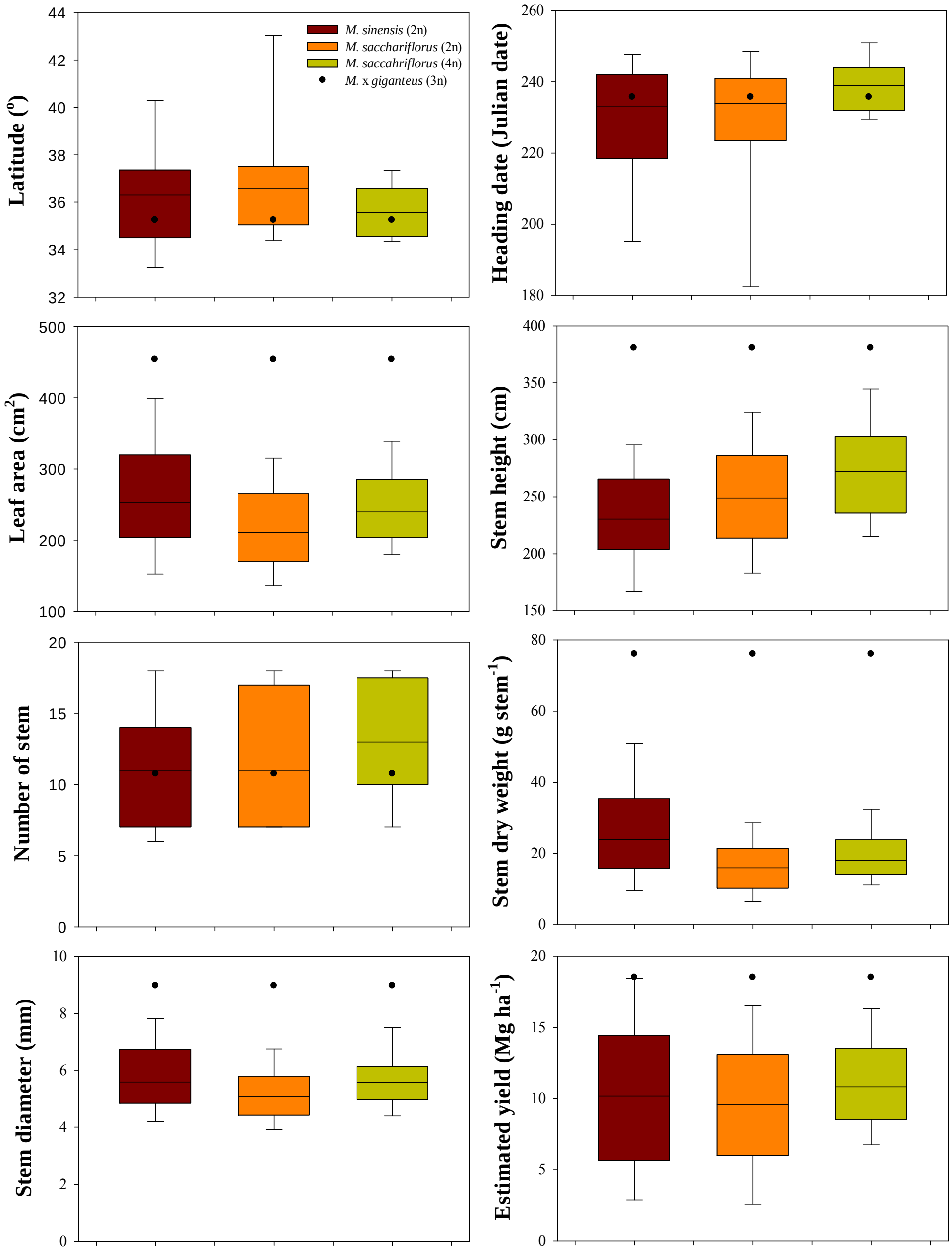


Figure 2-1. Box plot indicating median (line), interquartile range (boxes), and 5% to 95% percentile (whiskers) for each phenology and agronomic traits assessed in the 4th year *Miscanthus* after planting.

Relationship among the phenological traits, agronomic traits and biomass yield

To examine relationship among the accessions for phenological trait and agronomic traits are associated with biomass yield and latitudes of collecting sites, we conducted correlation analysis of phenological traits, agronomic traits, and biomass yield of *Miscanthus* species through the cultivation period and select the traits which showed high coefficient of correlations among the traits with the fourth year biomass yield.

Through the cultivation period, coefficient of correlation of traits which correlated with the fourth year biomass yield were gradually increased among the traits (Table 2-3 and Appendix 2-3, 2-4 and 2-5). Heading date, the second leaf length, the third leaf length, total leaf area, stem height, (total) stem dry weight, and stem diameter were highly correlated with latitude of collection sites and the fourth year biomass yield. Heading date and the 3rd leaf area were highly correlated with the fourth year biomass yield in early stage of planting (Appendix 2-3 and Table 2-3). Stem dry weight had the highest coefficient of correlated with the fourth year biomass yield in each species and pooled in all the year (Table 2-3 and Appendix 2-3, 2-4 and 2-5).

In the fourth year after planting, heading date (r = -0.714, $p < 0.01$), the second leaf length (r = -0.568, $p < 0.01$), the third leaf length (r = -0.552, $p < 0.01$), leaf area (r = -0.531, $p < 0.01$), stem height (r = -0.429, $p < 0.01$), stem dry weight (r = -0.551, $p < 0.01$), stem diameter (r = -0.582, $p < 0.01$) and estimated yield (r = -0.482, $p < 0.01$) were in significant negative correlation with latitude of collecting site, while correlation with other traits excepting latitude were showed positive correlations each other (Appendix 2-5).

Table 2-3. Summary of correlation analyses among the 2nd year agronomic traits, the 4th year biomass yield and latitude of collection site of *M. sinensis and M. sacchariflorus* accessions.

Traits	Latitude	Heading date	2nd leaf length	3rd leaf length	Total leaf area	Plant height	Stem dry weight	Stem diameter	Estimated yield
M. sinensis (*n* = 181)									
Heading date	-0.643**	1							
2nd leaf length	-0.471**	0.437**	1						
3rd leaf length	-0.415**	0.407**	0.926**	1					
Total leaf area	-0.320**	0.426**	0.991**	0.966**	1				
Stem height	-0.109	0.140*	0.505**	0.558**	0.534**	1			
Stem dry weight	-0.258**	0.598**	0.592**	0.576**	0.588**	0.271**	1		
Stem diameter	-0.604**	0.600**	0.443**	0.403**	0.426**	0.128*	0.717**	1	
4th year biomass yield	-0.190**	0.345**	0.440**	0.405**	0.437**	0.361**	0.527**	0.381**	1
M. sacchariflorus (*n* = 89)									
Heading date	-0.801**	1							
2nd leaf length	-0.554**	0.557**	1						
3rd leaf length	-0.541**	0.530**	0.966**	1					
Total leaf area	-0.509**	0.493**	0.878**	0.886**	1				
Stem height	-0.476**	0.464**	0.676**	0.690**	0.667**	1			
Stem dry weight	-0.503**	0.504**	0.397**	0.363**	0.398**	0.459**	1		
Stem diameter	-0.597**	0.614**	0.369**	0.304**	0.276**	0.382**	0.756**	1	
4th year biomass yield	-0.552**	0.514**	0.503**	0.471**	0.435**	0.463**	0.663**	0.448**	1
Pooled (*n* = 270, excluding *M.* × *giganteus*)									
Heading date	-0.702**	1							
2nd leaf length	-0.507**	0.492**	1						
3rd leaf length	-0.475**	0.470**	0.950**	1					
Total leaf area	-0.393**	0.427**	0.882**	0.869**	1				
Stem height	-0.211**	0.234**	0.428**	0.465**	0.497**	1			
Stem dry weight	-0.326**	0.359**	0.391**	0.386**	0.386**	0.283**	1		
Stem diameter	-0.599**	0.565**	0.530**	0.475**	0.436**	0.102*	0.750**	1	
4th year biomass yield	-0.244**	0.466**	0.462**	0.453**	0.435**	0.402**	0.554**	0.423**	1

* and ** indicate statistical significance at the 0.05 and 0.01 levels, respectively

Heading date, leaf area, stem diameter, and stem dry weight were main agronomic traits related with the biomass yield. Heading date was highly related with latitude (r^2 = 0.533) and stem diameter (r^2 = 0.285) in the fourth year (Figure 2-2). Stem diameter also highly related with total leaf area (r^2 = 0.590) in the fourth year (Figure 2-2). Stem dry weight which was strongly correlated with the fourth year biomass yield also showed high regression relationship with heading date (r^2 = 0.274), the 3rd leaf area (r^2 = 0.578), and stem diameter (r^2 = 0.830) (Figure 2-2). Especially, leaf area, stem diameter, and stem dry weight was strongly correlated each other. These relationships were naturally linked with heading date and latitude of collection sites.

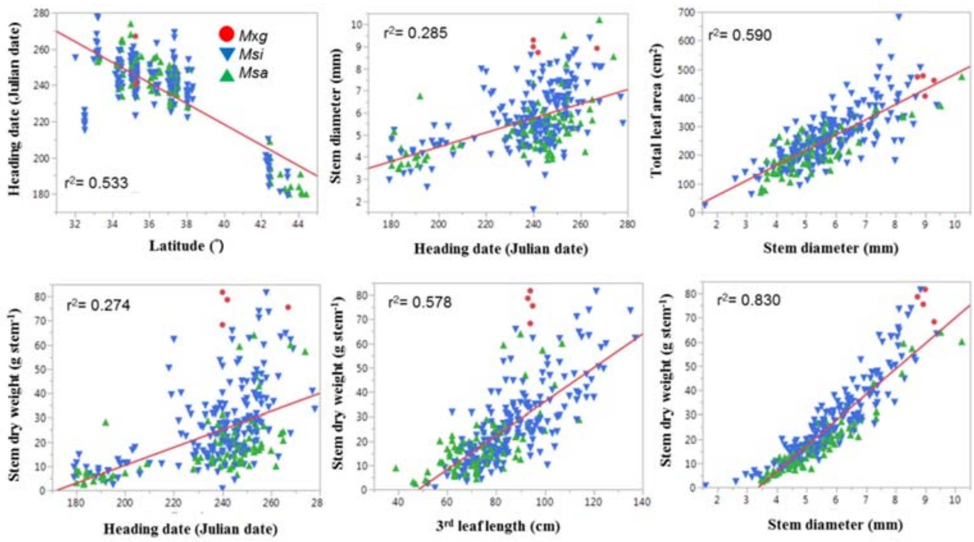


Figure 2-2. Relationship between main agronomic traits assessed in the 4th year after planting of pooled *Miscanthus*.

To examine specific relationship between the traits in the each *Miscanthus* species, we sorted each *Miscanthus* species by the collection sites. M. sinensis composed of six part of collection sites, as collected from Japan, Jeju island, Southern and central part of Korea, China, and Russia (Figure 2-3). Heading date and latitude of collection sites showed negative correlation (r^2 = 0.575). Some *M. sinensis* accessions from Japan and central part of Korea showed early heading date than collected with similar latitude. Interestingly, they collected high altitude than other accessions. Heading date of collected from central part of Korea (N 37° ~ 38°) showed diverse ranges than other part of *M. sinensis* (Table 2-3). Heading date and stem diameter showed positive correlation (r^2 = 0.327) (Table 2-3). Total leaf area and stem diameter also showed positive correlation (r^2 = 0.536). Stem dry weight with heading date, the third leaf area, and stem diameter were also showed positive correlations (r^2 = 0.327, 0.141 and 0.890, respectively) (Table 2-3). However, stem dry weight and the third leaf area were showed low correlation than other traits. Interestingly, *M. sinensis* collected from Japan distributed widely excepting relationship between heading date and latitude, while *M. sinensis* collected same regions excepting from Japan distributed similar space (Table 2-3). *M. sinensis* collected from low latitude was good performances to produce high biomass yield than *M. sinensis* collected from high latitude.

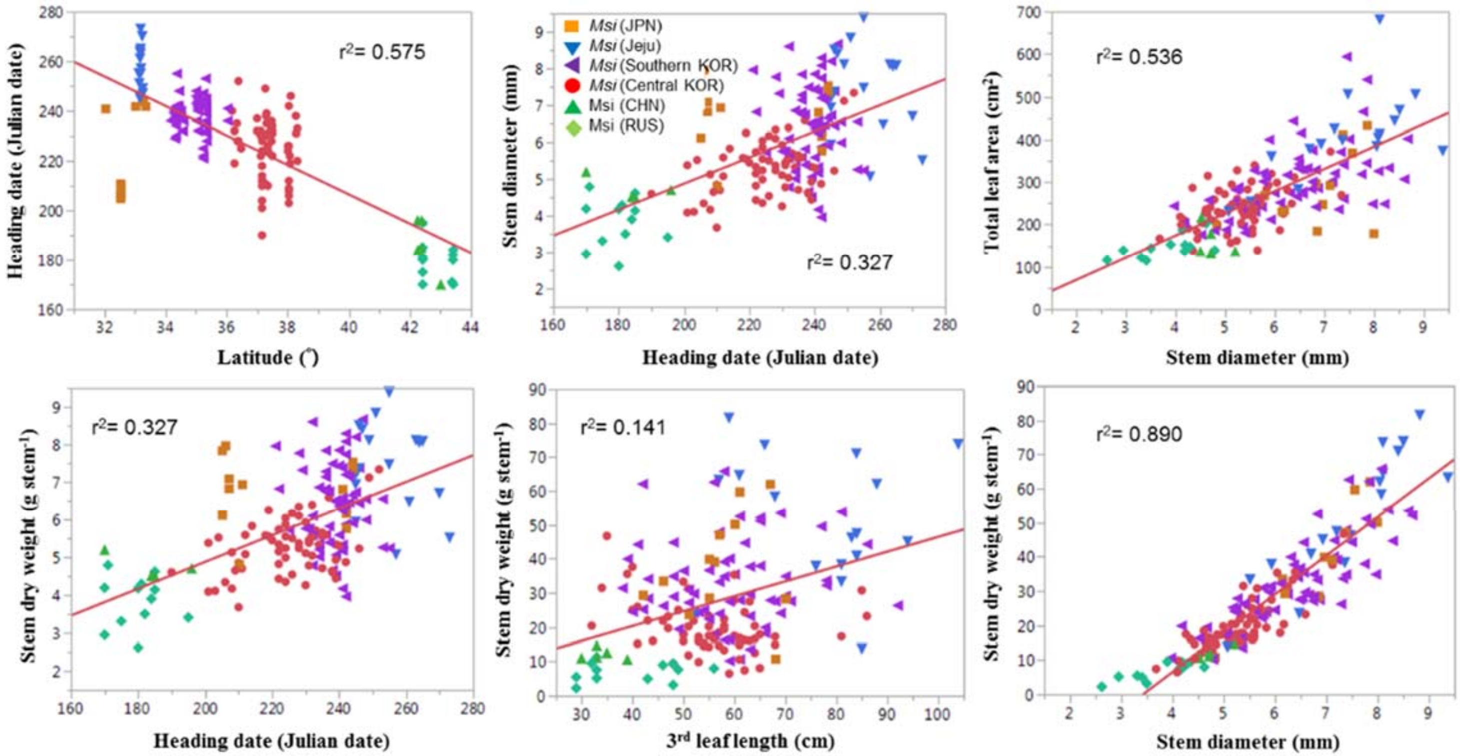


Figure 2-3. Relationship between main agronomic traits assessed in the 4th year after planting of *M. sinensis*.

M. sacchariflorus was sorted diploid, triploid, and tetraploid by ploidy level of chromosomes. Interestingly, diploid *M. sacchariflorus* was distributed from central part of Korea to Russia (N 35° 02' ~ N 44° 41'), while tetraploid *M. sacchariflorus* distributed from Japan to central part of Korea (N 33° 22' ~ N 37° 50') (Figure 2-1). *M. sacchariflorus* also showed negative correlation (r^2 = 0.810) between heading date and latitude (Figure 2-4). Relationship of total dry weight with stem diameter and stem dry weight with stem diameter were also positive correlations (r^2 = 0.651 and 0.890, respectively) (Figure 2-4) similar with *M. sinensis*. The other traits were showed different correlation by ploidy levels. Diploid *M. sacchariflorus* was showed higher correlation between the traits than tetraploid *M. sacchariflorus*. Supplementary, tetraploid *M. sacchariflorus*, coefficient of correlation among the agronomic traits was showed no significant relationship with the fourth year biomass yield (data not shown). It supposed to tetraploid *M. sacchariflorus* were collected from narrow regions, while diploid *M. sacchariflorus* were collected from wide regions that is because diploid *M. sacchariflorus* showed high correlations between the traits.

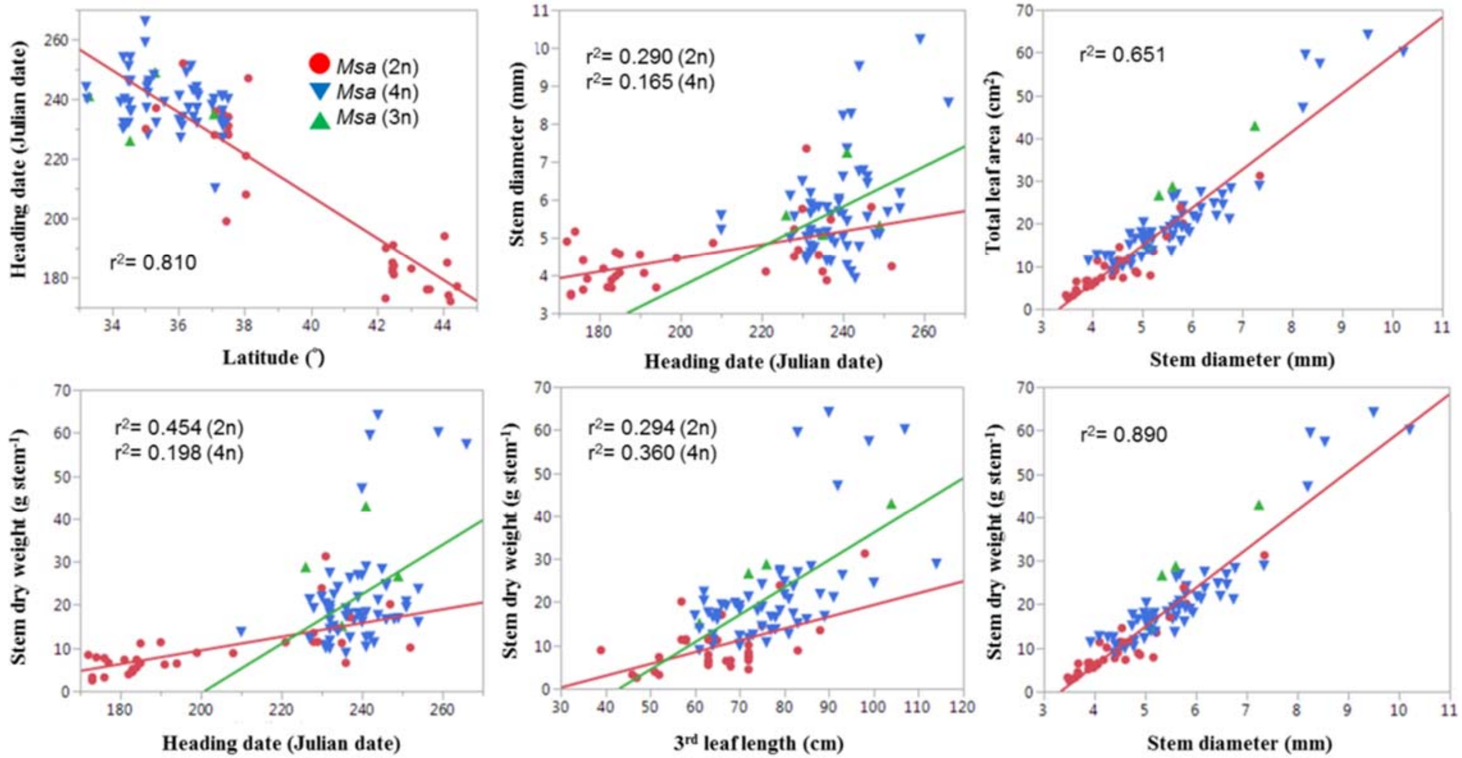


Figure 2-4. Relationship between main agronomic traits assessed in the 4th year after planting of *M. sacchariflorus*.

To examine the key traits that determining the fourth year biomass yield in early stage of *Miscanthus* planting, we summarized coefficient of correlation between the fourth year biomass yield and main agronomic traits related with biomass yield yearly planting. Heading date, the second leaf length, the third leaf length, leaf area, stem height, stem dry weight, stem number, stem diameter and estimated yield were significantly correlated with the fourth year biomass yield (Figure 2-5). In the first year, number of stem was not significant correlation with the fourth year biomass yield in both *M. sinensis* (r = 0.109, p<0.05) and *M. sacchariflorus* (r = 0.009, NS) (Figure 2-5). Correlation in the second and the third year also showed similar patterns with the first year correlation. Excepting number of stem and estimated yield, other traits were consistently correlated with the fourth year biomass yield in all planting years and species. Focusing the second year agronomic traits with the fourth year biomass yield of pooled *Miscanthus*, heading date (r = 0.466, p < 0.01), the second leaf length (r = 0.462, p < 0.01), the third leaf length (r = 0.453, p < 0.01), total leaf length (r = 0.435, p < 0.01), stem height (r = 0.402, p < 0.01), stem diameter (r = 0.423, p < 0.01) and stem dry weight (r = 0.554, p < 0.001) showed high coefficient of correlations (Figure 2-5). It suggests that these traits can predict high biomass yield of the fourth year in early stage of *Miscanthus* planting.

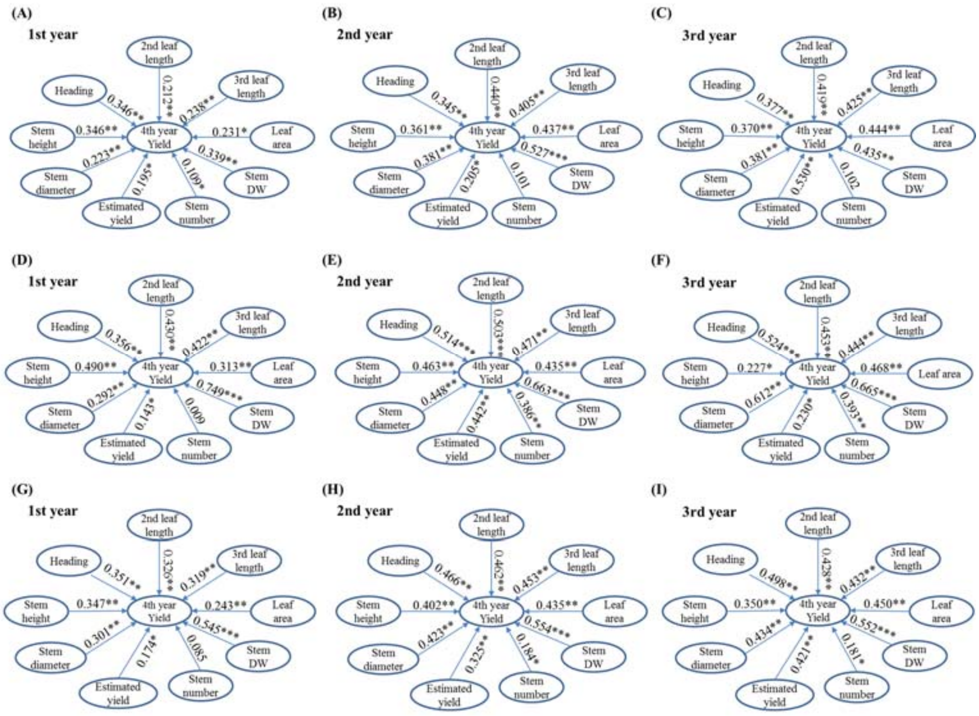


Figure 2-5. Correlation between main agronomic traits assessed in three years after planting and the 4th year biomass yield. *M. sinensis* (A, B and C), *M. sacchariflorus* (D, E and F), and Pooled (G, H and I). *, ** and *** indicate statistical significance at the 0.05, 0.01 and 0.001 levels, respectively.

Through the relationship among the phenological trait, agronomic traits and the fourth year biomass yield, there are significantly correlated between some traits (Figure 2-6). Latitude of collecting sites determined heading date of *Miscanthus* (Table 2-3 and Appendix 2-3, 2-4 and 2-5), and heading date was affected to duration of vegetative stage and positively correlated with agronomic trait such as leaf length, leaf area, stem height, stem dry weight, stem diameter, and biomass yield. Additionally, these main agronomic traits were significantly correlated with the fourth year biomass yield. To investigate association among the phenological trait, main agronomic traits and the fourth year biomass yield, we summarized correlations between the each trait assessed in the second year after planting (Figure 2-6). Correlation between heading date and stem height of *M. sinensis* showed low coefficient (r = 0.113, NS) (Figure 2-6A) than other traits. *M. sinensis* accessions collected from high and low latitude were the shortest stem height in early stage of planting, while accessions collected from mid latitude were taller than other accessions. Association between the stem dry weight and the fourth year biomass yield was the highest correlation in *M. sinensis* and *M. sacchariflorus* (r = 0.527 and 0.663, p<0.001, respectively) (Figure 2-6A and B). Pooled *Miscanthus* also showed high correlation between the stem dry weight and the fourth year biomass yield ($r = 0.554$, $p < 0.001$) (Figure 2-6C). High correlation with the fourth year biomass was the second leaf area (r = 0.462, $p < 0.01$), the third leaf area (r = 0.453, $p < 0.01$), total leaf area (r = 0.435, $p < 0.01$), stem diameter (r = 0.423, $p < 0.01$), stem height (r = 0.402, $p < 0.01$), and flag leaf area (r = 0.381, $p < 0.01$) followed in the pooled *Miscanthus* (Figure 2-6C). It proposes that these main agronomic traits with heading date were key traits to determine biomass yield in *Miscanthus* planting and to develop the new cultivar in the *Miscanthus* breeding.

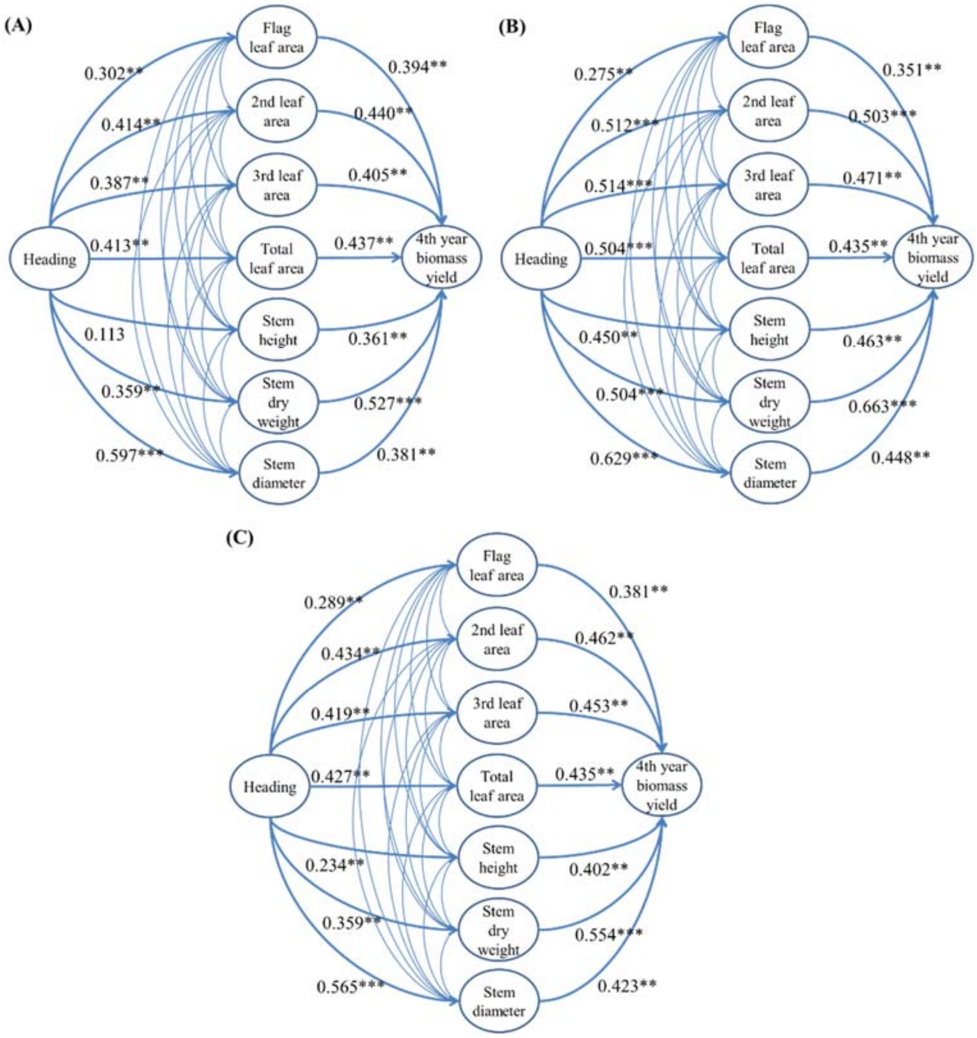


Figure 2-6. Associations among the phenological trait (heading date), main agronomic traits assessed in the 2nd year *Miscanthus* after planting, and the 4th year biomass yield. *M. sinensis* (A), *M. sacchariflorus* (B), and Pooled (C). ** and *** indicates statistical significance at the 0.01 and 0.001 level, respectively.

Discussion

Key traits determining *Miscanthus* biomass yield in early stage of *Miscanthus* planting

Producing high biomass yield is the main goal for *Miscanthus* cultivation. Many researches have focuses on how to produce more biomass and those traits which are associated with biomass yield productivity (Clifton-Brown and Lewandowski, 2002; Jezowski *et al*., 2011; Price *et al*., 2004; Robson *et al*., 2013a and b). As *Miscanthus* requires at least three years after planting for commercially viable biomass production, phenotyping of agronomic traits associated with biomass yield requires more than three years as well. Thus, this limits breeding a new Miscanthus cultivar with high biomass yield. Earlier estimation of biomass yield potential of a new candidate is important to shorten the periods for breeding and will facilitate *Miscanthus* breeding.

In our results, main agronomic traits which were highly correlated with biomass yield and observed in the early stage of *Miscanthus* include heading date, leaf length, leaf area, stem height, stem dry weight, and stem diameter. These traits were significant contributors in determining biomass yield in both *M. sinensis* and *M. sacchariflorus* (Figure 2-5), with particular high correlations of heading date and stem dry weight observed in the earlier stages with the fourth year biomass yield. It suggests that these two traits are key traits in determining biomass yield and can be assessed in earlier stages of *Miscanthus* than the stage for assessing biomass yield potential. These two traits can be selected as a target trait to improve biomass yield when screening *Miscanthus* accessions and breeding a new *Miscanthus* cultivar. Anzoua *et al.* (2012) investigated the relationship between morphological traits and biomass yield potential and revealed that biomass production was highly correlated with stem height and leaf length. Jezowski *et al.* (2011), Yan *et al.* (2011), and Zub *et al.* (2011) reported that stem diameter, stem height and heading date showed significant correlation with biomass yield. Clifton-Brown and Lewandowski (2002) suggested that at least 2 years field trials were required to predict biomass yield of *Miscanthus* and the first year data was insufficient to predict biomass yield of *Miscanthus*. Considering our results and Clifton-Brown and Lewandowski (2002) suggestion, it can be concluded that selected agronomic traits such as heading date, stem dry weight, and so on observed in the second year after planting may provide us with potential biomass yield of *Miscanthus* and can screen candidates with our target traits. Those key traits observed in the second year after planting can be used for screening potential candidates in *Miscanthus* breeding program.

Relationship among the phenological traits, agronomic traits and biomass yield

Robson *et al.* (2012, 2013a and 2013b) commented that biomass yield of *Miscanthus* was affected by complex association among the environmental factor, morphological traits and phenological traits. Significant association between the heading date and latitude of collecting site (Figure 2-2) suggests photoperiodic and thermal influences on heading and flowering date as reported similarly by Jensen *et al.* (2011) and Zhao *et al.* (2013). The wide range of heading date among *M. sinensis* accessions collected from the similar latitude of N 37° indicates genetic diversity in their reproductive responses to the same environment (Figure 2-2) (Lim *et al.*, 2014). Heading date also determined canopy duration, which was also highly correlated with biomass yield. The period between shoot emergence and heading determines the length of vegetative stage of *Miscanthus*, i.e., canopy duration. In our study, shoot emergence date was not diverse among the *Miscanthus* accessions, while heading date was very diverse mainly depending on latitude of collection sites, suggesting that canopy duration is mainly determined by heading date. Clifton-Brown *et al.* (2001) also found a similar result showing significant association between biomass yields and flowering timing. The presence of the significant relationship between latitude and agronomic traits suggests that accessions collected from different geographical latitude will provide more genetically diverse materials in agronomic traits for our breeding efforts, such as controlling *Miscanthus* flowering timing for artificial cross and breeding *Miscanthus* varieties adapting to various latitudinal locations.

In summary, our results showed that *Miscanthus* accessions collected in East Asia, mainly from Korea, had a wide range of variation in each agronomic trait which exhibited close relationship with biomass yield and geographical latitude of collecting sites. Our results will thus provide an opportunity to select proper parents for future breeding of *Miscanthus* species, aiming at high biomass yield with high regional adaptability.

References

Amini F, Saeidi G, Arzani A. 2008. Study of genetic diversity in safflower genotypes using agro-morphological traits and RAPD markers. Euphytica **163**: 21-30.

Anzoua KG, Suzuki K, Fujita S, Toma Y, Yamada T. 2015. Evaluation of morphological traits, winter survival and biomass potential in wild Japanese *Miscanthus sinensis* Anderss. populations in northern Japan. Grassland Science **61**: 83-91.

Chung, J.H. and Kim D.S. 2012. *Miscanthus* as a potential bioenergy crop in East Asia. Journal of Crop Science and Biotechnology **15**: 65-77.

Clifton-Brown JC, Lewandowski I, Andersson B, Basch G, Christian DG, Kjeldsen JB, Jørgensen U, Mortensen JV, Riche AB, Schwarz KU, Tayebi K, Teixeira F. 2001. Performance of 15 *Miscanthus* genotypes at five sites in Europe. Agronomy Journal **93**: 1013-1019.

Clifton-Brown JC, Lewandowski I. 2002. Screening *Miscanthus* genotypes in field trials to optimise biomass yield and quality in southern Germany. European Journal of Agronomy **16**: 97-110.

Clifton-Brown JC, Neilson B, Lewandowski I, Jones MB. 2000. The modelled productivity of *Miscanthus* × *giganteus* (GREEF et DEU) in Ireland. Industrial Crops and Products **12**: 97-109.

Courtney EJ, John KM, Ramil M, Janice S, Kenneth LM, Daniel RB, Hei L, and Jan EL. 2011. Genetic Variation in Biomass Traits among 20 Diverse Rice Varieties. Plant Physiology **155**: 157-168.

Danalatos NG, Archontoulis SV, and Mitsios I. 2007. Potential growth and biomass productivity of *Miscanthus* × *giganteus* as affected by plant density and N-fertilization in central Greece. Biomass and Bioenergy **31**: 145-152.

Dohleman, FG and Long SP 2009. More productive than maize in the Midwest: How does *Miscanthus* do it? Plant Physiology **150**: 2104-2115.

Hotz A, Kuhn W, Jodl S. 1996. Screening of different *Miscanthus* cultivars in respect of yield production and usability as a raw material for energy and industry. Biomass for Energy and the Environment: Proceedings of the Ninth European Bioenergy Conference, Copenhagen, Denmark, pp. 24-27.

Ings J, Mur LA, Robson PR, Bosch M. 2013. Physiological and growth responses to water deficit in the bioenergy crop *Miscanthus* × *giganteus*. Frontiers in Plant Science **4**: 1-12.

Jensen E, Farrar K, Thomas-Jones S, Hastings A, Donnison I, Clifton-Brown JC. 2011. Characterization of flowering time diversity in *Miscanthus* species. Global Change Biology Bioenergy **3**: 387-400.

Jezowski S, Glowacka K, Kaczmarek Z. 2011. Variation on biomass yield and morphological traits of energy grasses from the genus *Miscanthus* during the first years of crop establishment. Biomass and Bioenergy **35**: 814-821.

Kearsey MJ, Pooni HS. 1996. The genetical analysis of quantitative traits. London, UK: Chapman and Hall.

Lim SH, Yook MJ, Kim JW, Song JS, Zhang CJ, Nah GJ, Kim DS. 2014. Genetic diversity in agronomic traits associated with the biomass production of *Miscanthus* species collected in Northeast Asia. Plant Genetic Resources **12**: S137-S140.

McKendry P. 2002. Energy production from biomass (part 1): overview of biomass. Bioresource Technology **83**: 37-46.

Prasifka JR, Bradshaw JD, Meagher RL, Nagoshi RN, Steffey KL, Gray ME. 2009. Development and feeding of fall armyworm on *Miscanthus* × *giganteus* and switchgrass. Journal of Economic Entomology **102**: 2154–2159.

Price L, Bullard M, Lyons H, Anthony S, Nixon P. 2004. Identifying the yield potential of *Miscanthus* × *giganteus*: an assessment of the spatial and temporal variability of *M.* × *giganteus* biomass productivity across England and Wales. Biomass and Bioenergy **26**: 3-13.

Robson P, Farrar K, Gay AP, Jensen E, Clifton-Brown J, Donnison I. 2013a. Variation in canopy duration in the perennial biofuel crop *Miscanthus* reveals complex associations with yield. Journal of Experimental Botany **64**: 2373-2383.

Robson P, Mos M, Clifton-Brown J, Donnison I. 2012. Phenotypic Variation in Senescence in *Miscanthus*: Towards Optimising Biomass Quality and Quantity. Bioenergy Research **5**: 95-105.

Robson P, Jensen E, Hawkins S, White SR, Kenobi K, Clifton-Brown J, Donnison I, Farrar K. 2013b. Accelerating the domestication of a bioenergy crop: identifying and modelling morphological targets for sustainable yield increase in *Miscanthus*. Journal of Experimental Botany **64**: 4143-4155

Schwarz H, Liebhard P, Ehrendorfer K, and Ruckenbauer P. 1994. The effect of fertilization on yield and quality of *Miscanthus sinensis* '*Giganteus*'. Industrial Crops and Products **2**: 153-159.

Yan J, Chen WL, Luo F, Ma HZ, Meng AP, Li XW, Zhu M, Li SS, Zhou HF, Zhu WX, Han B, Ge S, Li JQ, Sang T. 2012. Variability and adaptability of *Miscanthus* species evaluated for energy crop domestication. Global Change Biology Bioenergy **4**: 49-60.

Zhao H, Wang B, He JR, Yang JP, Pan L, Sun DF, Peng JH. 2013. Genetic diversity and population structure of *Miscanthus sinensis* germplasm in China. PLoS One **8**: e75672.

Zub HW, Arnoult S, Brancourt-Hulmel M. 2011. Key traits for biomass production identified in different *Miscanthus* species at two harvest dates. Biomass and Bioenergy **35**: 637-651.

CHAPTER III. Relationship between agronomic traits and canopy structure in determining *Miscanthus* biomass yield

ABSTRACT

Canopy structure of monocot crop is determined by vertical and horizontal plant growths mainly represented by stem height and stem distribution, respectively, and determine crop biomass yield. *Miscanthus* species have diverse growth habits and agronomic traits depending on species and thus show high diversity in biomass yield potential. To develop methods to assess canopy structure of *Miscanthus* and to investigate *Miscanthus* canopy structure in determining biomass yield, the growth parameters of *M.* × *giganteus*, selected as a model *Miscanthus*, associated with canopy structure such as the spatial distribution of stems, stem height, and stem dry weight were assessed at harvest during the four years after planting, and then canopy area and volume were estimated. In the first and the second year, canopy area and volume of *M.* × *giganteus* increased due to slow expansion of stem growth, but from the third year after planting increased dramatically, resulting in dramatic biomass yield. Canopy structures of three *Miscanthus* species, *M. sinensis* (2*n*=2*x*=38), *M. sacchariflorus* (2*n*=4*x*=76), and triploid *Miscanthus* including *M.* × *giganteus* (2*n*=3*x*=57), were assessed and compared each other. *Miscanthus sinensis* showed high biomass per canopy area and canopy volume due to its tufted stem distribution, while *M. sacchariflorus* showed low biomass per canopy area and volume because of wide-spreading stem growth. Interestingly, triploid hybrid *Miscanthus* showed intermediate canopy development between *M. sinensis* and *M. sacchariflorus* and showed hybrid vigor in yearly growth rate related with biomass yield parameters such as stem height, number of stem, and stem dry weight. Correlation analyses revealed that canopy structure parameters such as canopy diameter ($r = 0.579$, $p < 0.001$), canopy height ($r = 0.559$, $p < 0.001$), number of stem ($r = 0.636$, $p < 0.001$), canopy area ($r = 0.523$, $p < 0.001$), and canopy volume ($r = 0.506$, $p < 0.001$) were highly correlated with biomass yield. These results thus suggest that canopy structure parameters of *Miscanthus* provide useful information for understanding *Miscanthus* growth and biomass production and can be used as target traits to breed high biomass yielding *Miscanthus* cultivars.

Key words: *Miscanthus,* canopy structure, canopy area, canopy volume, biomass yield

Introduction

In crop production, morphological, phenological, physiological and agronomic traits are important components determining plant productivity and these traits are strongly related with plant canopy structure. Canopy structure is characterized by the spatial arrangement of stems and their components such as leaves and other photosynthetic tissues (Russell *et al*., 1990). Biomass production of grass species is the function of developing above ground canopy with stem density and architecture. In above ground, stem height, stem densities, leaf area index (LAI), the angle of inclination of the leaf tip, leaf blade length and width, and internode length are important factors to develop plant canopy structure, which determine the biomass yield (Redfearn *et al*., 1997). These growth development characteristics are diverse depending on species and cultivars. Canopy structure parameters include growth development in plant morphology that establish during primary growth can be important determinants of potential productivity in perennial grasses (Redfearn *et al*., 1997). Canopy structure have a diverse usage such as crop-weed competition and weed controls using herbicide effects by canopy structure in wheat (Kim *et al*., 2010).

Diverse morphological traits such as leaf area, stem height, stem diameter, and stem density related with biomass yield (Clifton-Brown and Lewandowski, 2002; Jezowski *et al*., 2011; Price *et al*., 2004; Yan *et al.*, 2011; Zub *et al*., 2011). However, these results were focused on individual traits of *Miscanthus*. Understanding of canopy structure combined with morphological and physiological characteristics in association with dry matter accumulation could be a good tool for predicting crop biomass yield.

In this reason, it is expected that *Miscanthus* canopy structure parameters consisting of stem height, spatial stem distribution, and stem dry weight may play important roles in determining *Miscanthus* biomass and can be used as target traits for *Miscanthus* breeding. In addition, these parameters can be used to characterize Miscanthus vegetative growth and to evaluate its genetic diversity in canopy development and growth habit.

It is generally known that *Miscanthus* species have a unique growth habit depending on species; *M. sinensis* has bunch growth habit, while *M. sacchariflorus* has scattered growth habit. *Miscanthus* × *giganteus*, a hybrid between *M. sinensis* and *M. sacchariflorus*, has an intermediate growth habit. Three dimensional development of *Miscanthus* canopy is important for determining biomass yield in a unit area of field and thus biomass productivity. In this sense, new parameters representing canopy structure are essential in *Miscanthus* research on biomass production. However, no studies have ever investigated to characterize canopy structures of these three species and to relate canopy structure with biomass yield.

Therefore, this study was conducted to develop parameters representing canopy structure of *Miscanthus* species, to characterize canopy structures of *Miscanthus* species, and to relate canopy structure with *Miscanthus* biomass yield.

Materials & Methods

Assessment of canopy structure development of *M. × giganteus*

Miscanthus rhizome spreads radially from the main stem. New shoots emerge from the spread rhizomes. Degree of rhizome spreading and stem density are the main functions of canopy structure development in *Miscanthus*. To characterize and quantify *Miscanthus* canopy structure, *M. × giganteus* was selected as a model *Miscanthus* and planted on 27 May 2010 in a planting space of 100 cm × 100 cm in a field located at the Experimental Farm Station of Seoul National University, Suwon (N 37° 16’ 12.1”, E 126° 59’ 27.5”), Korea. Nitrogen fertilizer was applied at 60 kg N ha^{-1} $year^{-1}$ in early April and weeds were controlled by using herbicide and hand-weeded if necessary. We randomly selected 3 *M. × giganteus* plants and assessed stem distribution from the center of the individual plant (the main stem of the first year growth) at harvest every year for 4 years after planting *M. × giganteus* rhizomes. Individual stem length and stem dry weight were also measured at harvest.

The distribution of stem was recorded two-dimensionally in a x y coordinate from the center of the individual plant (Figure 2-1 and 3-2). Canopy area (CA) was then estimated using the following equation,

$$\text{Canopy area } (cm^2) = \pi \times r_i^2 \qquad (3.1)$$

where r_i is the maximum radius of two-dimensional stem distribution in the ith year. Canopy volume (CV) was then estimated by multiplying canopy area with maximum stem height (h) as follows,

$$\text{Canopy volume } (cm^3) = \pi \times r_i^2 \times h_i \qquad (3.2)$$

To assess growth rate in canopy structure parameters for 4 years after planting *Miscanthus*, canopy growth rate (CGR) was calculated using the following equation,

$$\text{Canopy growth rate } (CGR) = (W_2 - W_1) \ / \ (t_2 - t_1) \qquad (3.3)$$

where W_1 and W_2 are canopy structure values at the year t_1 and t_2 after planting, respectively.

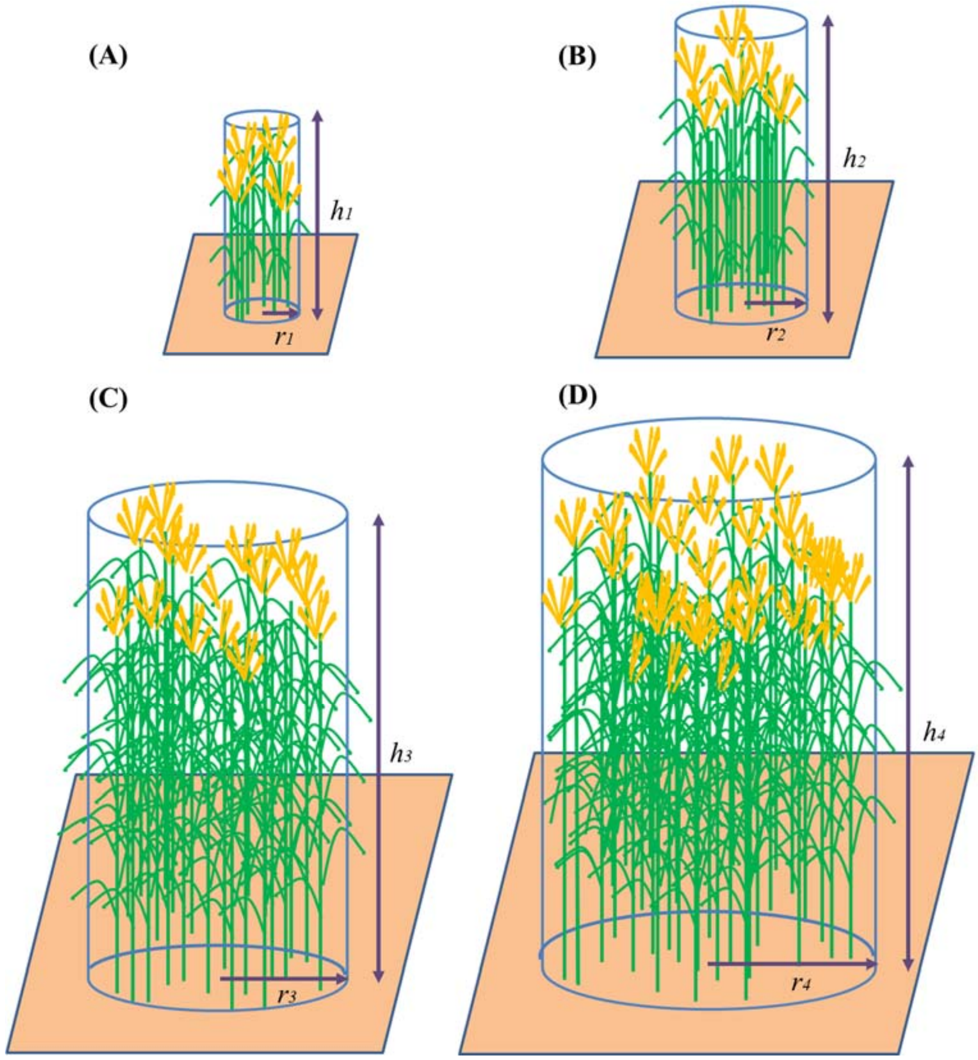


Figure 3-1. Canopy area and canopy volume of *M*. × *giganteus* assessed in the 1st (A), the 2nd (B), the 3rd (C), and the 4th year (D) after planting. The parameter *r* and *h* are maximum radius (1/2 × canopy diameter) and maximum stem height (canopy height), respectively.

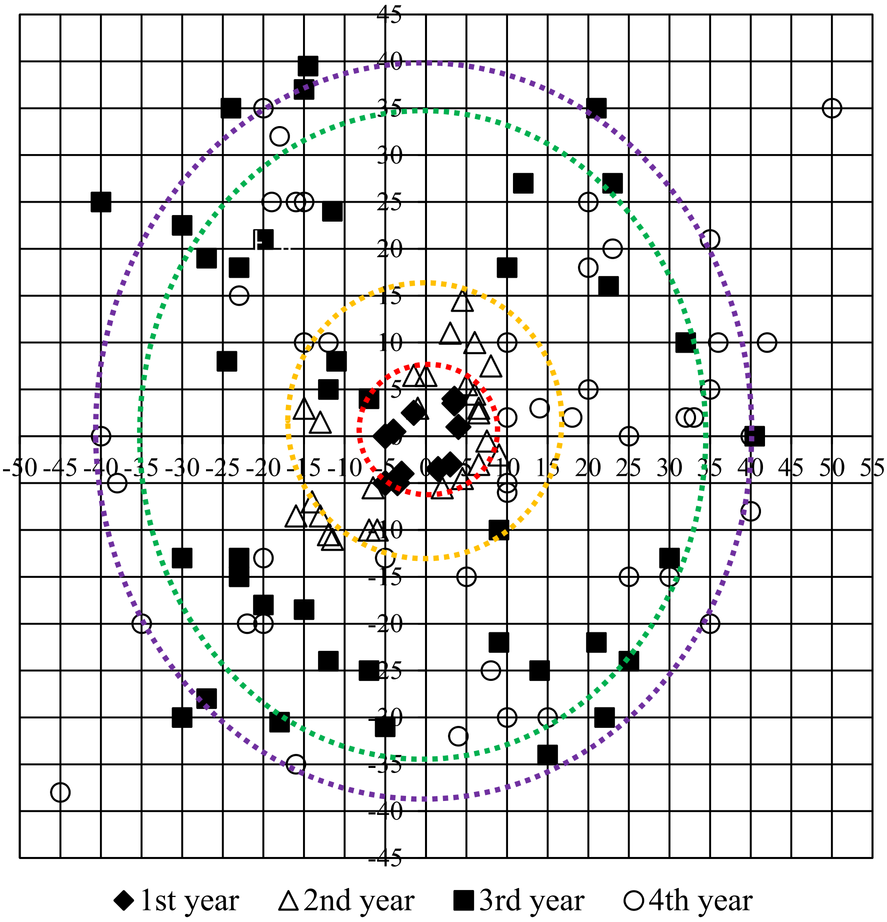


Figure 3-2. One example of stem distribution of *M.* × *giganteus* assessed in the 1st year (◆), the 2nd year (△), the 3rd year (■) and the 4th year (○) after planting.

Assessment of canopy structure of *Miscanthus* species

To examine canopy structure of *Miscanthus* species, 17 *Miscanthus* accessions composed of 8 *M. sinensis* ($2n=2x=38$), 6 *M. sacchariflorus* ($2n=4x=76$) and 3 *Miscanthus* hybrids ($2n=3x=57$) including *M.* × *giganteus* were selected. Their individual rhizomes were planted at a planting space of 100 cm × 100 cm in a field located at the Experimental Farm Station of Seoul National University, Yeoju (N 37° 15' 29.4", E 127° 32' 16.8"), Korea. Nitrogen fertilizer was applied at 60 kg N ha^{-1} $year^{-1}$ in early April and weeds were controlled by using herbicide and hand-weeded if necessary. All the accessions were arranged in a randomized block design with three replications. As described above, the spatial stem distribution and individual stem length and stem dry weight were measured at harvest. The distribution of stem was recorded two-dimensionally in a x y coordinate from the center of the individual plant (the main stem of the first year growth). Using the above equations 3-1, 3-2, and 3-3, canopy structure parameters were then estimated.

To relate between canopy structure parameters with agronomic traits in the 3rd year after planting *Miscanthus*, we assessed leaf traits such as leaf length and width of top 3 leaves including flag leaf, stem traits including stem diameter measured at the mid-point between the second or the third basal nodes using Vernier calipers in the 3rd year after planting. Leaf area was calculated using equation (2.1).

Distribution of stem number and stem dry weight

Stem distribution in a x y coordinate (Figure 3-2) was rearranged along a distance from the center of canopy area. Accumulated stem number and stem dry weight per unit distance were presented along the distance from the center of canopy area. The distributions of stem number and stem dry weight per unit distance were then fitted to the Gaussian function as follows,

Stem number or stem dry weight (g) =

$$\mathrm{a} \times exp\left[-\left[0.5 \times \left[\frac{(x-b)}{c}\right]^2\right]\right] \quad (3.4)$$

where a is the height of the curve's peak, b is the distance (x) of the peak from the center and c is standard deviation that controls the width of the curve.

Statistical analysis

Canopy structure parameters, agronomic traits and *Miscanthus* biomass yield were subjected to analysis of variance (ANOVA). Correlation coefficients were calculated among the canopy structure parameter, agronomic trait and biomass yield. To examine the relationships among the canopy structure parameter, agronomic traits and biomass yield, linear regression analysis was conducted. Non-linear regression analysis was also conducted to fit distributions of stem number and stem dry weight per unit distance to Gaussian function. All statistical analyses were performed by using JMP 12 software (SAS Institute, USA).

Results

Canopy structure development of *M. × giganteus* and its association with biomass

To characterize and quantify canopy structure development of *Miscanthus*, key parameters associated with canopy structure were chronologically measured in a model *Miscanthus*, *M. × giganteus*, for 4 years. As shown in Figure 3-2, two-dimensional stem distribution, maximum radius of canopy area, stem height, no. of stem, etc. were assessed in the first year through the fourth year of planting. *Miscanthus* grows horizontally and vertically in its canopy structure, so the radius of canopy area represents *Miscanthus*' horizontal growth, while stem height does *Miscanthus*' vertical growth. In the first year of planting, the maximum radius of its canopy area was 9.7 cm and gradually increased up to 38.1 cm in the fourth year (Table 3-1). Maximum stem height was 179 cm in the first year and increased to 377 cm in the fourth year. Three-dimensional canopy structure is determined by horizontal and vertical growths of *Miscanthus*, so the radius of canopy area and the stem height determine canopy volume. Canopy area and canopy volume were 318.4 cm^2 and 51,568 cm^3, respectively, in the first year of planting, and then increased to 4,728.1 cm^2 and 1,787,768 cm^3, respectively, in the fourth year (Table 3-1). The number of stems was 4.7 stems $plant^{-1}$ in the first year and increased to 46.7 stems $plant^{-1}$ in the fourth year, about 10 times increase. Dry biomass (g dry weight $plant^{-1}$) was increased from 28.6 g in the first year to 2,807.9 g, almost 100 times increase.

Canopy development in a unit area determines biomass productivity. Therefore, canopy development and growth per unit canopy area or unit canopy volume were estimated (Table 3-1). Number of stem per canopy area was decreased with time, 0.022 stem cm^{-2} in the first year and 0.011 stem cm^{-2} in the fourth year. Contrastingly biomass per canopy area and per canopy volume was increased with time. Biomass per canopy area was 0.17 g cm^{-2} in the first year and increased to 0.67 g cm^{-2} in the fourth year. Biomass per canopy volume was 0.0008 g cm^{-3} in the first year and increased to 0.0018 g cm^{-3} in the fourth year. Interestingly, biomass per canopy area or biomass per canopy volume in the third and the fourth year maintained steadily with no year difference, 0.61 ~ 0.67 g cm^{-2} and 0.0017 ~ 0.0018 g cm^{-3}, respectively, after more than 2 times increase in the third year in comparison with those in the second year. The significant increase in these parameters in the third year compared with the second year is mainly attributed to greater increase in biomass (3.92 times) than in canopy area (1.27 times) and canopy volume (1.65 times). On the contrary, the steady maintenance of them since the third year is attributed to similar increases in biomass (2.25 times), canopy area (2.33 times), and canopy volume (2.45 times) in the fourth year as compared with those in the third year. Biomass accumulation in a unit area or volume is important parameter to estimate biomass productivity of bioenergy crop. These two new parameters thus may be useful to characterize and quantify biomass productivity of *Miscanthus* and other perennial bioenergy grasses with different canopy development traits.

Table 3-1. Summary of canopy structure parameters of *M.* × *giganteus*. The values in the parentheses are standard errors (*df* = 6).

Year after planting year	Max. radius (cm)	Mean radius (cm)	Max. stem height (cm)	Mean stem height (cm)	Number of stem (stem plant^{-1})	Biomass (g plant^{-1})	Canopy area (cm^2)	Canopy Volume (cm^3)	Number of stem per canopy area (stem cm^{-2})	Biomass per canopy area (g cm^{-2})	Biomass per canopy volume (g cm^{-3})
1st	9.7 (2.0)	5.0 (1.1)	179.0 (24.4)	109.0 (16.5)	4.7 (0.3)	28.46 (9.95)	318.4 (116.3)	51568 (14502)	0.022 (0.011)	0.17 (0.13)	0.0008 (0.0005)
2nd	22.5 (0.8)	12.7 (0.3)	279.3 (26.0)	177.8 (26.1)	19.3 (0.3)	317.84 (76.99)	1599.6 (107.6)	444295 (38444)	0.012 (0.001)	0.21 (0.06)	0.0007 (0.0002)
3rd	25.4 (0.9)	16.6 (0.8)	360.0 (5.0)	282.3 (9.5)	30.3 (3.2)	1246.11 (167.99)	2031.8 (140.9)	731179 (48992)	0.015 (0.001)	0.61 (0.07)	0.0017 (0.0002)
4th	38.1 (5.2)	26.2 (2.6)	377.0 (2.0)	304.1 (1.5)	46.7 (0.9)	2807.94 (74.60)	4728.1 (1322.0)	1787768 (510390)	0.011 (0.002)	0.67 (0.14)	0.0018 (0.0004)

Horizontal growth of *Miscanthus* can be presented as the distribution of number of stems (Figure 3-3A) and stem dry weight (Figure 3-3B) from the center of the canopy to the outmost point of the canopy area. As described in Figure 3-2, all the stems established in the canopy area were marked in a x y coordinate, and the distance of each stem from the center was calculated. Then, the number of stems was plotted in each unit distance from the center. Interestingly, the number of stems distributed along the distance from the center in the function of the Gaussian distribution (normal distribution), and its distribution was gradually expanded with time (Figure 3-3A). The distribution of the stem dry weight was also followed the similar function of the Gaussian distribution, and was also gradually expanded with time (Figure 3-3B). The number of stem at the peak center of stem distribution (*a*) was 1.8 and 2.2 stems cm^{-1} in the first year and the fourth year, respectively, and the distance of the peak center of stem distribution (*b*) was 4.2 cm in the first year and increased to 26.8 cm in the fourth year (Table 3-2).. The biomass at the peak center of biomass distribution (*a*) was 14.6 g in the first year and increased 154.2 g in the fourth year, about 10 times increase, and the distance of the peak center of biomass distribution was 5.4 cm in the first year and increased to 27.8 cm in the fourth year (Table 3-2). The width of curve shape ($c \times 2$) representing dense area of *Miscanthus* canopy was 2.8 cm in the first year and increased to 18.6 cm in the fourth year (Table 3-2). In the case of biomass, it was 5.4 cm in the first year and increased to 25.0 cm in the fourth year (Table 3-2).

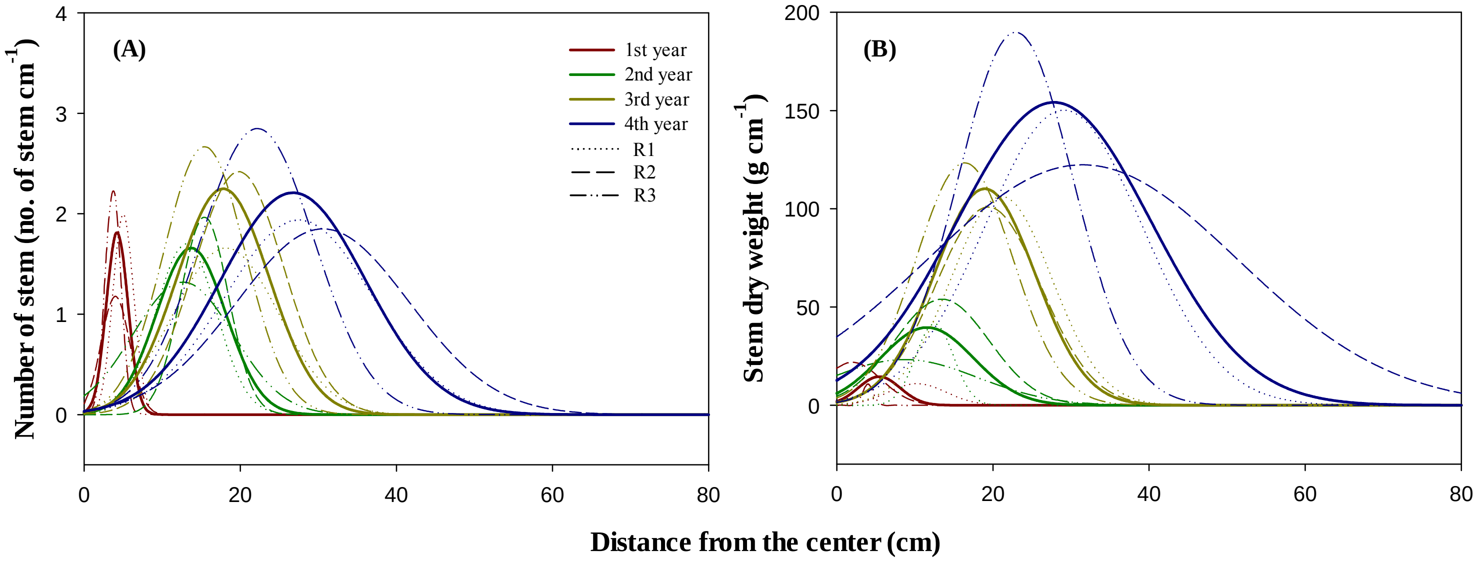


Figure 3-3. Simulated distribution of number of stem (A) and stem dry weight (B) per unit distance from the center of *M.* × *giganteus* for 4 years since the first planting in 2011. The simulated values were calculated using the Gaussian distribution curve (equation 3.4) and the parameter estimates (Table 3-1).

Table 3-2. Summary of stem dry weight and number of stem distribution parameters following Gaussian distribution curve in each planting year.

Distribution	Rep	1st year			2nd year			3rd year			4th year		
		a[1)]	*b*[2)]	*c*[3)]	*a*	*b*	*c*	*a*	*b*	*c*	*a*	*b*	*c*
Number of stem	R1	2.00 (*)[4)]	5.0 (*)	1.3 (*)	1.7 (0.3)	13.1 (0.9)	3.9 (0.9)	1.7 (0.5)	18.2 (2.7)	6.8 (3.0)	1.9 (0.3)	27.5 (1.5)	9.5 (1.6)
	R2	1.2 (0.2)	4.0 (0.4)	1.8 (0.5)	2.0 (0.5)	15.4 (0.8)	3.1 (0.8)	2.4 (0.3)	19.8 (0.9)	5.8 (1.0)	1.9 (0.3)	30.6 (1.8)	11.0 (1.9)
	R3	2.3 (0.9)	3.7 (0.2)	0.6 (0.3)	1.3 (0.4)	12.7 (2.1)	6.4 (2.4)	2.7 (0.4)	15.4 (0.9)	5.2 (0.9)	2.9 (0.4)	22.2 (1.1)	7.3 (1.3)
	Mean	1.8 (0.3)	4.2 (0.4)	1.4 (0.2)	1.7 (0.2)	13.7 (0.9)	4.5 (1.0)	2.3 (0.3)	17.8 (1.3)	5.9 (0.5)	2.2 (0.3)	26.8 (2.5)	9.3 (1.1)
Stem dry weight	R1	11.0 (7.4)	10.3 (1.6)	3.7 (2.4)	41.6 (6.9)	12.5 (0.5)	2.4 (0.5)	106.3 (28.3)	21.1 (3.2)	6.5 (3.4)	150.2 (15.3)	29.1 (1.2)	10.1 (1.5)
	R2	21.9 (*)	2.0 (*)	3.7 (*)	54.0 (14.3)	13.5 (2.1)	6.1 (2.5)	100.9 (13.2)	19.4 (1.2)	6.7 (1.5)	122.4 (14.1)	31.58 (3.9)	19.9 (7.8)
	R3	10.9 (*)	4.0 (*)	0.7 (*)	23.3 (5.6)	8.7 (4.4)	9. (6.0)	123.4 (16.7)	16.4 (1.0)	6.2 (1.3)	189.9 (25.2)	22.9 (1.1)	7.4 (1.5)
	Mean	14.6 (3.7)	5.4 (2.5)	2.7 (1.0)	39.6 (8.9)	11.6 (1.5)	6.00 (2.0)	110.2 (6.8)	19.0 (1.4)	6.5 (0.2)	154.2 (19.6)	27.8 (2.6)	12.5 (3.8)

[1)] a, the height of the curve's peak

[2)] b, the position of the center of the peak (mean)

[3)] c, standard deviation of curve

[4)] Standard errors (*df* = 6)

Comparison of canopy structures between *Miscanthus* species

Based on canopy assessment of *M.* x *giganteus*, canopy structures of other *Miscanthus* species with different growth habits, 8 *M. sinensis*, 6 *M. sacchariflorus* and 3 *Miscanthus* hybrids accessions, were also assessed to characterize canopy structure and relate canopy structure parameters with biomass yield.

The distribution of number of stem (Figure 3-4A, B, and C) and stem dry weight (Figure 3-4D, E, and F) in *M. sinensis*, *M. sacchariflorus*, and hybrid *Miscanthus* showed similar patterns with *M.* × *giganteus.* They also were following the Gaussian distribution curve, although they had differences in curve parameters. The distribution of number of stems and stem dry weight were clearly represented characteristic of *Miscanthus* species. *Miscanthus sinensis* had a narrow and high-peak distribution curve than other species (Figure 3-4A and D), while *M. sacchariflorus* had a wide and low-peak distribution curve than other *Miscanthus* species (Figure 3-4B and E). Interestingly, hybrid *Miscanthus* showed intermediate distribution curve patterns between *M. sinensis* and *M. sacchariflorus* (Figure 3-4C and F). Unusually, two *M. sacchariflorus* (M-154, M-265) showed extremely wide and low distribution curve pattern in both number of stem and stem dry weight. It caused by their fast growth rate of rhizomes. The number of stem at the peak center of stem distribution (*a*) was ranging from 0.8 to 3.2 stems plant^{-1} in each *M. sinensis* in the first year and increased ranging from 4.3 to 14.6 stems plant^{-1} in the third year (Table 3-3). While, *M. sacchariflorus* did not increased yearly like *M. sinensis* (Table 3-3). Except *M.* × *giganteus,* the number of stem at the peak center of stem distribution in hybrid *Miscanthus* was ranged from 2.8 to 3.1 stems plant^{-1} in the first year and increased 10.1

to 15.2 stems plant^{-1} in the third year (Table 3-3). However, the biomass at the peak center of biomass accumulation was increased in every year and all the accessions. The biomass at the peak center of biomass accumulation in *M. sinensis* was ranged from 4.4 to 34.8 g in each accession in the first year and increased ranged from 47.1 to 270.4 g in the third year, about 10 times increase (Table 3-4). *M. sacchariflorus*, except M-265, was ranged from 6.9 to 36.4 g in the first year and increased ranged from 30.8 to 60.8 g in the third year (Table 3-4). In case of M-265, it reduced biomass at the peak center of biomass accumulation. Hybrid *Miscanthus* also ranged 5.5 to 29.8 g in the first year and increased ranged from 80.3 to 108.6 g in the third year (Table 3-4). The distance of the peak center of stem distribution (*b*) of *M. sinensis* and *M. sacchariflorus* were ranged from 1.9 to 4.3 cm and 2.0 to 12.5 cm in the first year and increased ranging from 6.1 to 12.0 cm and 9.1 to 68.2 cm in the third year, respectively (Table 3-4). Hybrid *Miscanthus* was ranged from 1.9 to 7.0 cm in the first year and increased ranging from 18.0 to 19.5 cm in the third year (Table 3-4). The width of dense canopy ($c \times 2$) also increased through yearly in each species. The width of dense canopy of *M. sinensis* was ranged from 1.6 to 5.2 cm in the first year and increased ranging from 6.4 to 11.0 cm in the third year (Table 3-4). *M. sacchariflorus* was more widely increased canopy area. They were ranged from 4.4 to 11.4 cm in the first year and increased ranging from 14.6 to 187.0 cm in the third year, about 10 times increase (Table 3-4). The width of dense canopy of Hybrid *Miscanthus*, except *M. × giganteus,* was 3.6 cm in the first year and increased ranging from 16.0 to 17.2 cm in the third year (Table 3-4).

The first and the second year, the distribution of number of stem curve parameters were no differences among the species, while the third year parameters showed differences among the species. Number of stem at the peak center of stem distribution (*a*) was showed high value in *M. sinensis* and hybrid *Miscanthus* than *M. sacchariflorus*. However, the distance to the peak center of stem distribution (*b*) was showed reversal patterns. The distance to the peak center (*b*) of hybrid *Miscanthus* showed intermediate value (mean = 18.6) between *M. sinensis* (mean = 9.6) and *M. sacchariflorus* (mean = 29.2). The distribution of stem dry weight was more clearly represented differences among the species. Number of stems did not show any differences in the first and the second year, while stem dry weight showed significantly differences yearly and among the species. *M. sinensis* had the highest stem dry weight at the peak center of distribution curve (*a*), while *M. sacchariflorus* had the lowest one at the peak of distribution curve. On the other hand, the distance to the peak of distribution curve (*b*) was the longest in *M. sacchariflorus* and the shortest in *M. sinensis*. Hybrid *Miscanthus* showed intermediate values both parameters. The width of the dense canopy was also represented specific growth habit of *Miscanthus* species. *M. sinensis* had low growth rate of horizontal growth and it represented narrow width of dense canopy, while *M. sacchariflorus* had high growth rate of horizontal growth and it represented to widely width of dense canopy.

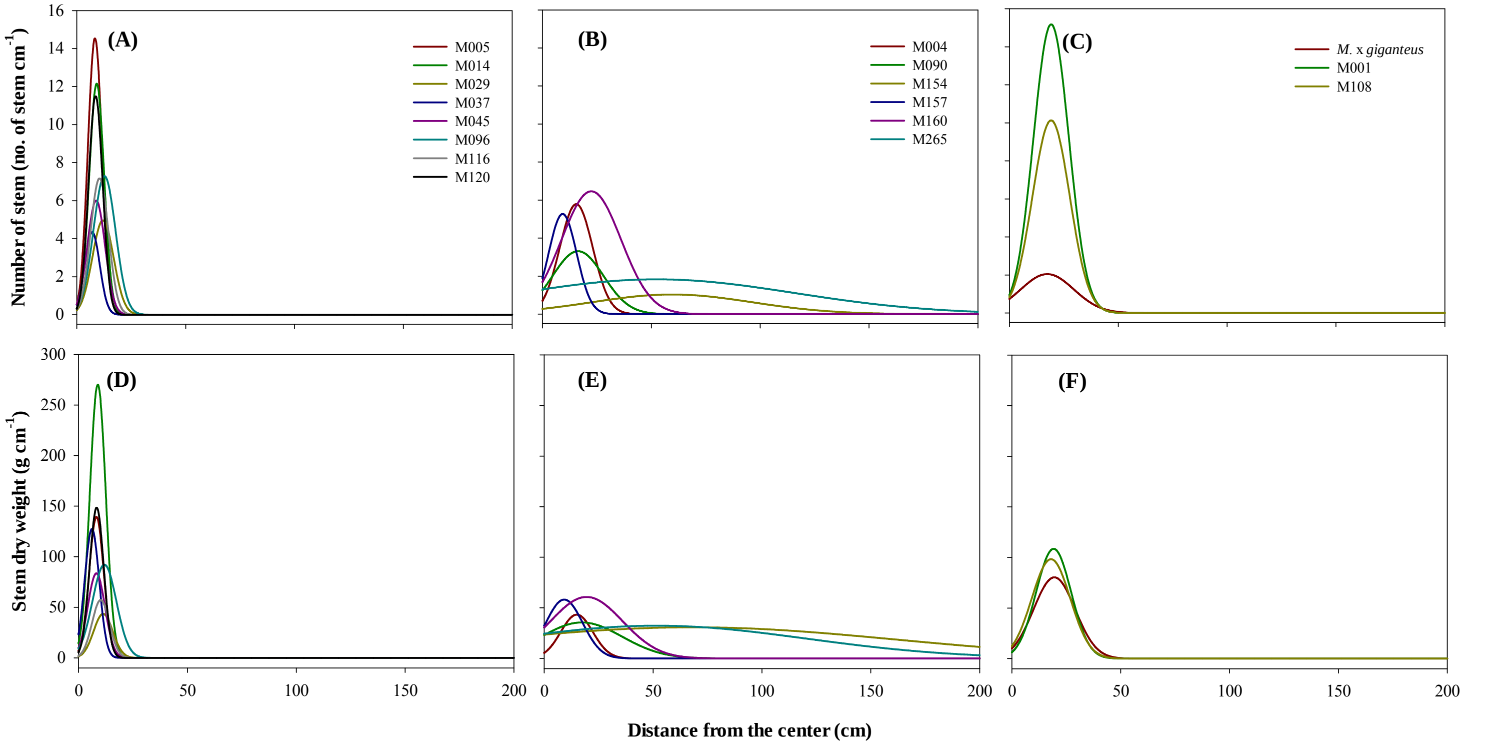


Figure 3-4. Distribution of number of stem (*M. sinensis* (A), *M. sacchariflorus* (B) and hybrid *Miscanthus* (C)) and stem dry weight (*M. sinensis* (D), *M. sacchariflorus* (E) and hybrid *Miscanthus* (F)) per unit distance from the center of *Miscanthus* accessions in the 3rd year after planting.

Table 3-3. Summary of number of stems distribution parameters following Gaussian distribution curve in each year after planting.

Species	Code	1st year			2nd year			3rd year		
		a[1)]	*b*[2)]	*c*[3)]	*a*	*b*	*c*	*a*	*b*	*c*
M. sinensis	M-005	3.2 (0.7)[4)]	4.1 (1.9)	0.9 (0.1)	7.1 (0.3)	8.2 (1.3)	3.4 (1.0)	14.6 (1.2)	8.3 (0.7)	3.3 (0.7)
	M-014	3.1 (0.6)	2.7 (0.1)	1.1 (0.1)	2.8 (0.7)	8.2 (2.0)	5.3 (2.0)	12.2 (3.2)	9.1 (1.6)	3.4 (0.8)
	M-029	0.8 (0.0)	2.6 (1.9)	4.3 (1.9)	1.3 (0.1)	12.1 (2.5)	8.4 (0.5)	5.0 (0.4)	12.0 (0.8)	4.9 (0.4)
	M-037	2.2 (0.7)	2.1 (0.2)	1.0 (0.1)	4.4 (1.2)	3.5 (0.1)	1.9 (0.5)	4.3 (0.9)	7.1 (0.5)	3.5 (0.5)
	M-045	1.7 (0.4)	2.6 (0.6)	1.9 (0.4)	5.1 (0.7)	5.4 (0.3)	2.9 (0.3)	6.0 (1.0)	8.9 (0.7)	4.1 (0.4)
	M-096	2.8 (1.1)	3.7 (1.2)	2.1 (1.0)	4.8 (1.5)	7.8 (0.4)	2.8 (0.3)	7.3 (0.5)	12.7 (1.3)	5.1 (0.5)
	M-116	1.5 (0.0)	2.8 (0.2)	2.6 (0.4)	3.1 (1.0)	10.3 (1.6)	4.7 (0.3)	7.2 (0.2)	10.4 (1.2)	4.1 (0.7)
	M-120	2.0 (0.3)	3.7 (1.0)	2.6 (1.0)	6.1 (0.3)	8.5 (1.0)	3.4 (0.3)	11.5 (1.4)	8.6 (0.1)	3.2 (0.3)
M. sacchariflorus	M-004	1.2 (0.1)	7.4 (1.4)	3.0 (1.0)	3.4 (0.8)	18.8 (4.7)	5.8 (1.1)	5.8 (1.3)	15.5 (2.5)	7.6 (3.5)
	M-090	1.5 (0.3)	12.0 (3.1)	4.9 (2.6)	2.4 (0.2)	7.7 (0.4)	8.0 (1.9)	3.3 (1.2)	16.3 (3.0)	11.7 (2.6)
	M-154	1.6 (0.4)	1.9 (0.9)	2.5 (0.2)	0.8 (0.1)	66.2 (8.3)	20.9 (0.9)	1.0 (0.2)	59.5 (3.4)	36.7 (7.2)
	M-157	1.9 (0.5)	6.6 (1.4)	3.5 (1.2)	6.4 (1.4)	5.4 (0.4)	2.5 (0.3)	5.3 (0.8)	9.2 (1.3)	6.5 (1.3)
	M-160	2.1 (0.1)	2.7 (0.9)	2.5 (0.3)	6.6 (1.8)	11.0 (2.7)	5.3 (1.7)	6.5 (2.3)	22.4 (6.4)	13.7 (4.6)
	M-265	3.8 (0.7)	4.6 (0.8)	2.9 (0.5)	2.1 (0.1)	9.3 (2.8)	18.3 (5.9)	1.8 (0.3)	52.4 (12.6)	63.5 (24.5)
Hybrid *Miscanthus*	$M \times g$	2.2 (0.3)	7.6 (2.4)	2.9 (1.1)	1.5 (0.1)	28.5 (8.8)	16.1 (6.3)	2.0 (0.4)	17.4 (3.6)	12.2 (2.5)
	M-001	3.1 (0.9)	4.8 (0.8)	2.4 (0.8)	5.8 (0.5)	15.1 (2.5)	5.4 (0.6)	15.2 (3.3)	19.2 (2.7)	8.1 (0.6)
	M-108	2.8 (0.9)	2.8 (0.1)	2.4 (0.6)	6.3 (1.5)	11.6 (1.6)	5.9 (2.5)	10.1 (1.0)	19.2 (2.3)	8.6 (1.7)

[1)] a, the number of stem at the peak center of stem distribution; [2)] b, the distance of the center of the peak (mean); [3)] c, standard deviation of curve; [4)] standard errors ($df = 32$)

Table 3-4. Summary of stem dry weight distribution parameters following Gaussian distribution curve in each year after planting.

Species	Code	1st year			2nd year			3rd year		
		a[1)]	*b*[2)]	*c*[3)]	*a*	*b*	*c*	*a*	*b*	*c*
M. sinensis	M-005	19.9 (5.8)[4)]	4.1 (1.9)	0.8 (0.1)	72.5 (4.9)	8.2 (1.2)	3.2 (1.1)	139.6 (10.5)	8.2 (0.7)	3.4 (0.7)
	M-014	34.8 (1.9)	2.6 (0.2)	1.0 (0.0)	60.7 (19.5)	7.5 (1.7)	4.8 (1.6)	270.4 (62.2)	8.8 (1.6)	3.6 (0.8)
	M-029	4.4 (0.0)	4.3 (1.7)	2.6 (0.2)	12.1 (0.4)	10.8 (0.9)	7.9 (0.4)	47.1 (3.5)	12.0 (0.8)	5.0 (0.6)
	M-037	34.5 (7.6)	1.9 (0.2)	1.0 (0.2)	101.2 (22.5)	2.9 (0.2)	1.9 (0.6)	127.5 (15.9)	6.1 (0.3)	3.3 (0.2)
	M-045	8.4 (2.0)	2.2 (1.0)	1.8 (0.6)	63.5 (12.6)	4.9 (0.3)	2.8 (0.3)	83.6 (15.5)	8.2 (0.8)	4.0 (0.4)
	M-096	27.9 (13.4)	2.3 (0.4)	2.0 (1.1)	58.1 (19.6)	7.0 (0.2)	3.0 (0.4)	92.2 (10.7)	11.9 (1.4)	5.5 (0.7)
	M-116	10.6 (0.4)	2.9 (0.3)	2.4 (0.7)	26.9 (7.3)	10.4 (1.8)	4.6 (0.1)	57.3 (4.3)	10.2 (1.4)	4.0 (0.8)
	M-120	22.9 (3.5)	3.1 (1.1)	2.4 (1.1)	74.8 (5.3)	7.8 (0.8)	3.4 (0.3)	148.7 (22.2)	8.3 (0.2)	3.2 (0.2)
M. sacchariflorus	M-004	6.9 (0.2)	7.6 (1.3)	5.7 (2.9)	25.3 (7.0)	18.9 (4.8)	6.1 (1.3)	43.3 (9.5)	14.9 (1.9)	7.3 (3.4)
	M-090	9.7 (2.0)	12.5 (3.8)	4.3 (2.7)	29.2 (4.7)	6.6 (1.8)	8.6 (1.6)	35.6 (11.4)	17.2 (4.1)	18.5 (6.0)
	M-154	11.1 (4.6)	2.0 (1.0)	2.2 (0.1)	20.5 (0.4)	56.3 (7.2)	33.9 (5.5)	30.8 (3.2)	68.2 (9.1)	93.5 (13.9)
	M-157	14.0 (2.7)	7.2 (1.5)	4.0 (1.3)	55.9 (12.8)	5.4 (0.4)	2.9 (0.3)	58.2 (9.3)	9.1 (1.6)	8.4 (0.8)
	M-160	9.0 (0.5)	2.6 (0.8)	2.5 (0.3)	68.1 (19.7)	10.6 (2.4)	5.4 (1.9)	60.8 (22.4)	19.4 (5.0)	16.6 (5.7)
	M-265	36.4 (4.0)	4.0 (0.5)	2.5 (0.3)	36.1 (1.3)	7.2 (1.7)	15.9 (6.8)	32.4 (1.9)	51.5 (12.4)	68.7 (24.3)
Hybrid *Miscanthus*	*M × g*	29.8 (3.7)	7.0 (2.0)	2.2 (0.5)	42.2 (6.2)	24.7 (6.0)	12.6 (3.5)	80.3 (19.5)	19.5 (5.0)	9.6 (1.6)
	M-001	5.5 (1.8)	3.8 (0.2)	1.8 (0.4)	40.9 (4.2)	15.0 (2.7)	5.4 (0.7)	108.6 (19.6)	19.2 (2.7)	8.0 (0.5)
	M-108	16.0 (4.1)	1.9 (0.4)	1.8 (0.8)	53.6 (12.8)	10.2 (1.2)	6.3 (2.5)	98.3 (14.9)	18.0 (2.3)	8.8 (1.6)

[1)] a, the biomass at the peak center of biomass accumulation; [2)] b, the distance of the center of the peak (mean); [3)] c, standard deviation of curve; [4)] standard errors (*df* = 32)

Maximum radius (canopy diameter) growth rate of *M. sinensis* was ranged from 2.40 to 7.32 cm year^{-1}, while *M. sacchariflorus* was ranged from 6.27 to 77.30 cm year^{-1} (Table 3-5). In case of hybrid *Miscanthus* was ranged from 15.64 to 16.21 cm year^{-1} (Table 3-5). Horizontal growth rate of *M. sinensis* (mean = 5.41 cm year^{-1}) was much lower than *M. sacchariflorus* (mean = 29.91 cm year^{-1}) and hybrid *Miscanthus* (mean = 15.91 cm year^{-1}) (Table 3-5). It suggested that growth rate of canopy diameter of *M. sacchariflorus* increased widely and rapidly every year. Growth rate of canopy diameter related with canopy area, so growth rate of canopy area also showed similar patterns in each *Miscanthus*. Canopy area of *M. sinensis* was ranged from 192 to 603 cm^2 year^{-1}, while *M. sacchariflorus* was ranged from 783 to 40,526 cm^2 year^{-1} (Table 3-5). And hybrid *Miscanthus* was ranged from 2,333 to 2,689 cm^2 year^{-1} (Table 3-5). In the growth of maximum stem height and number of stem, hybrid *Miscanthus* was ranged from 28.3 to 57.5 cm year^{-1} and 16.2 to 146.1 stems year^{-1}, respectively (Table 3-5). The growth rate of total dry weight also showed higher values in hybrid *Miscanthus*. However, the growth of total dry weight did not show significant difference among the species. The growth rate of canopy area and canopy volume of *M. sacchariflorus* (mean = 12,654 cm^2 and 2,781, 699 cm^3, respectively) were greater than *M. sinensis* (mean = 401 cm^2 and 76,813 cm^3, respectively) and hybrid *Miscanthus* (mean = 2,473 cm^2 and 616,667 cm^3, respectively) (Table 3-5). These results were represented morphological traits related with growth habit of *Miscanthus* species.

Table 3-5. Summary of canopy structure parameters of *Miscanthus* species

Species	Genotype	Ploidy level	Max radius (cm $year^{-1}$)	Mean radius (cm $year^{-1}$)	Max. height (cm $year^{-1}$)	Mean height (cm $year^{-1}$)	Number of stem (no. $year^{-1}$)	Total dry weight (g $year^{-1}$)	Canopy area (cm^2 $year^{-1}$)	Canopy volume (cm^3 $year^{-1}$)
M. sinensis (n = 8)	M-005	2n	5.29	2.22	16.7	5.5	56.0	557	324	45,000
	M-014	2n	7.32	3.19	21.7	5.8	43.3	1,058	510	98,500
	M-029	2n	3.97	2.75	68.1	44.6	26.4	264	312	90,000
	M-037	2n	5.51	2.50	23.1	10.3	16.0	517	292	48,000
	M-045	2n	6.98	2.69	25.0	3.6	27.4	383	574	98,000
	M-096	2n	4.59	4.13	15.8	3.4	38.5	524	397	75,000
	M-116	2n	7.20	3.61	30.0	9.5	33.5	262	603	90,000
	M-120	2n	2.40	2.40	31.7	15.2	41.3	550	192	70,000
	Mean		**5.41**	**2.94**	**29.0**	**12.2**	**35.3**	**683**	**401**	**76,813**
M. sacchariflorus (n = 6)	M-004	4n	6.88	2.75	41.7	22.5	48.6	359	817	215,000
	M-090	4n	6.27	0.31	40.0	23.5	43.3	464	783	200,000
	M-154	4n	77.3	31.4	32.5	10.1	41.3	877	40,526	9,990,000
	M-157	4n	9.07	3.30	30.0	14.1	31.5	390	1,224	310,000
	M-160	4n	22.64	10.35	55.0	18.6	77	756	4,346	995,000
	M-265	4n	57.3	23.86	27.9	16.3	28.5	661	28,225	4,980,000
	Mean		**29.91**	**11.99**	**37.9**	**17.5**	**45.0**	**585**	**12,654**	**2,781,667**
Hybrid *Miscanthus* (n = 3)	*M × g*	3n	16.21	8.37	57.5	27.4	16.2	527	2,689	970,000
	M-001	3n	15.88	7.78	30.0	24.5	146.1	1,064	2,333	390,000
	M-108	3n	15.64	7.59	28.3	8.9	102.8	1,072	2,398	490,000
	Mean		**15.91**	**7.91**	**38.6**	**20.3**	**88.4**	**888**	**2,473**	**616,667**
$LSD_{0.05}$*			11.07	4.95	11.9	8.2	23.4	669	6,227	1,365,429

* Least significant difference (LSD) values at the 0.05 level

The number of stem per canopy area (stem density) is important parameters related with biomass yield. Stem density of *M. sinensis* ranged from 0.05 to 0.17 stems cm^{-2} in the third year, while *M. sacchariflorus* was ranged from 0.01 to 0.05 stems cm^{-2} in the third year (Table 3-6). Stem density of hybrid *Miscanthus* was ranged from 0.02 to 0.06 stems cm^{-2} in the third year as an intermediate characteristic between *M. sinensis* and *M. sacchariflorus*. Biomass per canopy area also showed similar patterns among the species. Especially, *M. sinensis* showed the highest biomass per canopy area (mean = 1.44 g cm^{-2}) among the species. Biomass per canopy volume of *M. sinensis* ranged from 0.00167 to 0.01643 g cm^{-3} in the third year, while *M. sacchariflorus* ranged from 0.00065 to 0.00246 g cm^{-3} in the third year (Table 3-6). Hybrid *Miscanthus* (mean = 0.00205 g cm^{-3}) showed intermediate characteristic of *M. sinensis* (mean = 0.00764 g cm^{-3}) and *M. sacchariflorus* (mean = 0.00149 g cm^{-3}) (Table 3-6). Interestingly, M-014 and M-037 showed high values in biomass per canopy area and canopy volume. These *Miscanthus* were collected in Jeju island. *Miscanthus* collected in southern regions had a late heading date, so they had a long vegetative period that could produce high biomass yield. Canopy area and canopy volume of *M. sacchariflorus* and hybrid *Miscanthus* were greater than *M. sinensis*, while plant dry weight was showed no differences among *Miscanthus* species. Therefore, biomass per canopy area or canopy volume of *M. sinensis* was greater than the other species, it suggesting that *M. sinensis* had a greater advantage to produce more biomass in a unit space than the other *Miscanthus* species.

Table 3-6. Summary of canopy structure related with biomass yield of *Miscanthus* species.

Species	Genotype	Ploidy level	Number of stem /Canopy area (no. cm^{-2})	Biomass /Canopy area (g cm^{-2})	Biomass /Canopy volume (g cm^{-3})
M. sinensis	M-005	2n	0.17	1.29	0.00805
	M-014	2n	0.11	2.89	0.01643
	M-029	2n	0.03	0.28	0.00167
	M-037	2n	0.12	2.50	0.01592
	M-045	2n	0.11	1.14	0.00645
	M-096	2n	0.07	0.80	0.00430
	M-116	2n	0.05	0.38	0.00251
	M-120	2n	0.09	1.14	0.00575
	Mean		**0.09**	**1.44**	**0.00764**
M. sacchariflorus	M-004	4n	0.03	0.19	0.00094
	M-090	4n	0.02	0.21	0.00107
	M-154	4n	0.01	0.10	0.00065
	M-157	4n	0.05	0.47	0.00246
	M-160	4n	0.05	0.38	0.00196
	M-265	4n	0.03	0.30	0.00184
	Mean		**0.03**	**0.28**	**0.00149**
Hybrid *Miscanthus*	*M × g*	3n	0.02	0.32	0.00159
	M-001	3n	0.06	0.33	0.00208
	M-108	3n	0.06	0.46	0.00246
	Mean		**0.05**	**0.37**	**0.00205**
	$LSD_{0.05}$*		0.04	1.77	0.01015

* Least significant difference (LSD) values at the 0.05 level

To examine the relationship between canopy structure parameters and biomass yield, we conducted regression analysis between the parameters (Figure 3-5). Six canopy structure parameters, such as canopy diameter (r^2 = 0.516, $p < 0.001$), canopy height (r^2 = 0.341, $p < 0.001$), number of stems (r^2 = 0.844, $p < 0.001$), stem dry weight (r^2 = 0.299, $p < 0.001$), canopy area (r^2 = 0.463, $p < 0.001$) and canopy volume (r^2 = 0.377, $p < 0.001$) were positively correlated with biomass yield (Figure 3-5). Canopy diameter, canopy height and number of stems were highly correlated with biomass yield. *M. sinensis* showed tufted canopy structure through association with canopy area and canopy volume (Figure 3-5). Canopy area and canopy volume of *M. sinensis* showed lower than *M. sacchariflorus* and hybrid *Miscanthus*. Nevertheless, *M. sinensis* showed higher biomass yield than that of the other species. It suggested that *M. sinensis* had an advantage to produce biomass yield per unit area and volume. *M. sacchariflorus* and hybrid *Miscanthus* developed large canopy area and canopy volume. Maximum stem height showed high correlation with biomass yield, while mean stem height did not show correlation with biomass yield.

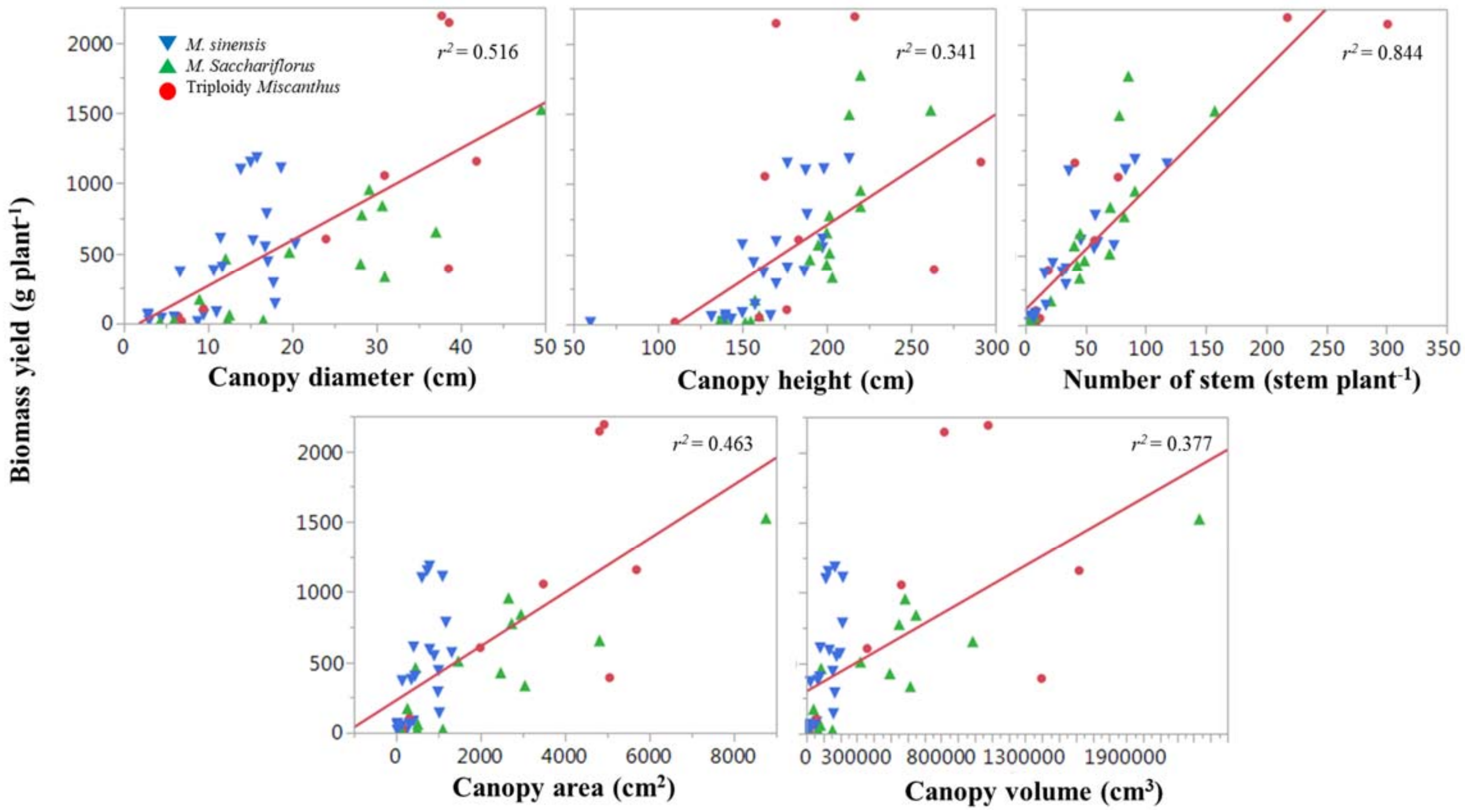


Figure 3-5. Relationship between canopy structure parameters and biomass yield of *Miscanthus* species.

Relationship among the agronomic trait, canopy structure development and biomass yield

To examine relationships among the agronomic trait, canopy structure and biomass yield, we conducted association among the 3rd year traits with each species (Figure 3-6, 3-7 and 3-8). They showed different relationships between the species with leaf area, stem diameter, number of stems and stem dry weight contributors to four canopy structure parameters, which were highly correlated with biomass yield. Correlation analyses of the canopy structure parameters with agronomic traits and biomass yields revealed that canopy diameter, canopy height, canopy area and canopy volume were significantly related with biomass yield but the extent of significance of these relationships depends on *Miscanthus* species. Biomass yield of *M. sinensis* significantly correlated with canopy volume (r = 0.476, p < 0.05), canopy area (r = 0.444, p < 0.05), and canopy diameter (r = 0.434, p < 0.05). Canopy area was strongly correlated with biomass yield of *M. sinensis*. Additionally, number of stem (r = 0.694, p < 0.01) was strongly correlated with canopy area than other agronomic traits (Figure 3-6). The number of stem (r^2 = 0.844, p < 0.001) was also strongly correlated parameter with biomass yield (Figure 3-5). It suggested that the number of stem was important parameter to determine *Miscanthus* biomass yield.

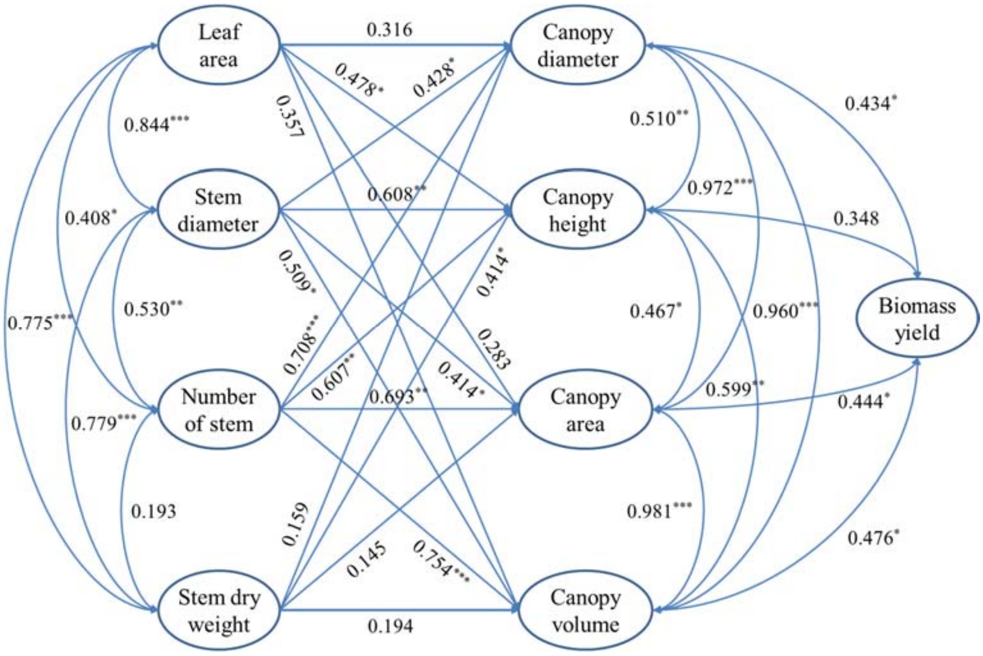


Figure 3-6. Association among the 3rd year agronomic traits, canopy structure parameters and biomass yield of *M. sinensis* ($2n = 2x = 38$). *, ** and *** indicate statistical significance at the 0.05, 0.01 and 0.001 levels, respectively.

M. sacchariflorus had more highly correlated agronomic traits with canopy structure parameters such as canopy diameter, canopy height, canopy area and canopy volume (Figure 3-7). Canopy height (r = 0.854, p < 0.001), canopy diameter (r = 0.791, p < 0.001), and canopy volume (r = 0.743, p < 0.01) were highly correlated with biomass yield of *M sacchariflorus*. In case of *M. sacchariflorus*, canopy height was strongly correlated with biomass yield. And the number of stem (r = 0.663, p < 0.01) also highly correlated with canopy height than other agronomic traits in *M. sacchariflorus*. This pattern also showed in association among the traits of *M. sinensis*. *M. sinensis* was also highly correlated with the number of stem which was important canopy structure to determine the biomass yield. It suggests that the number of stem is important agronomic traits to determine the main canopy structure which is to determine the biomass yield in *M. sinensis* and *M. sacchariflorus*.

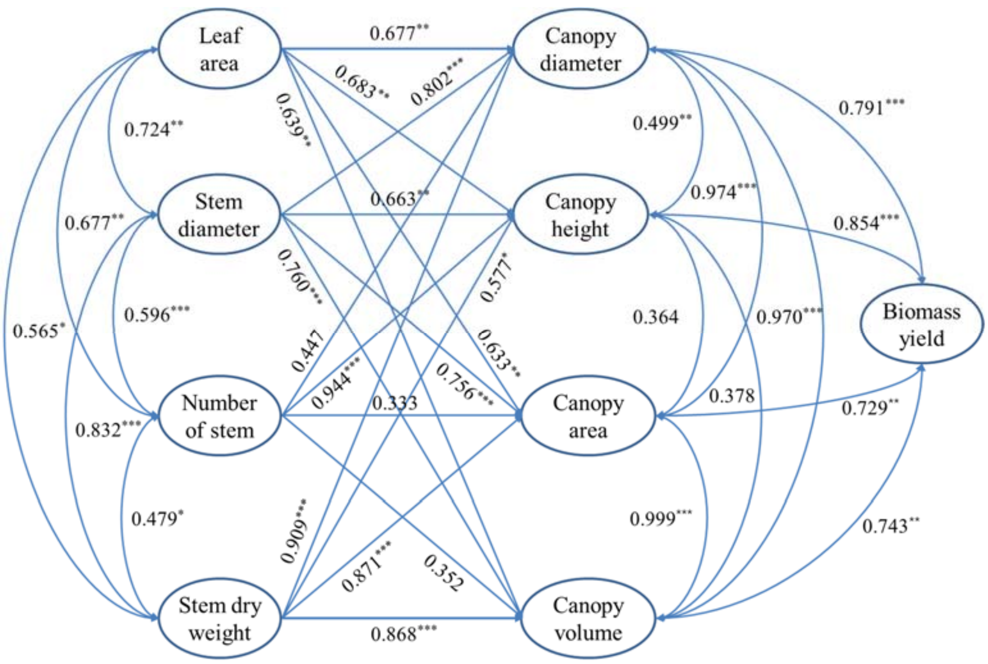


Figure 3-7. Association among the 3rd year agronomic traits, canopy structure parameters and biomass yield of *M. sacchariflorus* ($2n = 4x = 76$). *, ** and *** indicate statistical significance at the 0.05, 0.01 and 0.001 levels, respectively.

Biomass yield of hybrid *Miscanthus* was correlated with canopy diameter (r = 0.761, $p < 0.05$), canopy area (r = 0.749, $p < 0.05$), and canopy volume (r = 0.552, no significant) (Figure 3-8). Association among the traits of hybrid *Miscanthus* was showed differences than the other species. *M. sinensis* and *M. sacchariflorus* did not showing negative correlation between the each traits, while hybrid *Miscanthus* showed negative correlations between some of traits (Figure 3-8). Furthermore, key traits to determine the biomass yield of hybrid Miscanthus was canopy diameter which was highly correlated with leaf area (r = 0.729, $p < 0.05$) (Figure 3-8). The number of stem was key traits to determine the main canopy structure related with biomass yield in *M. sinensis* and *M. sacchariflorus*. However, leaf area was key traits to determine the main canopy structure related with biomass yield in hybrid *Miscanthus*.

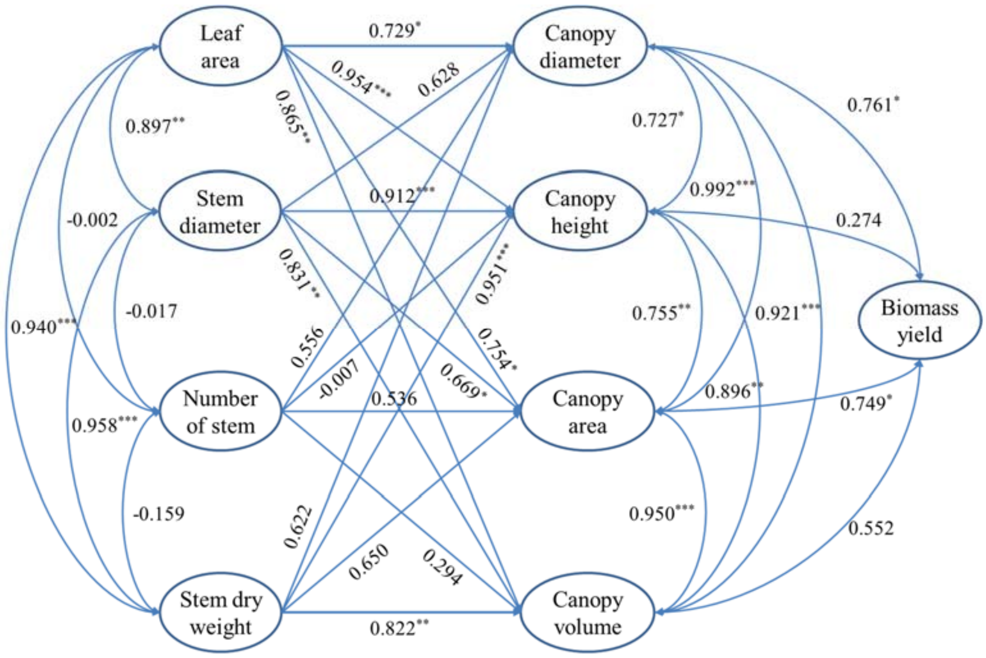


Figure 3-8. Association among the 3rd year agronomic traits, canopy structure parameters and biomass yield of hybrid *Miscanthus* ($2n = 3x = 57$). *, ** and *** indicate statistical significance at the 0.05, 0.01 and 0.001 levels, respectively.

Discussion

Relationship between species and canopy structure development

Through this study, we could understand canopy structure developments of *Miscanthus* species. Diverse parameters such as radius, stem height, the number of stem, and dry weight of stem were related with each canopy parameters. Furthermore, these canopy structure parameters showed differences among the species. *M. sinensis* had tufted stems because of its sympodial rhizome, while *M. sacchariflorus* scattered stems because of its monopodial and rhizomatous rhizome (Sun *et al.*, 2010). These morphological traits were affected to horizontal expanding such as radius (canopy diameter) and canopy area of *Miscanthus* species. Hybrid *Miscanthus* was showed intermediate canopy structure parameters between *M. sinensis* and *M. sacchariflorus*. Chae *et al.* (2014), Yook *et al.* (2014) and Lim *et al.* (2014) reported hybrid *Miscanthus* had intermediate morphological traits between *M. sinensis* and *M. sacchariflorus*. Interestingly, parameter related with biomass yield such as stem height, number of stems and stem dry weight of hybrid *Miscanthus* showed more vigorous than other species (Figure 3-5). Atkinson (2009) commented that diploid *M. sinensis* hybrid had the highest yields compared to *M.* × *giganteus*. To increase biomass yield efficiently, biomass yield per canopy area and canopy volume were very important. Among the *Miscanthus* species, canopy area and canopy volume of *M. sinensis* were lower than other species, while biomass yields per canopy area and canopy volume were higher than other species (Figure 3-5). It means that canopy structure of *M. sinensis* had an advantage than other species to produce more biomass yield in the same size of the field. It should be a useful target species and key parameters to breed new *Miscanthus* cultivar.

Relationship among the agronomic trait, canopy structure development and biomass yield

We found that canopy structure parameters were important traits to determine biomass yield of *Miscanthus*. To improve canopy structure parameters, we should understand the relationships between agronomic traits and canopy structure parameters. In case of *M. sinensis*, leaf area and stem diameter highly correlated with canopy structure parameters. Stem height and number of stems are included in canopy structure of *M. sinensis* also highly correlated with agronomic traits of *M. sinensis* (Figure 3-6). Clifton-Brown *et al.* (2013) reported that *M. sinensis* had wide ranges in stem height and stem diameter which related with biomass yield potential. The more complex correlation of agronomic traits of *M. sacchariflorus* with canopy structure parameters was observed. Leaf area and stem diameter strongly correlated with canopy structure parameters. Clifton-Brown *et al.* (2013) also commented that *M. sacchariflorus* showed enormous phenotypic variation. Hybrid *Miscanthus* was showed intermediate characteristics between *M. sinensis* and *M. sacchariflorus*. The number of stem and total dry weight of hybrid *Miscanthus* did not showing correlation with any agronomic traits. It would be caused by hybrid vigor. The number of stem of hybrid *Miscanthus* was rapidly increased through planting year. Interestingly, the number of stem per canopy area, biomass per canopy area, and biomass per canopy volume showed difference among the species, while these parameters did not showing any correlation with agronomic trait and biomass yield. Biomass per canopy area and canopy volume were important parameters determining the biomass yield of each species. Robson *et al.* (2012, 2013a and 2013b) and Clifton-Brown *et al.* (2013) reported that biomass yield is a complex trait and can be considered as either the composite of several agronomic traits or the result of various agronomic traits. Interestingly, canopy volume followed by canopy diameter and canopy area were the most determinant of biomass yield in *M. sinensis*, while canopy height followed by canopy diameter and canopy volume in *M. sacchariflorus* and canopy area followed by canopy diameter and canopy volume in hybrid *Miscanthus* were the most determinant of biomass yield, respectively. Our results indicated that these canopy structure parameters such as maximum radius (canopy diameter), canopy height, canopy area, and canopy volume related with biomass yield were strongly correlated with the number of stem, leaf area, and stem diameter as agronomic traits can be key traits to determine biomass yield and target traits to breed new *Miscanthus* cultivar.

References

Atkinson CJ. 2009. Establishing perennial grass energy crops in the UK: A review of current propagation options for *Miscanthus*. Biomass and Bioenergy **33**: 752-759.

Chae WB, Hong SJ, Gifford JM, Rayburn AL, Sacks EJ, Juvik JA. 2014. Plant morphology, genome size, and SSR markers differentiate five taxonomic groups among accessions in the genus *Miscanthus*. Global Change Biology Bioenergy **6(6)**: 646-660.

Christian DG, Yates NE, Riche AB. 2005. Establishing *Miscanthus sinensis* from seed using conventional sowing methods. Industrial Crops and Products **21**: 109-111.

Chung JH, Kim DS. 2012. *Miscanthus* as a potential bioenergy crop in East Asia. Journal of Crop Science and Biotechnology **15**: 65-77.

Clifton-Brown JC, Lewandowski I. 2002. Screening *Miscanthus* genotypes in field trials to optimize biomass yield and quality in southern Germany. European Journal of Agronomy **16**: 97-110.

Clifton-Brown J, Robson P, Davey C, Farrar K, Hayes C, Huang L, Jensen E, Jones L, Hinton-Jones M, Maddison A. 2013. Breeding Miscanthus for bioenergy. Bioenergy Feedstocks: Breeding and Genetics 67-81

Ehlert D, Horn HJ, Adamek R. 2008. Measuring crop biomass density by laser triangulation. Computers and Electronics in Agriculture **61(2)**: 117-125.

Ehlert D, Dammer KH. 2006. Widescale testing of the Crop-meter for site-specific farming. Precision Agriculture, http://dx.doi.org/10.1007/s11119-006-9003-z.

Hodkinson TR, Renvoize S. 2001. Nomenclature of *Miscanthus* × *giganteus* (*Poaceae*). Kew Bulletin **56**: 759-760.

Hodkinson TR, Chase MW, Takahashi C, Leitch IJ, Bennett MD, Renvoize SA. 2002. The use of DNA sequencing (ITS and trnL-F), AFLP, and fluorescent in situ hybridization to study allopolyploid *Miscanthus* (*Poaceae*). America Journal of Botany **89**: 279-286.

Jezowski S, Glowacka K, Kaczmarek Z. 2011. Variation on biomass yield and morphological traits of energy grasses from the genus *Miscanthus* during the first years of crop establishment. Biomass and Bioenergy **35**: 814-821.

Jørgensen U, Muhs HJ. 2001. *Miscanthus* breeding and improvement. In: Jones MB WM, editor. *Miscanthus* for Energy and Fibre. London, UK: James & James Ltd., p. 68-85.

Kim DS, Marshall EJP, Brain P, Caseley JC. 2011. Effects of crop canopy structure on herbicide deposition and performance. Weed Research **51**: 310-320

Lafferty J, Lelley T. 1994. Cytogenetic studies of different *Miscanthus* species with potential for agricultural use. Plant Breeding **113**: 246-249.

Lim SH, Yook MJ, Kim JW, Song JS, Zhang CJ, Nah GJ, Kim DS. 2014. Genetic diversity in agronomic traits associated with the biomass production of *Miscanthus* species collected in Northeast Asia. Plant Genetic Resources **12**: S137-S140.

Price L, Bullard M, Lyons H, Anthony S, Nixon P. 2004. Identifying the yield potential of *Miscanthus* × *giganteus*: an assessment of the spatial and temporal variability of *M*. × *giganteus* biomass productivity across England and Wales. Biomass and Bioenergy **26**: 3-13.

Redfearn DD, Moore KJ, Vogel KP, Waller SS, and Mitchell RB. 1997. Canopy architecture and morphology of switchgrass populations differing in forage yield. Agronomy Journal **89(2)**: 262-269.

Robson P, Farrar K, Gay AP, Jensen E, Clifton-Brown J, Donnison I. 2013a. Variation in canopy duration in the perennial biofuel crop *Miscanthus* reveals complex associations with yield. Journal of Experimental Botany **64**: 2373-2383.

Robson P, Mos M, Clifton-Brown J, Donnison I. 2012. Phenotypic Variation in Senescence in *Miscanthus*: Towards Optimising Biomass Quality and Quantity. Bioenergy Research **5**: 95-105.

Robson P, Jensen E, Hawkins S, White SR, Kenobi K, Clifton-Brown J, Donnison I, Farrar K. 2013b. Accelerating the domestication of a bioenergy crop: identifying and modelling morphological targets for sustainable yield increase in *Miscanthus*. Journal of Experimental Botany **64**: 4143-4155.

Russell G, Marshall B, Jarvis PG. 1990. Plant canopies: their growth, form and function: Cambridge University Press.

Sun Q, Lin Q, Yi Z, Yang Z, Zhou F. 2010. A taxonomic revision of *Miscanthus* s. l. (*Poaceae*) from China. Botanical Journal of the Linnean Society **164**: 178–220.

Yan J, Chen WL, Luo F, Ma HZ, Meng AP, Li XW, Zhu M, Li SS, Zhou HF, Zhu WX, Han B, Ge S, Li JQ, Sang T. 2012. Variability and adaptability of *Miscanthus* species evaluated for energy crop domestication. Global Change Biology Bioenergy **4**: 49-60.

Yook MJ, Lim SH, Song JS, Kim JW, Zhang CJ, Lee EJ, Ibaragi Y, Lee GJ, Nah GJ, Kim DS. 2014. Assessment of genetic diversity of Korean *Miscanthus* using morphological traits and SSR markers. Biomass and Bioenergy **66**: 81-92.

Zub HW, Arnoult S, Brancourt-Hulmel M. 2011. Key traits for biomass production identified in different *Miscanthus* species at two harvest dates. Biomass and Bioenergy **35**: 637-651.

APPENDIX 1. Summary of morphological trait in *Miscanthus* accessions.

Organs	Traits	Pattern	State (score or unit)
Leaf	Leaf cross section shape		Flat (0) Flat-rolled (1) Flat-v shape (2) V-shape (3)
	Ligule hair		No ligule hair (0) Little ligule hair (1) Mid ligule hair (2) Many ligule hair (3)
	Auricle		Absent (0)/ present (1)
	Leaf hair		Absent (0)/ present (1)
	Collar hair		Absent (0)/ present (1)
	Leaf sheath hair		Absent (0)/ present (1)
	Leaf dry weight at harvest		Dry weight of leaf blade and leaf sheath at harvest (g)
Culm	Growth habit		Bunch type (0) Scattered type (1) Intermediate type (2)

APPENDIX 1. Continued

Organs	Traits	Pattern	State (score or unit)
Cukm	Branch Stem diameter		Absent (0)/ present (1) Stem diameter assessed in the 3rd year after planting (mm)
	New shoot emergence		Absent (0)/ present (1)
Floret	Awn length		Awn length assessed in the 3rd year after planting (mm)
	No. of stigma and anther /color		Number of stigma and anther Stigma (purple) Anther (yellow)
Panicle	Panicle shape Panicle length		Diamond-shape (0) Flat (1) Rolled (2) Sector-shape (3) Assessed in the 3rd year after planting (cm)
Spikelet	Ratio between callus hair and spikelet		Assessed in the 3rd year after planting Callus hair/spikelet
	Touch of callus hair		Silky(0)/ rough (1)

APPENDIX 2-1. Range of variation assessed in the 1st year phenological traits and agronomic traits of *Miscanthus* accessions.

Traits	Unit	*M. sinensis* (n = 181)				*M. sacchariflorus* (2n; n = 32)				*M. sacchariflorus* (4n; n = 57)				*M.* × *giganteus*
		Min	Max	Mean	Median	Min	Max	Mean	Median	Min	Max	Mean	Median	
Shoot Emergence	Julian date	66.0	156.0	97.1	93.0	85.0	109.0	102.4	104.0	93.0	110.0	103.8	104.0	115.0
Heading date	Julian date	178.0	283.0	239.8	244.0	181.0	257.0	212.5	192.5	239.0	271.0	251.4	251.0	261.5
Flag leaf area	cm^2	0.6	148.5	9.2	6.6	0.9	28.6	5.4	2.0	0.7	38.4	8.1	6.1	28.0
2nd leaf area	cm^2	7.0	117.6	39.3	36.4	3.4	92.4	22.8	17.3	8.6	96.9	35.6	33.6	79.1
3rd leaf area	cm^2	9.0	128.9	47.8	44.0	3.9	96.8	25.2	18.7	12.1	120.0	42.8	37.1	94.2
Total leaf area	cm^2	16.6	299.5	96.4	90.2	8.8	211.1	53.4	38.9	24.5	221.1	86.6	76.7	201.4
Stem height	cm	35.0	205.0	128.4	125.0	60.0	180.0	114.5	111.3	95.0	175.0	127.3	125.0	160.0
No. of stem (0.01 m^2)	Number	1.0	26.5	6.3	5.0	1.0	40.0	8.9	5.8	2.7	28.0	10.3	9.0	3.4
Total stem DW	g $stem^{-1}$	0.8	41.3	12.8	10.7	1.3	13.2	4.5	3.5	1.8	37.1	7.4	6.1	16.7
Stem diameter	mm	0.8	5.5	3.4	3.4	1.7	3.8	2.4	2.3	1.8	5.2	3.0	3.0	4.7
Stem density	g cm^{-3}	0.2	0.8	0.4	0.4	0.3	0.8	0.5	0.5	0.3	0.8	0.5	0.5	0.4
Estimated yield	Mg ha^{-1}	0.01	7.8	1.1	0.8	0.04	4.4	0.7	0.3	0.1	5.6	1.0	0.7	0.8

APPENDIX 2-2. Range of variation assessed in the 3rd year phenological traits and agronomic traits of *Miscanthus* accessions.

Traits	Unit	*M. sinensis* (n = 181)				*M. sacchariflorus* (2n; n = 32)				*M. sacchariflorus* (4n; n = 57)				*M.* × *giganteus*
		Min	Max	Mean	Median	Min	Max	Mean	Median	Min	Max	Mean	Median	
Shoot Emergence	Julian date	79.0	91.0	87.5	88.0	83.0	89.0	87.0	87.0	85.0	91.0	88.0	88.0	89.0
Heading date	Julian date	178.0	275.0	234.3	239.0	179.0	251.0	209.1	199.0	231.0	265.0	245.1	244.0	255.8
Flag leaf area	cm^2	1.5	77.9	16.9	13.2	2.2	44.2	9.0	5.2	1.8	31.1	12.6	11.4	48.2
2nd leaf area	cm^2	9.2	241.1	107.7	104.7	27.7	130.1	65.4	62.1	64.0	192.7	100.2	94.3	187.7
3rd leaf area	cm^2	11.1	234.7	114.2	110.3	24.5	129.0	68.1	61.1	73.3	190.8	104.8	101.1	186.7
Total leaf area	cm^2	21.7	553.7	238.8	225.0	67.0	268.3	142.5	128.9	145.4	388.8	217.6	207.5	422.6
Stem height	cm	46.7	302.7	200.5	205.7	143.3	265.7	193.7	190.8	150.0	303.3	227.1	224.0	362.0
No. of stem (0.01 m^2)	Number	3.0	30.0	9.7	9.0	6.0	24.0	9.6	6.0	3.0	18.0	10.5	11.0	8.8
Total stem DW	g $stem^{-1}$	1.5	71.8	22.6	19.9	2.6	21.0	7.8	6.1	5.9	71.9	17.2	14.7	52.5
Stem diameter	mm	1.4	10.1	6.0	6.0	3.0	6.8	4.2	4.0	3.6	9.0	5.3	5.1	8.4
Stem density	g cm^{-3}	0.1	0.6	0.3	0.2	0.2	0.4	0.3	0.3	0.2	0.5	0.3	0.3	0.2
Estimated yield	Mg ha^{-1}	0.2	29.8	8.2	7.6	1.3	17.9	5.5	3.1	2.4	27.4	11.0	10.6	11.5

APPENDIX 2-3. Summary of correlation analyses among the 1st year agronomic traits, the 4th year biomass yield and latitude of collection site of *M. sinensis and M. sacchariflorus* accessions.

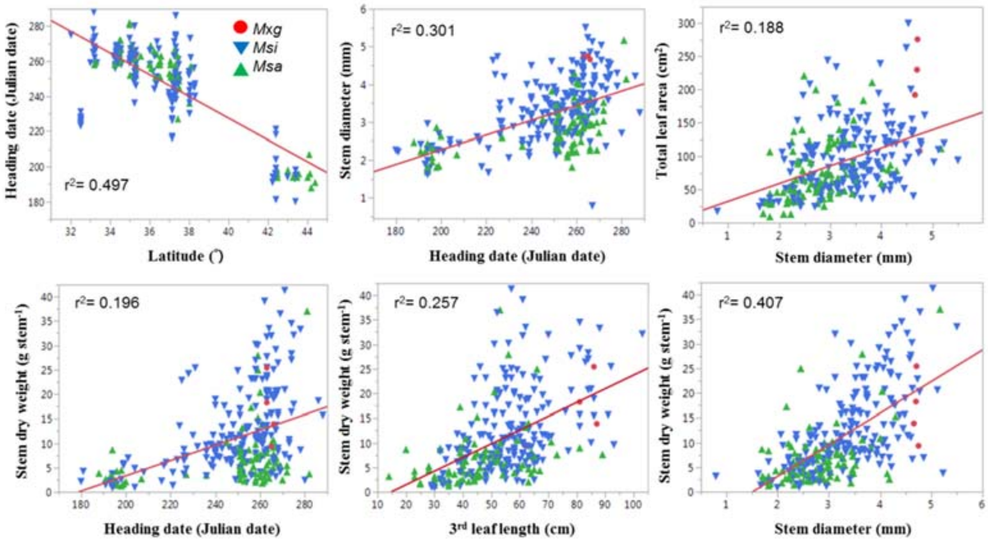
Traits	Latitude	Heading date	2nd leaf length	3rd leaf length	Total leaf area	Plant height	Stem dry weight	Stem diameter	Estimated yield
M. sinensis (*n* = 181)									
Heading date	-0.666**	1							
2nd leaf length	-0.457**	0.327**	1						
3rd leaf length	-0.442**	0.363**	0.915**	1					
Total leaf area	-0.263**	0.335**	0.959**	0.939**	1				
Stem height	-0.241**	0.240**	0.260**	0.301**	0.322**	1			
Stem dry weight	-0.490**	0.547**	0.369**	0.419**	0.387**	0.231**	1		
Stem diameter	-0.606**	0.564**	0.361**	0.350**	0.351**	0.259**	0.633**	1	
4th year biomass yield	-0.357**	0.346**	0.212**	0.238**	0.231**	0.346**	0.339**	0.223**	1
M. sacchariflorus (*n* = 89)									
Heading date	-0.837**	1							
2nd leaf length	-0.513**	0.523**	1						
3rd leaf length	-0.511**	0.555**	0.921**	1					
Total leaf area	-0.444**	0.462**	0.862**	0.883**	1				
Stem height	-0.491**	0.539**	0.548**	0.520**	0.437**	1			
Stem dry weight	-0.284**	0.315**	0.261**	0.250**	0.226**	0.300**	1		
Stem diameter	-0.577**	0.553**	0.477**	0.394**	0.392**	0.505**	0.453**	1	
4th year biomass yield	-0.329**	0.356**	0.430**	0.422**	0.313**	0.490**	0.749**	0.292**	1
Pooled (*n* = 270, excluding *M. × giganteus*)									
Heading date	-0.731**	1							
2nd leaf length	-0.494**	0.461**	1						
3rd leaf length	-0.485**	0.494**	0.911**	1					
Total leaf area	-0.344**	0.379**	0.830**	0.786**	1				
Stem height	-0.332**	0.329**	0.415**	0.426**	0.355**	1			
Stem dry weight	-0.442**	0.448**	0.435**	0.451**	0.356**	0.291**	1		
Stem diameter	-0.597**	0.534**	0.531**	0.484**	0.405**	0.322**	0.639**	1	
4th year biomass yield	-0.351**	0.351**	0.326**	0.319**	**0.243****	0.347**	0.545**	0.301**	1

APPENDIX 2-4. Summary of correlation analyses among the 3rd year agronomic traits, the 4th year biomass yield and latitude of collection site of *M. sinensis and M. sacchariflorus* accessions.

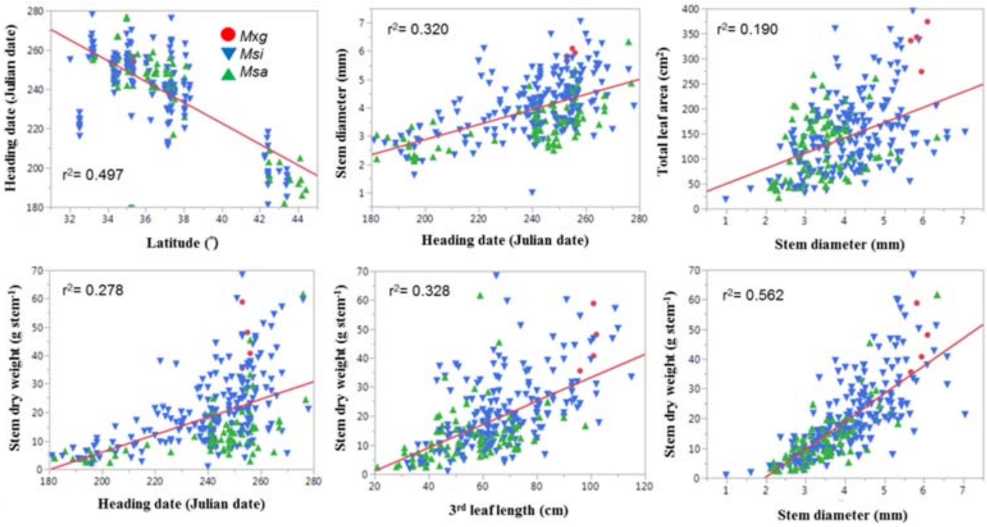
Traits	Latitude	Heading date	2nd leaf length	3rd leaf length	Total leaf area	Plant height	Stem dry weight	Stem diameter	Estimated yield
M. sinensis (*n* = 181)									
Heading date	-0.674**	1							
2nd leaf length	-0.608**	0.598**	1						
3rd leaf length	-0.600**	0.574**	0.951**	1					
Total leaf area	-0.473**	0.607**	0.983**	0.978**	1				
Stem height	-0.211**	0.318**	0.401**	0.408**	0.400**	1			
Stem dry weight	-0.575**	0.561**	0.737**	0.739**	0.748**	0.322**	1		
Stem diameter	-0.604**	0.600**	0.730**	0.722**	0.744**	0.421**	0.866**	1	
4th year biomass yield	-0.459**	0.377**	0.419**	0.425**	0.444**	0.370**	0.435**	0.381**	1
M. sacchariflorus (*n* = 89)									
Heading date	-0.823**	1							
2nd leaf length	-0.539**	0.541**	1						
3rd leaf length	-0.515**	0.503**	0.951**	1					
Total leaf area	-0.683**	0.655**	0.826**	0.857**	1				
Stem height	-0.425**	0.344**	0.177*	0.226*	0.414**	1			
Stem dry weight	-0.502**	0.522**	0.600**	0.615**	0.700**	0.415**	1		
Stem diameter	-0.597**	0.605**	0.680**	0.684**	0.721**	0.288**	0.882**	1	
4th year biomass yield	-0.516**	0.524**	0.453**	0.444**	0.468**	0.227*	0.665**	0.612**	1
Pooled (*n* = 270, excluding *M.* × *giganteus*)									
Heading date	-0.732**	1							
2nd leaf length	-0.583**	0.514**	1						
3rd leaf length	-0.568**	0.494**	0.966**	1					
Total leaf area	-0.540**	0.590**	0.829**	0.819**	1				
Stem height	-0.260**	0.288**	0.188**	0.173**	0.339**	1			
Stem dry weight	-0.550**	0.523**	0.666**	0.662**	0.687**	0.342**	1		
Stem diameter	-0.599**	0.566**	0.758**	0.757**	0.763**	0.297**	0.884**	1	
4th year biomass yield	-0.458**	0.498**	0.428**	0.432**	0.450**	0.350**	0.552**	0.434**	1

APPENDIX 2-5. Summary of correlation analyses among the 4th year agronomic traits, the 4th year biomass yield and collection site latitude of *M. sinensis and M. sacchariflorus* accessions.

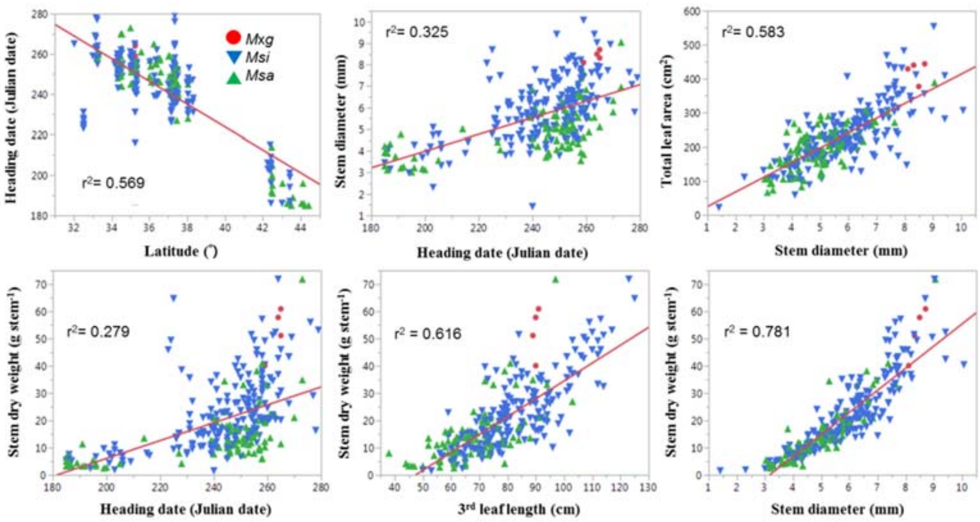
Traits	Latitude	Heading date	2nd leaf length	3rd leaf length	Total leaf area	Plant height	Stem dry weight	Stem diameter	Estimated yield
M. sinensis (*n* = 181)									
Heading date	-0.674**	1							
2nd leaf length	-0.605**	0.598**	1						
3rd leaf length	-0.597**	0.574**	0.951**	1					
Total leaf area	-0.484**	0.607**	0.983**	0.978**	1				
Stem height	-0.406**	0.318**	0.401**	0.408**	0.400**	1			
Stem dry weight	-0.551**	0.561**	0.737**	0.739**	0.748**	0.322**	1		
Stem diameter	-0.577**	0.600**	0.730**	0.722**	0.744**	0.421**	0.866**	1	
Estimated yield	-0.433**	0.377**	0.419**	0.425**	0.444**	0.370**	0.435**	0.381**	1
M. sacchariflorus (*n* = 89)									
Heading date	-0.787**	1							
2nd leaf length	-0.488**	0.555**	1						
3rd leaf length	-0.457**	0.503**	0.954**	1					
Total leaf area	-0.629**	0.626**	0.846**	0.871**	1				
Stem height	-0.581**	0.522**	0.632**	0.640**	0.666**	1			
Stem dry weight	-0.546**	0.548**	0.585**	0.609**	0.759**	0.734**	1		
Stem diameter	-0.570**	0.552**	0.712**	0.717**	0.801**	0.754**	0.939**	1	
Estimated yield	-0.599**	0.545**	0.351**	0.339**	0.439**	0.317**	0.460**	0.368**	1
Pooled (*n* = 270, excluding *M.* × *giganteus*)									
Heading date	-0.714**	1							
2nd leaf length	-0.568**	0.504**	1						
3rd leaf length	-0.552**	0.481**	0.968**	1					
Total leaf area	-0.531**	0.552**	0.836**	0.821**	1				
Stem height	-0.429**	0.405**	0.438**	0.405**	0.476**	1			
Stem dry weight	-0.551**	0.544**	0.638**	0.641**	0.738**	0.632**	1		
Stem diameter	-0.582**	0.549**	0.743**	0.744**	0.768**	0.576**	0.911**	1	
Estimated yield	-0.482**	0.415**	0.430**	0.423**	0.453**	0.367**	0.468**	0.407**	1



APPENDIX 2-6. Relationship between main agronomic traits assessed in the 1st year after *Miscanthus* planting.



APPENDIX 2-7. Relationship between main agronomic traits assessed in the 2nd year after *Miscanthus* planting.



APPENDIX 2-8. Relationship between main agronomic traits assessed in the 3rd year after *Miscanthus* planting.

ABSTRACT IN KOREAN

한국 억새의 유전적 다양성과 바이오매스 수량 잠재성

임수현

작물생명과학전공

식물생산과학부

서울대학교 농업생명과학대학

억새는 국내 자생 다년생 C_4 식물로서 높은 바이오매스 수량성과 함께 환경 적응성이 높아 유럽과 미국에서는 바이오에너지 작물로서 활발히 연구되고 있다. 그러나 억새의 자생지로 서 다양한 억새 유전자원을 가지고 있는 한국에서는 억새를 바이오에너지용 작물로서의 연구는 전무한 실정이다. 따라서 본 연구는 국내 및 동아시아지역에서 수집된 참억새(*M. sinensis*)와 물억새(*M. sacchariflorus*)를 경기도 수원과 여주에 위차한 서울대학교 농업생명과학대학 억새유전자원포장에 재식하고 4년에 걸쳐 한국 억새의 형태적 특성, 농업적 형질 등을 평가하여 한국 억새의 유전적 다양성 및 바이오매스 수량성을 평가하고자 실시되었다.

형태적 특성을 통한 유전적 다양성 평가를 위해 한국을 비롯한 중국, 일본과 러시아에서 수집된 184종의 참억새 수집종과 93종의 물억새 수집종을 비롯한 277종의 억새유전자원이 사용되었으며 비교종으로 *M. × giganteus*, *M. floridulus*와 *M. lutarioriparius* 가 사용되었다. 잎, 줄기,

화기 및 이삭과 관련된 20개의 형태적 특성을 조사하여 계통분석을 수행하였다. 그 결과 영화에서 까락의 유무, 줄기생장특성, 수확기 새싹 출아와 소수와 기모의 길이 비율이 참억새와 물억새를 구분짓는 핵심 형질로 확인되었으며, 그 외 다른 형질들은 종속 또는 같은 그룹내에서 유전적 다양성을 보였다. 참억새와 물억새 각각 5개의 그룹으로 분류되었으며 *M. floridulus* 는 참억새 IIa 그룹에 *M.* × *giganteus* 와 *M. lutarioriparius* 는 물억새 V 그룹에 포함되는 것을 확인할 수 있었다. 주좌표 분석을 통하여 참억새 그룹과 물억새 그룹 사이에 위치한 7종의 참억새와 17종의 물억새를 포함한 24종의 중간형 억새 수집종을 찾아낼 수 있었다. 24종의 중간형 억새들은 참억새와 물억새의 형태적 특성의 중간형을 보이고 있었으며, 염색체 수 조사 결과 1종의 참억새와 4종의 물억새에서 3배체를 확인할 수 있었는 데 이는 참억새와 물억새의 자연교잡에 의한 것으로 생각된다.

바이오매스 수량과 관련된 기후학적 형질, 농업적 형질들의 상호연관성 평가가 2010년부터 4년간 수원에 위치한 억새 유전자원 포장에서 수행되었다. 기후학적 형질, 농업적 형질, 바이오매스 수량과 억새가 수집된 지역의 위도간의 상관분석 결과 출수기, 잎 생육 형질, 줄기 생육 형질이 바이오매스 수량과 밀접한 연관성이 있는 것으로 확인되었다. 수집지 위도는 출수기와 정의 상관관계를 보였으며, 출수기는 바이오매스 수량과 정의 상관관계를 보였다. 수집지의 위도와 농업적 형질들간의 유의적 관계는 서로 다른 지역에서 수집된 억새유전자원들의 육종을 위한 다양한 유전적 재료를 확보할 수 있다. 줄기에서 최상위 잎들의 (지엽, 2번째 잎, 3번째 잎) 형질은 줄기두께와 줄기건물중과 정의 상관관계를 보였으며, 이는 바이오매스 수량과도 정의 상관관계를 보였다. 또한 2년차 재배된 억새의 농업적 형질들은 4년차 바이오매스 수량과 정의 상관관계

를 보였다. 특히, 2년차 억새의 엽면적, 줄기두께와 줄기 건물중은 4년차 바이오매스 수량과 유의한 상관관계를 보였으며, 이는 2년차에 조사된 농업적 형질을 통해 억새 유전자원들의 높은 바이오매스 수량성을 예측하여 스크리닝 할 수 있음을 시사한다. 일반적으로 기후학적 형질들과 농업적 형질과 바이오매스 수량과의 연관성이 있음에도 불구하고 가끔 낮은 상관관계가 관측되기도 하는데 이는 연구에 수행된 억새 수집종들의 바이오매스 수량 잠재성과 관련된 기후학적 형질 및 농업적 형질들이 다른 형태로 연관이 되면서 높은 유전적 다양성에 기인한다는 것을 내포하고 있다.

억새의 바이오매스 형성과 높은 바이오매스 수량을 위한 육종에서의 이상적인 초관의 확립을 위해서 2012년부터 3년간 경기도 여주에 위치한 억새유전자원 포장에서 참억새 8종, 물억새 6종 3배체 억새 3종을 포함한 억새 수집종 17종의 초관구조형성 연구가 수행되었다. 매년 초관의 지름, 초관의 높이, 초관 면적 및 부피와 함께 개체당 줄기개수와 줄기 건물중이 조사되었으며, 초관 면적당 줄기개수, 초관 면적당 줄기 건물중 및 초관 부피당 줄기건물중이 계산되었다. 초관구조와 관련된 모든 변수들의 선형회귀분석을 통한 연간 초관구조 생장률이 예측되었다. 물억새와 3배체 억새들의 뿌리줄기가 빠르게 생장하는데 비해 참억새의 경우 뿌리줄기의 생육특성에 의해 상대적으로 초관 면적 및 부피의 발달이 느린 것을 확인할 수 있었다. 단위 면적 및 부피당 바이오매스 축적의 경우 참억새의 밀집된 생육특성에 의해 물억새보다 바이오매스 밀도가 높은 것을 확인 할 수 있었고, 3배체 억새는 참억새와 물억새의 중간적인 형태인 것을 확인할 수 있었다. 이는 초관발달 형질들이 억새 종에 의존하여 서로 다른 특징을 보이며 이를 통해 억새 종을 식별하는데 유용한 지표가 될 것으로 생각된다. 초관형성 형질들과 농업적 형질들의

상관관계 분석을 통하여 초관 지름, 초관 높이, 초관 면적 및 부피가 바이오매스 수량과 유의적 연관성이 높은 것을 확인할 수 있었으나, 유의성 정도는 억새 종에 따라 상이한 것을 확인할 수 있었다. 참억새의 초관 부피는 초관 지름과 초관 면적과 함께 바이오매스 수량을 결정 짓는 요인으로 나타났고, 반면에 물억새의 경우 초관 높이가 초관 지름과 초관 부피와 함께 바이오매스를 결정짓는 요인으로 확인되었다. 3배체 억새는 초관 지름이 초관 면적과 초관 부피와 함께 바이오매스를 결정짓는 요인으로 확인되었다. 이를 통해 참억새와 3배체 억새의 바이오매스 생산성은 초관구조의 수평적 발달이 중요하고, 물억새의 경우에는 초관의 수직적 확장이 중요함을 알 수 있다. 따라서, 군락형성의 변수들의 도입 및 조사는 높은 바이오매스 생산성을 가진 새로운 억새 품종 육종을 위한 억새의 이상적인 초관확립을 위한 단서를 제공했다.

주요어: 농업적형질, 바이오매스 수량, 억새, 유전적 다양성, 육종, 초관구조형성, 형태적 형질

학번: 2008-21273