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Biomass yield in a genetically diverse *Miscanthus sinensis* germplasm panel evaluated at five locations revealed individuals with exceptional potential

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Abstract

To breed improved biomass cultivars of *Miscanthus × giganteus*, it will be necessary to select the highest-yielding and best-adapted genotypes of its parental species, *Miscanthus sinensis* and *Miscanthus sacchariflorus*. We phenotyped a diverse clonally propagated panel of 569 *M. sinensis* and nine natural diploid *M. × giganteus* at one subtropical (Zhuji, China) and five temperate locations (Sapporo, Japan; Leamington, Ontario, Canada; Fort Collins, CO; Urbana, IL; and Chuncheon, Korea) for dry biomass yield and 14 yield-component traits, in trials grown for 3 years. Notably, dry biomass yield of four *Miscanthus* accessions exceeded 80 Mg/ha in Zhuji, China, approaching the highest observed for any land plant. Additionally, six *M. sinensis* in Sapporo, Japan and one in Leamington, Canada also yielded more than the triploid *M. × giganteus* ‘1993-1780’ control, with values exceeding 20 Mg/ha. Diploid *M. × giganteus* was the best-yielding group at the northern sites. Genotype-by-environment interactions were modest among the five northern trial sites but large between Zhuji, and the northern sites. *M. sinensis* accessions typically yielded best at trial sites with latitudes similar to collection sites, although broad adaptation was

observed for accessions from southern Japan. Genotypic heritabilities for third year yields ranged from 0.71 to 0.88 within locations. Compressed circumference was the best predictor of yield. These results establish a baseline of data for initiating selection to improve biomass yield of *M. sinensis* and *M. ×giganteus* in a diverse set of relevant geographies.

KEYWORDS

biomass yield, genotype-by-environment effects, germplasm, *Miscanthus ×giganteus*, *Miscanthus sinensis*, multi-location field trials

1 | INTRODUCTION

Miscanthus is a C₄ perennial grass native to East Asia, and a promising biomass crop for many applications, including lignocellulosic ethanol production, gasification or direct combustion to generate electricity or heat, producing paper, building materials, biodegradable plastic, animal bedding, mulch, and livestock feed (Acikel, 2011; Clifton-Brown & Lewandowski, 2002; Heaton, Clifton-Brown, Voigt, Jones, & Long, 2004; Johnson, Tucker, Barnes, & Kirwan, 2005; Sacks, Juvik, Lin, Stewart, & Yamada, 2013). All of these applications depend on high-yielding cultivars in order to be commercially viable. Nearly all *Miscanthus* biomass production currently uses a single high-yielding clone of *M. ×giganteus* (Głowacka et al., 2015), despite its insufficient winter hardiness in USDA zone 5b environments and colder (<−26.1°C average annual minimum air temperature; Dong, Green et al., 2018), as well as the risk of disease and pest susceptibility associated with monoculture (Ahonsi et al., 2010; Arnoult & Brancourt-Hulmel, 2015; Bradshaw, Prasifka, Steffey, & Gray, 2010; Clifton-Brown & Lewandowski, 2002; Prasifka et al., 2009). We refer to this clone as *M. ×giganteus* ‘1993-1780’ after the accession number of the type specimen at Kew Royal Botanic Gardens Herbarium (Hodkinson & Renvoize, 2001); it is also commonly referred to as *M. ×giganteus* ‘Illinois’ in North America (Głowacka et al., 2015). Although *M. ×giganteus* ‘1993-1780’ is a triploid, the species name can refer to hybrids of any ploidy between *Miscanthus sinensis* and *Miscanthus sacchariflorus* (McNeill et al., 2012; Sacks et al., 2013).

Of all temperate-adapted *Miscanthus* species, *M. sinensis* has the broadest native range (Clifton-Brown, Chiang, & Hodkinson, 2008; Dwiyantri, Stewart, & Yamada, 2013; Sacks et al., 2013), suggesting high genetic diversity for environmental adaptation. In previous population genetics studies, we identified six major genetic groups of *M. sinensis*, with three in China, one in China and Korea, and two in Japan (Clark et al., 2014), with the South Japan (S Japan) group being further subdivided into S Japan and Central Japan in a subsequent study (Clark et al., 2015). Ornamental *M. sinensis*

cultivars represent a narrow portion of the genetic diversity of the species, originating almost exclusively from two small regions in southern Japan with subsequent introgression from diploid *M. ×giganteus* ‘Purpurascens’ in about half the cultivars currently marketed (Clark et al., 2014, 2015, 2018). US-naturalized *M. sinensis* were derived from non-admixed ornamentals (Clark et al., 2014, 2015). Despite this narrow genetic base, and artificial selection for short stature (Kaiser, Clark, Juvik, Voigt, & Sacks, 2015), ornamental *M. sinensis* have been used in the breeding of new biomass cultivars simply due to their availability in Europe and North America (Clifton-Brown et al., 2008). However, it is also widely recognized that better yields can be obtained with a broader germplasm base than that represented by the ornamental cultivars (Clark et al., 2014; Clifton-Brown et al., 2001, 2008; Jensen et al., 2011). The selection of high-yielding *M. sinensis* clones derived from crosses indicates the potential for yield improvement through breeding (Arnoult & Brancourt-Hulmel, 2015; Clifton-Brown et al., 2001).

To date, few studies have evaluated large, diverse germplasm panels of *M. sinensis* for yield, and these field trials were conducted only at single locations (Nie et al., 2016; Slavov et al., 2014; Zhao et al., 2013). In contrast to the single-location studies, previous multi-location trials of *M. sinensis* have included relatively few genotypes, yet consistently found significant genotype-by-environment (G × E) effects on yield (Arnoult & Brancourt-Hulmel, 2015; Clifton-Brown et al., 2001; Kaiser et al., 2015; Yan et al., 2012).

In the current study, we present field evaluations of the largest and most genetically diverse panel of *M. sinensis* evaluated to date, with phenotypic data from three field trial locations in East Asia and three in North America. In particular, we phenotyped 569 *M. sinensis* genotypes previously characterized for population structure (Clark et al., 2014), representing six genetic groups from nearly the entirety of the species’ natural geographic range. Biomass yield and 14 yield-component traits of *M. sinensis* were studied to (a) determine the range of genotypic diversity for yield in this species, and how performance varied with location of origin and genetic group, (b) quantify G × E effects and how well

performance at one trial site predicted performance at other trial sites, and (c) identify yield-component traits that are strong predictors of yield.

2 | MATERIALS AND METHODS

2.1 | Plant materials and field trials

In total, 589 *Miscanthus* accessions were studied (Data S1 and S2). We previously assigned the 566 *M. sinensis* and three *M. floridulus* genotypes evaluated in the current study to one of the eight genetic groups (hereafter referred to as *M. sinensis* for a total of 569 genotypes) (Clark et al., 2014): 77 Ornamental, 38 US naturalized, 28 S Japan, 84 N Japan, 157 Korea/N China, 25 Sichuan, 75 Yangtze-Qinling, and 85 SE China/tropical. Due to the small number of individuals in the S Japan group, for the purpose of this study we did not divide it into the S Japan and Central Japan groups identified by Clark et al. (2015). Six of the genetic groups for *M. sinensis* were identified previously via discriminant analysis of principle components (Jombart, Devillard, & Balloux, 2010) and by the software Structure (Falush, Stephens, & Pritchard, 2003); the ornamental group and the US naturalized group were found to be subsets of the S Japan group but we label them independently to denote their unique provenances (Clark et al., 2014, 2015, 2018). Moreover, about half the accessions in the ornamental group had most of their ancestry from *M. sinensis* and $\leq 30\%$ ancestry from diploid *M. sacchariflorus*, presumably the result of plant breeding efforts to introgress greater winter hardiness via crosses with diploid *M. xgiganteus* ‘Purpurascens’, while the other half were pure *M. sinensis* (Clark et al., 2014, 2015, 2018). Collection locations of all the natural and naturalized accessions are listed in Data S1 and those collected in Asia are shown in Figure 1. In addition to the *M. sinensis* accessions, we phenotyped seven diploid and three tetraploid *M. sacchariflorus* accessions from Korea and China, eight natural diploid *M. xgiganteus* from China, the ornamental diploid *M. xgiganteus* ‘Purpurascens’, and the biomass cultivar triploid *M. xgiganteus* ‘1993-1780’. All accessions were maintained as clonal stock plants in pots at a greenhouse in Ontario, Canada and vegetatively propagated. Ramets of each accession were distributed to each field trial location during January–March of 2012.

In the early summer of 2012, field trials were planted at five northern locations (Sapporo, Japan by Hokkaido University (HU); Leamington, Ontario, Canada by New Energy Farms (NEF); Fort Collins, Colorado, USA by Colorado State University (CSU); Urbana, Illinois, USA by the University of Illinois (UI); and Chuncheon, Korea by Kangwon National University (KNU)); plus one southern location (Zhuji, China by Zhejiang University (ZJU)) (Table 1). Except for CSU, which has an arid climate, supplemental irrigation was only provided in year 1 to facilitate establishment. Nitrogen

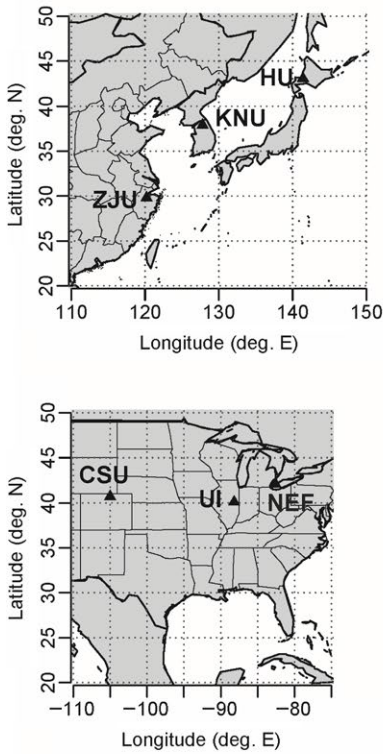
fertilizer was applied to the field trials in the spring in the following amounts: 100 kg/ha at HU, NEF, and UI each year; none at CSU; 80 kg/ha at KNU each year; and 14 kg/ha at ZJU in the first year only. Not all accessions were successfully established at all six locations (Table 1 and Data S1). Further, at the two locations in the US we were limited largely to ornamental and US naturalized accessions due to quarantine limitations on importation of *Miscanthus* from Asia (Table 1 and Data S1). In spring of 2013, an additional planting was made at UI to include plant materials that had been newly released from USDA quarantine. Field trials at each site had from three to four replications in a randomized complete block design with single-plant plots equally spaced within and between rows on 1.5 m centers (Table 1). Harvesting was conducted in late autumn or early winter, after dormancy or the first killing freeze led to dry-down, with stems being cut 15–20 cm from the ground. To determine dry biomass yield, at some sites the entire plant was oven dried before weighing, while at other sites the fresh weight was measured, then a subsample was weighed before and after oven drying in order to estimate dry weight of the whole plant.

2.2 | Phenotypic data collection and analyses

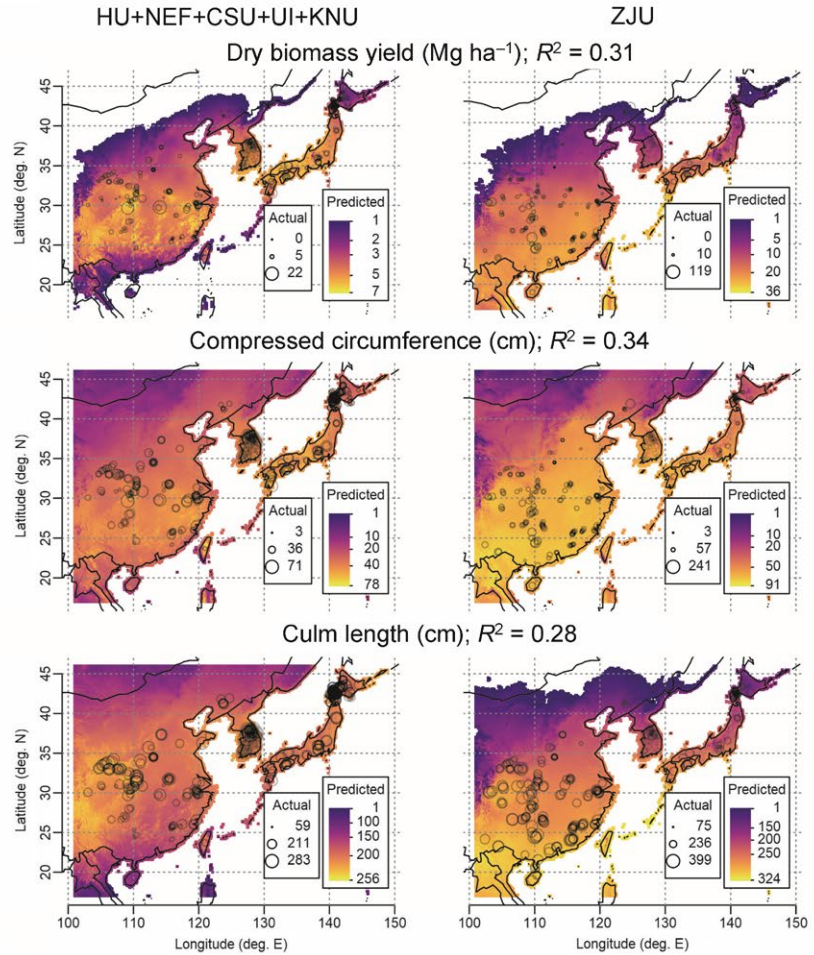
Biomass yield (dry weight) and 14 yield-component traits were measured in the second and third year after planting at each trial location (Table 2). Additionally, phenotypic data were collected in the fourth year at KNU for all traits and at HU for biomass yield only. Only data on establishment were taken during the first year because year 1 yields of *Miscanthus* are not expected to be strongly associated with yield in years 2 and 3 but data from years 2 and 3 are typically strongly correlated (Clifton-Brown et al., 2001). At UI, year 2 was 2013 for the trial planted in 2012, and 2014 for the trial planted in 2013. To estimate dry biomass yield per area, yield per plant was divided by the plot area (2.25 m²) and expressed as Mg/ha. For plants on which basal circumference exceeded the square plot perimeter (mostly *M. sacchariflorus* accessions in years 3 and 4), we instead estimated plot area from basal circumference, assuming a circular footprint. Data on flowering time and winter hardiness were measured but are not presented here.

For each trait \times year combination, phenotypic values were transformed using the Box–Cox method (Box & Cox, 1964) as implemented in the R package MASS (Venables & Ripley, 2002) in order to make the data approximate a normal distribution. Linear models for determining optimum lambda values for Box–Cox (where the transformation to be performed is $\log(x)$ if $\lambda = 0$, and $(x^\lambda - 1)/\lambda$ otherwise) were fit in R with field trial location, genotype, and their interaction as fixed effects, using the 569 *M. sinensis* individuals from the field trial. Lambda values ranging from -2 to 2 , at intervals of 0.1, were tested. Least square means (LS means) were estimated for all entries using the R package

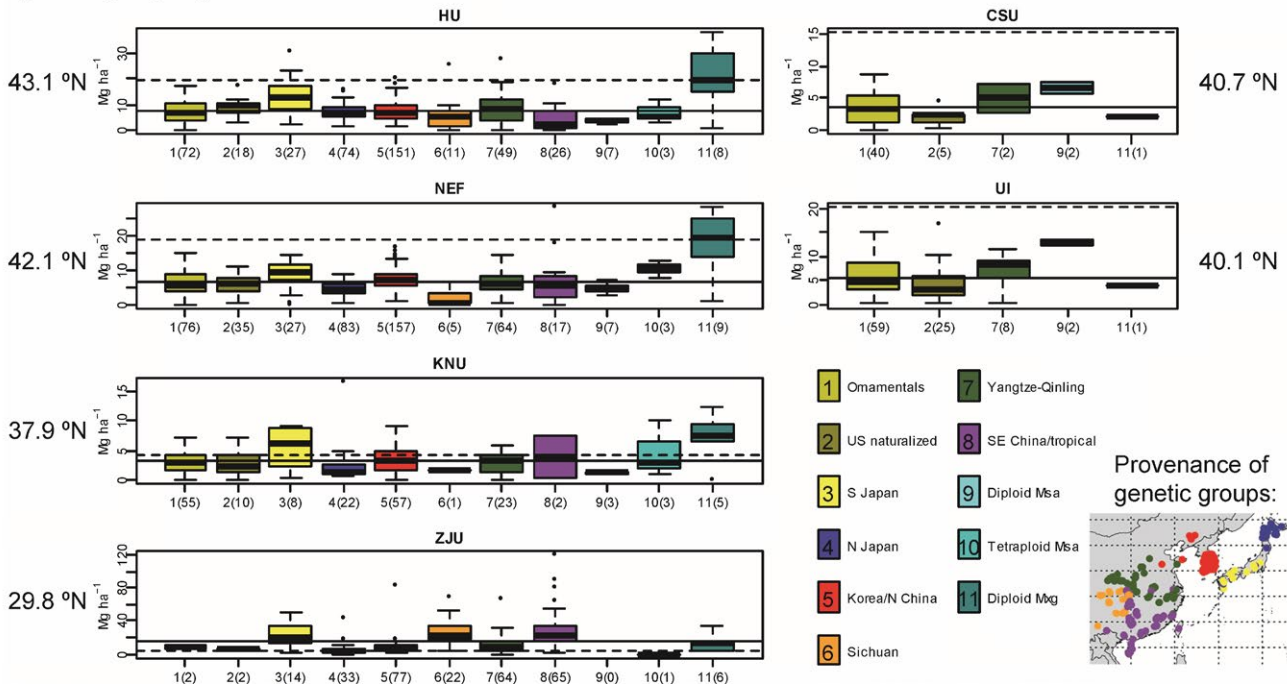
(a) Field trial locations



(b) Actual and predicted phenotypic values mapped to collection locations



(c) Dry biomass yield (Mg ha⁻¹) of genetic groups by field trial location



lsmeans (Lenth, 2016) with linear models fit in R using genotype and replication as fixed effects for within-location estimates, and using genotype and replication within

location as fixed effects for multi-location estimates. Back-transformed LS means were then calculated and presented in Table 2, Table S2 and Data S1.

FIGURE 1 Variation in year 3 yield and yield-component traits among *Miscanthus* accessions and genetic groups. (a) Field trial locations in Asia and North America. (b) Least-squared means of *M. sinensis* genotypes in the study for dry biomass yield (Mg/ha; 10 Mg/ha = 2,250 g/plant), compressed circumference (cm), and culm length (cm), at five northern locations (HU, NEF, UI, CSU, and KNU; 37.9–43.1° N; left column) or one southern location (ZJU; 29.8° N; right column) in year 3 mapped to the collection locations in Asia. Circle diameters are proportional to trait values, as shown in the legend for each plot. Colors indicate predicted trait values at UI (left column) and ZJU (right column) from bioclimatic variables using linear modeling (Equation 7). The maximum predicted value within the geographic range shown is indicated at the bottom of the color scale. Note that in the northern field trial locations, accessions collected below 30° N tended to perform poorly relative to accessions from further north, and that few of the southeast China accessions survived in the northern trial locations. In contrast, the accessions with the most southern provenance typically were the best performers at the southern field trial location. Notable exceptions to both trends can also be seen. (c) Boxplots of dry biomass yield. Each boxplot represents one field trial location, with latitude indicated. Genotypes are shown by genetic groups. Dashed horizontal lines indicate yield of the *M. ×giganteus* ‘1993-1780’ control, and solid lines indicate mean yield across all *M. sinensis*. Boxes span from the first to third quartile for each group. Whiskers extend to the minimum and maximum values, or to the first and third quartile ±1.5 times the box length, respectively, whichever is shorter. Points indicate genotypes with values outside the range spanned by the whiskers. The number of genotypes with data for each genetic group in each location is indicated in parentheses after the group number. Msa = *M. sacchariflorus*; Mxg = *M. ×giganteus*; all other groups are *M. sinensis*

Analyses of variance (ANOVA) were conducted with random effects models using the R package lme4 (Bates, Mächler, Bolker, & Walker, 2015) to estimate variance components for *M. sinensis* only. Box–Cox transformed phenotypic values (Y) were used for all models. Models were fit for each location \times trait \times year combination with replication (R) and genotype (G) as random effects. The model is given below, where b is the intercept, β is a vector of coefficients, and ε is random error.

$$Y = b + \beta_1 R + \beta_2 G + \varepsilon \quad (1)$$

Additionally, multi-location models were fit for all locations and for the five northern locations. Multi-location models were only fitted when data for a given trait \times year combination were available for all locations in a given combination of locations. Replication within location (L), genotype, and genotype \times location, and location were included as random effects in the multi-site models:

$$Y = b + \beta_1 RL + \beta_2 G + \beta_3 GL + \beta_4 L + \varepsilon \quad (2)$$

Genotypic (broad-sense) heritabilities were estimated as:

$$H^2 = \frac{\sigma_G^2}{\sigma_G^2 + \sigma_{GL}^2/n_L + \sigma_e^2/n_{LR}} \quad (3)$$

where σ_G^2 is the variance attributed to genotype (i.e., accession), σ_{GL}^2 is the variance attributable to genotype \times location interaction (omitted for single-site models), σ_e^2 is the residual variance, n_L is the number of field trial locations, and n_{LR} is the total number of replications across all field trial locations (Holland, Nyquist, & Cervantes-Martínez, 2002).

Genetic correlations between pairs of locations were estimated as:

$$r_G = \frac{\sigma_{G(\text{site1} \times \text{site2})}}{\left(\sigma_{G(\text{site1})}^2 \times \sigma_{G(\text{site2})}^2\right)^{0.5}} \quad (4)$$

where $\sigma_{G(\text{site1} \times \text{site2})}$ is the covariance of genotype means between locations and $\sigma_{G(\text{site1})}^2$ and $\sigma_{G(\text{site2})}^2$ are the components of variance among genotypes at locations 1 and 2, respectively (Burdon, 1977).

Genetic correlations between traits measured on the same *M. sinensis* plants were estimated as:

$$r_G^* = \frac{\sigma_{G(\text{trait1} \times \text{trait2})}}{\left(\sigma_{G(\text{trait1})}^2 \times \sigma_{G(\text{trait2})}^2\right)^{0.5}} \quad (5)$$

where $\sigma_{G(\text{trait1} \times \text{trait2})}$ is the genotypic component of covariance for traits 1 and 2 [calculated as $(\sigma_{G(\text{trait1} + \text{trait2})}^2 - \sigma_{G(\text{trait1})}^2 - \sigma_{G(\text{trait2})}^2)/2$], and $\sigma_{G(\text{trait1})}^2$ and $\sigma_{G(\text{trait2})}^2$ are the genotypic variance components for traits 1 and 2, respectively (see equation 9 of Howe, Saruul, Davis, & Chen, 2000). The ‘trait1+trait2’ indicates a calculated trait that is the sum the values for both traits at each field plot.

An additional random effects ANOVA was conducted on year 3 dry biomass yield data to estimate variance components for location, replication, genetic group (D), genetic group \times location, genotype within genetic group, and genotype \times location. In order to obtain significance estimates to test hypotheses that yield was dependent on these effects, this ANOVA was conducted in SAS 9.4 using the MIXED procedure.

$$Y = b + \beta_1 L + \beta_2 RL + \beta_3 D + \beta_4 DL + \beta_5 GD + \beta_6 GDL + \varepsilon \quad (6)$$

To predict performance in a given environment based on provenance of *M. sinensis* germplasm, linear models were fit for dry biomass yield, compressed circumference, and culm length in year 3 based on the ‘universal response function’ method of Wang, O’Neill, and Aitken (2010). Bioclimatic data were obtained from WorldClim v2 (Fick & Hijmans, 2017), including mean annual temperature (BIO1), maximum temperature of the warmest month (BIO5), annual precipitation (BIO12), and precipitation of the warmest quarter (BIO18). Minimum temperature of

TABLE 1 Field trial locations for phenotypic evaluations of *Miscanthus sinensis* and *Miscanthus* controls

Trial site code	Location	Latitude	Longitude	Elevation (m)	Hardiness zone ^a	Year planted	Total number of genotypes	Number of <i>M. sinensis</i> genotypes	Number of replicates	Number of years ^b
HU	Sapporo, Japan	43.1	141.4	11	7b	2012	529	509	4	4
NEF	Leamington, Ontario, Canada	42.1	-82.6	196	6b	2012	487	467	4	3
CSU	Fort Collins, Colorado, USA	40.7	-105.0	1,556	5b	2012	61	57	4	3
UI	Urbana, Illinois, USA	40.1	-88.2	230	5b	2012	112	108	4	3
						2013	92	84	4	2
KNU	Chuncheon, S Korea	37.9	127.8	75	6b	2012	206 ^c	194 ^d	3	4
ZJU	Zhuji, China	29.8	120.2	58	9a	2013	290	282	3	3
Minimum air temperature preceding winter (°C)										
Growing degree days (d °C)^f										
							Year 1	Year 2	Year 3	Year 4
HU	133	200	244	215	1,193	1,462	1,492	1,404	-13.5	-10.1
NEF	124	223	190	888	1,556	1,251	1,251	-18.0	-27.0	Humic andosol
CSU	83	210	185	800	1,421	1,269	1,269	-22.8	-29.9	Brookston clay sand spot phase ^g
UI 2012	126	210	192	1,055	1,789	1,651	1,651	-15.9	-25.2	Fort Collins loam ^h
UI 2013	185	192		1,718	1,651			-25.2		Drummer silty clay loam ⁱ
KNU	117	221	228	211	1,068	2,133	2,153	-23.1	-15.6	Drummer silty clay loam
ZJU	262	267	281	3,209	3,173	3,159	3,159	-5.0	-4.0	2:3 Alfisol:Inceptisol

Note. HU = Hokkaido University; NEF = New Energy Farms; CSU = Colorado State University; UI = University of Illinois at Urbana-Champaign; KNU = Kangwon National University; ZJU = Zhejiang University.

^aUSDA hardiness zone estimate based on plantmaps.com. ^bPhenotypic data were collected in years 2–4 only; year 1 was just an establishment year. ^cAt KNU, a set of 86 genotypes was planted in all three replications, but in one replication an additional 120 genotypes were included for a total of 206. ^dAt KNU, a set of 82 genotypes was planted in all three replications, but in one replication an additional 112 genotypes were included for a total of 192. ^eMeasured from planting date (year 1) or first date of spring emergence (years 2–4) to last date of autumn dormancy. ^fFor each day during the growing season, a number from 0 to 20 was calculated indicating the number of °C by which the average daily temperature exceeded 10°C. These were summed across the days of the growing season as estimated for growing season length. ^gRichards, Caldwell, and Morwick (1949). ^hUS Dept. of Agriculture – Natural Resources Conservation Service (2005). ⁱUS Dept. of Agriculture – Natural Resources Conservation Service (2015).

the coldest month (BIO6) was initially included, but was dropped from the model for not having a significant effect when BIO1 and BIO5 were included. Because latitude is known to influence *Miscanthus* yields via flowering time (Clifton-Brown et al., 2001; Jensen et al., 2011), latitude was also included in the model. For each of the five independent variables (four bioclimatic variables plus latitude), the second degree polynomial of the value at the germplasm collection location was included, as well as the value at the collection location multiplied by the value at the field trial location. Effects due to field trial location alone were omitted from the model because they resulted in negative yield predictions, likely because field trial locations differed in terms of management as well as climate. Models were run across all individual plots, with Box–Cox-transformed phenotypes as dependent variables. The models were then used to map predicted phenotypic values at UI and ZJU for hypothetical collection sites at 0.1 degree (6 min) intervals across East Asia. The equation for the models is given below, where Y is a Box–Cox transformed phenotype, b is a model coefficient, ε is random error, i is the collection location (provenance) of germplasm, j is the field location, $BIO1$, $BIO5$, $BIO12$, and $BIO18$ are the bioclimatic variables described above, and Lat is latitude.

$$\begin{aligned}
 Y_{ij} = & b_0 + b_1BIO1_i + b_2BIO1_i^2 + b_3BIO1_iBIO1_j + b_4BIO5_i \\
 & + b_5BIO5_i^2 + b_6BIO5_iBIO5_j + b_7BIO12_i + b_8BIO12_i^2 \quad (7) \\
 & + b_9BIO12_iBIO12_j + b_{10}BIO18_i + b_{11}BIO18_i^2 \\
 & + b_{12}BIO18_iBIO18_j + b_{13}Lat_i + b_{14}Lat_i^2 + b_{15}Lat_iLat_j + \varepsilon_{ij}
 \end{aligned}$$

All data and code are available in the Illinois Data Bank, https://doi.org/10.13012/B2IDB-0790815_V2.

3 | RESULTS

3.1 | Yield performance

The commercially important biomass cultivar control, triploid *M. × giganteus* ‘1993-1780’, typically yielded more at the northern trial sites (LS means for year 3 dry biomass yields were 19.7, 18.7, 15.2, and 20.4 Mg/ha at HU, NEF, CSU and UI, respectively; Figure 1 and Table 3) than at the southern site (3.8 Mg/ha at ZJU), though performance at KNU was also low (4.2 Mg/ha) although compressed circumference and culm length at KNU were similar to those at other northern sites (Data S2). In comparison to the northern locations in the current study, prior studies reported similar third year yields for triploid *M. × giganteus* of 13.7–37.3 Mg/ha in IL, USA (Arundale et al., 2014; Heaton, Dohleman, & Long, 2008; Kaiser et al., 2015) and 13.8–37.8 Mg/ha in England, Germany and Portugal (Clifton-Brown et al., 2001), though the prior trials were

planted at higher density (1–2 plants m⁻²) than the current study (1 plant 2.25 m⁻²). Thus, data from this and prior studies indicate that small and large plots provide similar estimates of *Miscanthus* biomass yield when expressed in Mg/ha (Dong, Liu et al., 2018; Kaiser et al., 2015; Zhao et al., 2013). Consistent with the poor performance of *M. × giganteus* ‘1993-1780’ at ZJU, we have previously observed this genotype to have low yields at similar latitudes in the southern coastal plain of the US due primarily to early flowering (e.g., in August, unpublished).

Among the *M. sinensis* genotypes, large and highly significant differences in yield and yield components were observed at each location (Tables 4 and 5). For example, over all trial sites, LS means for *M. sinensis* genotypes in year 3 ranged from 0.004–119 Mg/ha for dry biomass yield, 4–708 culms per plant, 2–241 cm for compressed circumference, and 28–399 cm for culm length (Data S1). Nineteen *M. sinensis* genotypes were observed to have year 3 yields exceeding 20 Mg/ha at HU or NEF, more than the highest-producing planting of the *M. × giganteus* ‘1993-1780’ control in this study, and/or 50 Mg/ha at ZJU (Table 3). Three *M. sinensis* genotypes at HU and one at NEF exceeded 25 Mg/ha, and four exceeded 80 Mg/ha at ZJU (Table 3). Average yield of *M. sinensis* in year 3 at the southern trial location, ZJU, was 15.4 Mg/ha, which was 2–5-fold greater than at the five northern locations (Figure 1, Table 4). Location main effects for year 3 yield of *M. sinensis* accounted for 23% of the total variation if all trial locations were included in the ANOVA and 20% for just the northern trial locations (Table 5, Equation 6). However, genotype × location interactions were modest among the five northern trial sites (37.9–43.1° N) for year 3 yield but large between ZJU (29.8°N) and the northern sites (Table 5). The combined genetic group × location and genotype within genetic group × location effects were only 10% of the total variation for yield in the five northern locations but were 32% of the total variation when ZJU was included in the analysis (Table 5). Similarly, in years 2 and 3, genetic correlations for yield between pairs of locations were always negligible between ZJU and northern locations (except for a moderately negative correlation between UI and ZJU in year 2) but generally much higher among northern locations (Table 6, Equation 4). Lower genetic correlations for yield among the northern trial locations in year 3 relative to year 2 may have been due to drought stress at HU in year 3 and an unusually cold winter prior to year 3 at UI that caused winter damage in some genotypes (Table 1 and Figure S1). Genetic correlations among sites tended to be high (>0.7) for culm node number, culm dry weight, and diameter of basal internode (Data S3).

The *M. sinensis* genotypes that yielded best at a given trial location typically originated from a latitude that was similar to or more southern by ~5° than the trial location, though there were notable exceptions (Figure 1). Additionally, the *M. sinensis* genotypes that had the longest culms at a given

TABLE 2 Yield and yield-component traits measured in multi-location field trials of *Miscanthus sinensis*

Trait	Abbreviation	Description ^a and notes
Dry biomass yield (g/plant or Mg/ha)	Yld	Single-plant plots on 1.5 m centers were harvested in late autumn by cutting the stems 15–20 cm above the soil surface. Samples were dried at 60°C until constant weight. Estimates are reported per area based on plot dimensions (2.25 m ²) or per plant.
Compressed circumference (cm)	CC	Stems were compressed at the middle height of the plant such that all the culms were in close contact without air gaps; then the circumference of the compressed bundle was measured.
Basal circumference (cm)	BC	Circumference of the base of the plant, without compression.
Compressed circumference/ basal circumference	CC/BC	Compressed circumference divided by basal circumference, to estimate the proportion of the plant's footprint filled by stems.
Culm length (cm)	CmL	Length of the tallest culm in late autumn, measured from the base of the stem to the tip of the panicle if present, otherwise to the highest part of the highest leaf.
Culm node number	CmNdN	Number of nodes on the tallest culm of each plant in late autumn.
Internode length (cm)	IntL	Culm length divided by the number of nodes for the tallest culm of each plant in late autumn.
Culm dry weight (g)	CmDW	Mass of the tallest culm of each plant in late autumn, after removal of leaves and drying at 60°C until constant weight achieved. (Not recorded at KNU in year 3 or 4 or at ZJU.)
Culm volume (cm ³)	CmV	Estimated from culm length, culm diameter at first internode, and culm diameter at last internode, assuming the stem was shaped like the frustum of a cone: $CmL * \pi * [(DBI/2)^2 + (DBI/2)*(DTI/2) + (DTI/2)^2]/3$
Culm density (g cm ⁻³)	CmDW/V	Culm dry weight divided by culm volume. (Not estimated for KNU in year 3 or 4 or at ZJU.)
Diameter of basal internode (mm)	DBI	Measured on the tallest culm of each plant in late autumn.
Diameter of topmost internode (mm)	DTI	Measured on the tallest culm of each plant in late autumn.
Total number of culms	TCmN	Counted for each plant.
Proportion of reproductive culms	RCmN/TCmN	Number of reproductive culms divided by the total number of culms. (Not estimated at HU in year 2 or at KNU.)
Culms per footprint (# cm ⁻²)	TCmN/A	The total number of culms divided by the area of the plant's footprint. The footprint area was estimated from the basal circumference, assuming a circular base.

^aAll traits were measured at the end of the growing season.

trial location were typically from within ~10° south of the testing site (especially the Yangtze-Qinling group for the northern trial locations, and the Sichuan and SE China/tropical groups for ZJU; Figure 1). One of the largest exceptions to this geographic pattern was PMS-496 from 41.9° N in northeast China (Korean/N China genetic group), which yielded 86.4 Mg/ha in year 3 at ZJU, yet most accessions from this northern group yielded poorly at ZJU (Figure 1, Table 3). Linear modeling using bioclimatic variables (Equation 7) predicted that the highest yielding *M. sinensis* for temperate regions could be collected at high elevations from ~24–32° N in China (e.g., PMS-014; Table 3) followed by S Japan, whereas the highest yielding *M. sinensis* for subtropical regions could be collected from the Ryukyu Islands, Taiwan and the Philippines (primarily due to large predicted culm length), and to a lesser extent coastal southeast China (Figure 1b and Figure S2). Notably, our model

predicted the presence of *M. sinensis* (yields greater than zero) along coastal southeast Russia, where it has been collected recently (Hodkinson et al., 2016), despite our study lacking genotypes of that provenance. Model terms and coefficients are provided in Table S1. Among the *M. sinensis* genetic groups, the S Japan group was especially notable for having produced relatively high and stable yields over all trial locations (Figure 1). For example, year 3 yields of JM0232.001 from the Hiruzen highlands in southwestern Honshu were 21.8, 11.4, and 35.6 Mg/ha at HU, KNU, and ZJU, respectively (Table 3). However, a comparison of all genetic groups over all species (not just within *M. sinensis*) revealed that the diploid *M. ×giganteus* genotypes collected in China from 29° to 37° N were the best-yielding entries at the northern trial sites HU, NEF and KNU, and some of these genotypes out-yielded the triploid *M. ×giganteus* '1993-1780' control where it performed best, at NEF

TABLE 3 Least-squared means for dry biomass yield (Mg/ha) of the best performing *Miscanthus sinensis* genotypes grown at five northern field trial locations (HU, NEF, CSU, UI, and KNU); 37.9–43.1° N) or one southern location (ZJU; 29.8° N) in year three; data shown for genotypes that exceeded 20 Mg/ha at HU or NEF or 50 Mg/ha at ZJU. Yields for all *M. sacchariflorus* and *M. xgiganteus* genotype controls are also shown for comparison

Species	Genotype	Genetic group	Latitude	Longitude	HU	NEF	CSU	UI	KNU	ZJU	Northern trial locations	All trial locations
<i>M. sinensis</i>	JM0119.001	S Japan	33.56	133.38	12.3	0.2				50.9	3.9	9.3
	JM0125.001	S Japan	31.53	131.24	30.9	0.4				7.5	15.9	11.1
	JM0232.001	S Japan	35.05	133.72	20.7	10.6				34.2	11.7	15.1
	JM0232.002	S Japan	35.05	133.72	23.0	12.6					11.9	12.7
	JM0361.002	S Japan	33.02	131.06	21.0	7.8					9.1	9.7
	PMS-014	Sichuan	29.66	109.12	25.4					40.2	20.6	26.3
	PMS-053	Sichuan	30.22	103.48						69.6		57.4
	PMS-054	Sichuan	30.08	103.08						51.7		41.1
	PMS-257	SE China/tropical	23.26	104.42						52.8		42.5
	PMS-314	Yangtze-Qinling	26.53	119.63				8.5	4.0	66.1	7.6	19.9
	PMS-397	SE China/tropical	24.40	109.98						63.7		51.6
	PMS-399	SE China/tropical	24.39	110.13	0.3					81.1	0.1	35.1
	PMS-404	SE China/tropical	24.69	110.47						54.1		43.2
	PMS-427	Yangtze-Qinling	29.77	114.04	27.6					18.2	22.3	17.9
	PMS-496	Korea/N China	41.91	124.67	2.2	3.8				81.9	2.3	13.0
	PMS-558	SE China/tropical	28.35	109.55						53.8		43.5
PMS-576	SE China/tropical	25.77	109.59						88.8		74.7	
PI417947	SE China/tropical	-6.00	144.00						119.1		101.2	
PI423566	SE China/tropical	24.00	121.00	1.1	28.2					7.0	7.6	

(Continues)

TABLE 3 (Continued)

Species	Genotype	Genetic group	Latitude	Longitude	HU	NEF	CSU	UI	KNU	ZJU	Northern trial locations	All trial locations
<i>M. xgiganteus</i> triploid	'1993-1780'	<i>M. xgiganteus</i>	35.44 ^a	139.64	19.7	18.7	15.2	20.4	4.2	3.8	14.9	14.5
<i>M. xgiganteus</i> diploids	PMS-111	<i>M. xgiganteus</i>	32.96	108.32	15.8	28.1	7.6	17.8	7.6	17.8	17.8	18.7
	PMS-162	<i>M. xgiganteus</i>	35.71	112.61	21.3	28.3	20.7	20.7	9.5	12.7	20.7	21.8
	PMS-279	<i>M. xgiganteus</i>	29.10	117.08	36.0	14.0	19.1	19.1	12.5	14.9	19.1	16.6
	PMS-300	<i>M. xgiganteus</i>	30.87	120.14	23.8	19.1	23.8	17.6	12.5	33.4	17.6	15.7
	PMS-430	<i>M. xgiganteus</i>	29.90	114.29	38.3	23.1	24.6	24.8	6.5	4.9	24.8	25.9
	PMS-459	<i>M. xgiganteus</i>	36.22	120.54	17.4	24.6	17.4	16.7	6.5	4.3	16.7	11.5
	PMS-467-8	<i>M. xgiganteus</i>	37.17	121.74	14.5	17.7	14.5	13.0	4.3	16.7	13.0	10.5
	PMS-533	<i>M. xgiganteus</i>	31.02	108.87	1.2	1.2	16.7	0.7	16.7	16.7	0.7	5.5
<i>M. xgiganteus</i>	'Purpurascens'	<i>M. xgiganteus</i>		0.8	0.8	1.9	2.3	3.9	0.0	2.0	2.0	2.2
<i>M. sacchariflorus</i>	KMS053	<i>M. sacchariflorus</i>	35.42	129.06	5.5	10.7	1.0	5.5	1.0	0.3	5.5	4.7
	KMS062	<i>M. sacchariflorus</i>	36.34	129.06	3.3	7.9	3.0	4.0	3.0	4.0	4.0	4.4
	PMS-071	<i>M. sacchariflorus</i>	40.39	117.01	4.7	7.2	7.2	5.2	7.2	5.2	5.2	5.6
	PMS-074	<i>M. sacchariflorus</i>	40.13	116.17	4.0	3.4	3.4	2.5	3.4	2.5	2.5	2.8
	PMS-075	<i>M. sacchariflorus</i>	40.13	116.18	4.4	6.0	7.6	13.5	1.5	6.5	6.5	7.1
	PMS-076	<i>M. sacchariflorus</i>	40.16	116.05	4.3	4.9	4.9	3.4	10.0	3.4	3.4	3.7
	PMS-077	<i>M. sacchariflorus</i>	40.15	115.90	2.1	5.2	5.2	3.3	10.0	3.3	3.3	3.6
	PMS-458	<i>M. sacchariflorus</i>	36.17	120.50	12.4	12.5	12.5	10.4	10.0	10.4	10.4	11.1
	PMS-512	<i>M. sacchariflorus</i>	41.33	123.69	4.3	3.0	3.0	2.4	0.8	0.8	2.4	2.7
	'Hortico'	<i>M. sacchariflorus</i>		2.4	2.4	4.7	5.6	12.4	1.2	4.5	4.5	5.0

Note. Original data were Box-Cox transformed before estimating least-squared means; back-transformed values are shown here. Genotypes outperforming the triploid *M. xgiganteus* '1993-1780' control at a given site are highlighted in bold. Collection locations are indicated for wild accessions.

HU = Hokkaido University; NEF = New Energy Farms; CSU = Colorado State University; UI = University of Illinois at Urbana-Champaign; KNU = Kangwon National University; ZJU = Zhejiang University.

^a Assuming origination from Yokohama, Japan.

TABLE 4 Least-squared means \pm SE within each trial location in year three for yield and yield-component traits of *M. sinensis* genotypes grown at five northern field trial locations (HU, NEF, CSU, UI, and KNU; 37.9–43.1° N) or one southern location (ZJU; 29.8° N)

Trait	HU	NEF	CSU	UI	KNU	ZJU	Northern locations	All locations
Dry biomass yield (Mg/ha)	7.8 \pm 0.2	6.5 \pm 0.2	3.5 \pm 0.4	5.7 \pm 0.4	3.2 \pm 0.2	15.4 \pm 1.0	5.1 \pm 0.1	7.6 \pm 0.4
Dry biomass yield (g/plant)	1744 \pm 50	1,477 \pm 35	793 \pm 80	1,289 \pm 89	723 \pm 40	3,494 \pm 223	1,157 \pm 29	1728 \pm 80
Compressed circumference (cm)	26.1 \pm 0.5	37.3 \pm 0.5	31.1 \pm 1.7	37.5 \pm 1.6	56.7 \pm 2.6	60.0 \pm 1.7	35.0 \pm 0.5	40.2 \pm 0.7
Basal circumference (cm)	97.2 \pm 1.4	139.6 \pm 4.4	98.3 \pm 4.7	145.3 \pm 3.8	130.3 \pm 3.6	159.8 \pm 2.7	123.9 \pm 1.9	131.1 \pm 1.7
Compressed circumference/basal circumference	0.27 \pm 0.00	0.28 \pm 0.00	0.32 \pm 0.01	0.26 \pm 0.01	0.44 \pm 0.02	0.36 \pm 0.01	0.29 \pm 0.00	0.31 \pm 0.00
Culm length (cm)	218.2 \pm 1.8	210.6 \pm 1.7	151.3 \pm 6.8	198.5 \pm 4.2	212.9 \pm 3.0	241.1 \pm 3.4	202.9 \pm 1.6	217.7 \pm 1.7
Culm node number	11.9 \pm 0.1	11.3 \pm 0.1	10.0 \pm 0.4	10.7 \pm 0.3	10.5 \pm 0.2	13.8 \pm 0.2	10.9 \pm 0.1	11.7 \pm 0.1
Internode length (cm)	18.8 \pm 0.2	19.6 \pm 0.2	15.6 \pm 0.7	19.3 \pm 0.5	21.0 \pm 0.4	18.3 \pm 0.3	19.0 \pm 0.2	19.0 \pm 0.2
Culm dry weight (g)	10.5 \pm 0.2	13.9 \pm 0.3	8.6 \pm 0.4	11.2 \pm 0.6	44.1 \pm 2.3	54.7 \pm 2.0	12.2 \pm 0.3	12.2 \pm 0.3
Culm volume (cm ³)	45.6 \pm 1.0	47.8 \pm 1.0	24.1 \pm 2.2	42.4 \pm 2.2	44.1 \pm 2.3	54.7 \pm 2.0	44.0 \pm 0.9	49.4 \pm 1.1
Culm density (g cm ⁻³)	0.23 \pm 0.00	0.30 \pm 0.00	0.35 \pm 0.02	0.27 \pm 0.01	6.7 \pm 0.2	5.4 \pm 0.1	0.27 \pm 0.00	0.27 \pm 0.00
Diameter of basal internode (mm)	6.4 \pm 0.1	5.7 \pm 0.0	5.4 \pm 0.1	5.6 \pm 0.1	6.7 \pm 0.2	5.4 \pm 0.1	6.2 \pm 0.0	6.2 \pm 0.1
Diameter of topmost internode (mm)	3.4 \pm 0.0	4.7 \pm 0.0	3.2 \pm 0.1	4.5 \pm 0.1	2.6 \pm 0.1	4.7 \pm 0.1	3.9 \pm 0.0	4.2 \pm 0.0
Total number of culms	123.0 \pm 3.7	112.5 \pm 2.8	130.7 \pm 8.7	170.5 \pm 10.0	118.3 \pm 5.2	213.9 \pm 6.4	112.9 \pm 2.3	123.5 \pm 2.2
Proportion of reproductive culms	0.58 \pm 0.01	0.69 \pm 0.01	0.45 \pm 0.02	0.55 \pm 0.02	0.53 \pm 0.01	0.53 \pm 0.01	0.50 \pm 0.01	0.53 \pm 0.01
Culms per footprint (# cm ⁻²)	0.18 \pm 0.01	0.08 \pm 0.00	0.20 \pm 0.02	0.11 \pm 0.01	0.11 \pm 0.01	0.11 \pm 0.00	0.10 \pm 0.00	0.10 \pm 0.00

Note. Data from individual plots were Box-Cox transformed, then used to estimate least-squared means for each genotype; back-transformed values shown.

HU = Hokkaido University; NEF = New Energy Farms; CSU = Colorado State University; UI = University of Illinois at Urbana-Champaign; KNU = Kangwon National University; ZJU = Zhejiang University.

TABLE 5 Partitioning of variance for dry biomass yield (Mg/ha) in year three among field trial locations, genetic group, genotypes within groups and their interactions for 569 *Miscanthus sinensis* genotypes (Equation 6)

Source	All locations				ZJU excluded			
	df	Variance	Prop. total variance	p	df	Variance	Prop. total variance	p
Location	5	1.01	0.23	0.0905	4	0.64	0.20	0.0915
Rep within location	15	0.58	0.01	0.0108	13	0.06	0.02	0.0157
Genetic group	7	0.05	0.01	0.3901	7	0.42	0.13	0.0549
Genetic group × location	25	0.93	0.20	0.0008	18	0.05	0.01	0.0636
Genotype within genetic group	548	0.70	0.15	<0.0001	489	0.57	0.18	<0.0001
Genotype within genetic group × location	902	0.56	0.12	<0.0001	690	0.29	0.09	<0.0001
Residual		1.33	0.28	<0.0001		1.21	0.37	<0.0001

Note. All effects were treated as random. Data were transformed by the Box–Cox method before fitting the model.

The field trial locations were HU = Hokkaido University; NEF = New Energy Farms; CSU = Colorado State University; UI = University of Illinois at Urbana-Champaign; KNU = Kangwon National University; ZJU = Zhejiang University. *df* = degrees of freedom, estimated using the Satterthwaite method for fixed effects model; all other estimates based on random effects model. *P* = significance.

and HU (Figure 1 and Figure S3, Table 3, Data S2). The diploid *M. ×giganteus* genotypes that outperformed triploid *M. ×giganteus* ‘1993-1780’ also had compressed circumferences exceeding that of *M. sinensis* and *M. ×giganteus* ‘1993-1780’ across all sites where they were planted (Figure S3). Culm lengths of the diploid *M. ×giganteus* genotypes, however, were similar to those of *M. sinensis* (Figure S3).

Though some diploid *M. ×giganteus* hybrids had high yields at ZJU, the best performing groups at this southern trial location were the *M. sinensis* SE China/tropical, Sichuan basin, and S Japan (Figure 1, Table 3). Genotypes in the SE China/tropical and Sichuan basin groups were also especially tall at ZJU, with culm lengths of eight genotypes between 3.5 and 4.0 m (Figure 1, Figures S3 and S4; Data S1 and S2). In contrast to the ZJU results, relatively few genotypes of the *M. sinensis* SE China/tropical group and Sichuan basin group survived in the northern trial locations and most that did survive in the north performed poorly there. However at HU (43.1° N), PMS-014 was the second highest yielding *M. sinensis* genotype in year 3 (25.4 Mg/ha) and it was present in three replicates, which was exceptional given that it originated from the Sichuan group (29.7° N); PMS-014 also yielded well at ZJU, with 40.2 Mg/ha but it did not survive at NEF (Table 3). Similarly, PI423566 from the SE China/tropical group was the highest yielding *M. sinensis* entry at NEF with 28.2 Mg/ha in year 3, essentially in a tie for top yield with two diploid *M. ×giganteus* hybrids (Table 3).

Among the ornamental cultivars, there was a significant negative association between year 3 yield and the proportion of ancestry from *M. sacchariflorus* (via diploid *M. ×giganteus* ‘Purpurascens’) at HU but not at the other trial locations (Figure S5a); however, a similar negative association

was observed at CSU, but too few non-hybrid individuals survived for the association to be significant (Figure S5a). Similarly, there was a negative association among the ornamental cultivars between the proportion of ancestry from *M. sacchariflorus* and culm length at HU, NEF, CSU, UI, and KNU (Figure S5b). In contrast, the total number of culms had a significant positive association with proportion of *M. sacchariflorus* ancestry at NEF, KNU, and UI for the ornamental cultivars (Figure S5c). Therefore, *M. sacchariflorus* ancestry did not affect yield at NEF, UI, or KNU because although hybrid ornamentals had shorter culms than the non-hybrids, they had more of them. At HU and CSU, hybrid and non-hybrid ornamental genotypes had similar numbers of culms, but because culms of hybrid ornamentals were shorter, they had lower yields than the non-hybrids. Less water availability at HU in year 3 and CSU in general (Figure S1) may have preferentially reduced the number of culms in the hybrids with greatest *M. sacchariflorus* ancestry, as in nature this species is typically found in riparian environments. A similar genotype-by-environment effect observed by Kaiser et al. (2015) was also associated with drought at a previous trial in southern Illinois, USA where hybrid ornamentals performed poorly.

3.2 | Heritabilities, and genetic correlations between years and traits

Genotypic heritabilities for most traits and locations were high, suggesting strong potential for improvement via clonal selection within *M. sinensis* (Table 7, Equation 3). For example, genotypic heritabilities for yield among the trial locations in year 2 ranged from 0.49 at KNU to 0.85 at ZJU, and in year 3 from 0.71 at HU to 0.88 at ZJU. Over all northern

TABLE 6 Genetic correlations among five northern field trial locations (HU, NEF, CSU, UI, and KNU; 37.9–43.1° N) and one southern location (ZJU; 29.8° N) for dry biomass yield (Mg/ha) in years two and three for *Miscanthus sinensis* accessions

	HU	NEF	CSU	UI	KNU	ZJU
Year 2						
HU		0.68	1.03	0.53	0.89	0.01
NEF	426		0.64	0.45	0.48	0.15
CSU	56	57		1.00	1.62	
UI	105	113	55		0.90	−0.47
KNU	187	183	46	74		−0.03
ZJU	242	200	4	18	67	
Year 3						
HU		0.61	0.77	0.22	0.48	0.11
NEF	400		0.40	0.66	0.58	0.08
CSU	44	47		0.47	0.94	
UI	77	87	43		0.51	
KNU	166	170	39	62		0.14
ZJU	192	197	4	10	62	

Note. Genetic correlations are shown in the top halves of the matrices; lower halves of the matrices indicate the number of individuals with yield data in common between each pair of sites. Correlation values are omitted for pairs of sites with fewer than 15 individuals in common. Genetic correlation was estimated as the genotypic covariance between two sites divided by the square root of the product of the genetic variance at each site (Equation 5).

HU = Hokkaido University; NEF = New Energy Farms; CSU = Colorado State University; UI = University of Illinois at Urbana-Champaign; KNU = Kangwon National University; ZJU = Zhejiang University.

trial sites, heritability for yield was 0.78 in year 2 and 0.84 in year 3 (Table 7). Genetic correlations between year 2 and year 3 for each of the traits were moderate to strong (Table 8, Equation 5). For yield, genetic correlations between year 2 and year 3 ranged from 0.58 at UI to 0.95 at ZJU, and were 0.76 across all five northern sites. Traits with consistently strong genetic correlations between years 2 and 3 at each trial site were: culm length, culm node number, internode length, culm dry weight, culm volume, diameter of basal internode, and diameter of topmost internode.

Genetic correlations between yield-component traits and yield in year 3 were mostly moderate, with some exceptions (Table 9, Equation 5). Among all yield component traits, compressed circumference had the highest correlation with yield, ranging, in year 3, from 0.91 at UI to >0.99 at KNU. In comparison, year 3 genetic correlations between culm length and yield ranged from 0.46 at NEF to 0.88 at ZJU, with 0.62 over all five northern trial sites. Mostly low or negligible year 3 genetic correlations with yield were observed for internode length, total culm number, and the proportion of reproductive culms (RCmN/TCmN). Negligible to moderate negative year 3 genetic correlations were observed between culms per footprint (TCmN/A) and yield at each trial location.

Genotypes with relatively many culms typically compensated to varying degrees with thinner, less voluminous, and lighter culms than genotypes with fewer culms (Data S4, Equation 5). At the northern trial locations, culm basal and topmost diameters, culm volume, and culm dry weights were negatively correlated with the total number of culms in year 3 (Data S4). Genetic correlations in year 3 between total number of culms and diameter of topmost internode were negative but weak at all locations (−0.02 at ZJU and from −0.27 at HU to −0.40 at CSU for the northern trial locations). However, for diameter of basal internode, year 3 genetic correlations with total number of culms were strongly negative at CSU, UI, and KNU (−0.69, −0.72, and −0.80, respectively) and weakly negative at HU and NEF (−0.38 and −0.44, respectively). Year 3 genetic correlations between culm volume and total number of culms were negative and weak at HU, NEF, CSU, and UI (−0.28 to −0.49) and strongly negative at KNU (−0.70). Similarly, year 3 genetic correlations between culm dry weight and total number of culms were negative and weak at HU, NEF, CSU, and UI (−0.28 to −0.47). However, genetic correlations between total number of culms and culm length were negligible. Moreover, negative year 3 genetic correlations between basal circumference and number of culms per footprint were strong at NEF (−0.73) and CSU (−0.80), moderate at HU (−0.51) and UI (−0.42), but negligible at KNU (−0.16) and ZJU (−0.33), indicating that genotypes with larger basal circumferences typically had fewer stems per area (more space between stems and/or thicker stems) than those with smaller footprints (Data S4).

4 | DISCUSSION

4.1 | Yield potential, adaptation and implications for breeding

A key finding of this study has been the identification of which *M. sinensis* genetic groups yield the most, and in which production environments. Given that the *M. sinensis* genetic groups originate from known geographies (Figure 1, Data S1), the results of the current study are expected to provide useful guidance to researchers regardless of whether their *M. sinensis* genotypes of interest were included in the present study. Moreover, our estimates of genotype-by-location interactions and genetic correlations among trial locations for *M. sinensis* yield indicated that information from one northern trial location may be expected to be moderately informative about performance at other northern trial locations (e.g., HU and NEF), whereas performance of genotypes at southern trial locations may be substantially different from and not well predicted by performance at northern trial locations and vice versa. Notably, we found that the S Japan *M. sinensis* group had high and stable yields over the northern and southern trial locations tested, and was the overall best *M. sinensis* group

TABLE 7 Genotypic heritabilities of Box-Cox transformed yield and yield-component traits in *Miscanthus sinensis* genotypes grown at five northern field trial locations (HU, NEF, CSU, UI, and KNU; 37.9–43.1° N) or one southern location (ZJU; 29.8° N) (Equation 3)

Trait	HU	NEF	CSU	UI	KNU	ZJU	Northern trial locations	All trial locations
Year 2								
Dry biomass yield (g/plant)	0.60	0.81	0.62	0.92	0.49	0.85	0.78	0.72
Compressed circumference (cm)	0.65	0.78	0.71	0.91	0.61	0.87	0.70	0.65
Basal circumference (cm)	0.72	0.81	0.80	0.89	0.68	0.83	0.80	0.79
Compressed circumference/basal circumference	0.71	0.78	0.72	0.80	0.65	0.83	0.73	0.72
Culm length (cm)	0.81	0.89	0.92	0.95	0.75	0.89	0.89	0.71
Culm node number	0.80	0.86	0.77	0.95	0.68	0.89	0.92	0.80
Internode length (cm)	0.82	0.86	0.87	0.96	0.59	0.83	0.87	0.81
Culm dry weight (g)	0.77	0.88	0.84	0.96	0.33	0.92	0.92	0.82
Culm volume (cm ³)	0.83	0.88	0.87	0.95	0.53	0.92	0.92	0.82
Culm density (g cm ⁻³)	0.81	0.83	0.74	0.89	0.84	0.91	0.91	0.82
Diameter of basal internode (mm)	0.80	0.87	0.84	0.95	0.27	0.83	0.93	0.90
Diameter of topmost internode (mm)	0.83	0.84	0.69	0.94	0.17	0.89	0.88	0.80
Total number of culms	0.67	0.82	0.63	0.90	0.53	0.77	0.77	0.77
Proportion of reproductive culms		0.84	0.64	0.92		0.72	0.85	0.45
Culms per footprint (# cm ⁻²)	0.74	0.81	0.84	0.88	0.65	0.77	0.89	0.88
Year 3								
Dry biomass yield (g/plant)	0.71	0.83	0.74	0.74	0.76	0.88	0.84	0.77
Compressed circumference (cm)	0.68	0.80	0.72	0.69	0.61	0.81	0.71	0.70
Basal circumference (cm)	0.66	0.91	0.70	0.79	0.44	0.81	0.73	0.71
Compressed circumference/basal circumference	0.63	0.79	0.73	0.60	0.59	0.79	0.66	0.69
Culm length (cm)	0.84	0.92	0.96	0.94	0.90	0.90	0.92	0.81
Culm node number	0.87	0.90	0.85	0.91	0.32	0.82	0.93	0.90
Internode length (cm)	0.79	0.90	0.83	0.93	0.30	0.68	0.89	0.83
Culm dry weight (g)	0.88	0.93	0.39	0.93		0.95	0.95	0.89
Culm volume (cm ³)	0.86	0.93	0.94	0.93	0.83	0.92	0.93	0.89
Culm density (g cm ⁻³)	0.74	0.92	0.56	0.74		0.86	0.86	0.86
Diameter of basal internode (mm)	0.85	0.90	0.94	0.90	0.65	0.87	0.92	0.92

(Continues)

TABLE 7 (Continued)

Trait	HU	NEF	CSU	UI	KNU	ZJU	Northern trial locations	All trial locations
Diameter of topmost internode (mm)	0.77	0.91	0.84	0.91	0.78	0.87	0.92	0.83
Total number of culms	0.82	0.78	0.64	0.75	0.59	0.70	0.80	0.78
Proportion of reproductive culms	0.74	0.78	0.74	0.79	0.79	0.83	0.66	0.00
Culms per footprint (# cm ⁻²)	0.79	0.78	0.91	0.87	0.50	0.76	0.83	0.83

Note. HU = Hokkaido University; NEF = New Energy Farms; CSU = Colorado State University; UI = University of Illinois at Urbana-Champaign; KNU = Kangwon National University; ZJU = Zhejiang University.

at the northern trial sites, which will be useful for breeding biomass cultivars that have broad adaptation. Among all the non-ornamental *M. sinensis* groups, the S Japan group had on average the greatest total number of culms in year 3 at all locations tested (Data S2), although year 3 genetic correlations between total culm number and yield at each trial location were mostly low (Table 9). In contrast to the wild accessions from S Japan, the S Japan-derived ornamental group, which mostly included individuals selected for short stature, was with few exceptions not advantageous for yield (Figure 1), yet most prior breeding of *Miscanthus* in the US and Europe has been based on the ornamental germplasm. For subtropical environments (hardiness zone 8 or warmer) such as ZJU, we expect that the *M. sinensis* SE China/tropical, Sichuan basin, and S Japan groups will be the most promising source of *M. sinensis* parents to breed improved biomass cultivars. To the best of our knowledge, this is the first study to identify differences in yield potential among *M. sinensis* genetic groups for different production environments.

In addition to quantifying differences among *M. sinensis* genetic groups for yield, we also identified specific genotypes with outstanding yield potential for different production environments. At HU and NEF, two of the northern trial locations, where the biomass cultivar control triploid *M. × giganteus* '1993-1780' yielded well (~20 Mg/ha), we observed six *M. sinensis* genotypes at HU and one at NEF with greater yield than the control in year 3 (20.7–30.9 Mg/ha; Table 3); such high yields have commercial potential. Similarly, high yields (15.0–22.4 Mg/ha) have been previously reported for *M. sinensis* genotypes that were collected from Honshu, Japan and evaluated in Sweden, Denmark and Portugal (Clifton-Brown et al., 2001), and from a Chinese germplasm panel evaluated at a trial in Wuhan, China in which rare individuals had high year 2 yields (~20–38 Mg/ha) (Zhao et al., 2013). However, at our southern trial location, ZJU, dry biomass yields of the seven most productive *M. sinensis* genotypes (63.7–119.1 Mg/ha; Table 3) were similar to maximum experimental dry matter yields obtained for sugarcane in Australia, Hawaii, and Louisiana (Bischoff et al., 2008; Tew & Cobill, 2008) and for the C₄ grass *Echinochloa polystachya* growing in the Amazon floodplain with no water or nutrient limitation, which has been suggested to represent the maximum productivity of a C₄ crop (Piedade, Junk, & Long, 1991). Such high yields in *Miscanthus* have not been reported previously. The exceptionally high yields estimated for some *M. sinensis* genotypes at ZJU were likely due to a combination of highly conducive growing conditions and small-plot bias. In small plots and especially single-plant plots, a tall individual can lean out and take canopy space from neighboring plots containing shorter plants, which would otherwise be unavailable if the tall genotype had been grown in a large monoculture. Additionally, the growing conditions that favored high biomass production at ZJU included

TABLE 8 Genetic correlations between years 2 and 3 for each trait measured on *Miscanthus sinensis* genotypes grown at five northern field trial locations (HU, NEF, CSU, UI, and KNU; 37.9–43.1° N) or one southern location (ZJU; 29.8° N)

Trait	HU	NEF	CSU	UI	KNU	ZJU	Northern trial locations	All trial locations
Dry biomass yield (g/plant)	0.85	0.69	0.77	0.58	0.68	0.95	0.76	0.99
Compressed circumference (cm)	0.69	0.53	0.65	0.66	0.94	0.74	0.92	1.23
Basal circumference (cm)	0.67	0.67	0.89	0.83	0.91	0.87	0.90	1.00
Compressed circumference/basal circumference	0.56	0.61	0.58	0.67	0.78	0.68	0.93	1.14
Culm length (cm)	0.90	0.85	0.91	0.94	0.76	0.89	0.94	1.02
Culm node number	0.84	0.97	1.02	0.89	1.20	0.90	0.94	0.98
Internode length (cm)	0.76	0.88	0.98	0.89	0.76	0.68	0.92	0.91
Culm dry weight (g)	0.91	0.90	1.19	0.90			0.95	0.95
Culm volume (cm ³)	0.92	0.92	0.90	0.92	0.92	0.95	0.98	1.01
Culm density (g cm ⁻³)	0.66	0.78	0.45	0.66			0.88	0.88
Diameter of basal internode (mm)	0.91	0.90	0.84	0.87	0.99	0.99	1.01	0.99
Diameter of topmost internode (mm)	0.94	0.82	1.05	0.88	0.98	0.96	0.90	0.95
Total number of culms	0.85	0.83	0.95	0.72	0.59	0.50	0.85	0.67
Proportion of reproductive culms		0.93	0.55	1.15		0.38	0.93	0.86
Culms per footprint (# cm ⁻²)	0.56	0.85	0.94	0.86	0.96	0.70	0.82	0.78

Note. All traits were Box–Cox transformed. Genetic correlation was estimated as the genetic covariance of two traits divided by the square root of the product of the genetic variance of each trait (Equation 5).

HU = Hokkaido University; NEF = New Energy Farms; CSU = Colorado State University; UI = University of Illinois at Urbana-Champaign; KNU = Kangwon National University; ZJU = Zhejiang University.

high soil fertility and a high water table associated with the land having previously been in rice production, a growing season of ~10 month duration with a large number of growing degree days (USDA hardiness zone 9; Table 1), and a carefully managed trial. Thus, exceptionally high yields were achieved for some entries at ZJU; however, determining exactly how exceptional the yield potential is for each of these entries will require further testing in large-plot trials. Overall, the results of this study suggest that some *Miscanthus* genotypes, when grown in a humid subtropical climate such as in Zhejiang province China or the southern coastal plain of the US, may be able to achieve dry matter yields similar to that of sugarcane grown in the tropics.

For subtropical production environments where light to moderate freezes are common, select *Miscanthus* genotypes or intergeneric hybrids between *Miscanthus* and *Saccharum* (i.e., miscanes), may have an adaptive advantage over sugarcane. In Tifton, GA (31.5° N), Knoll et al. (2013) observed

that two new energycane selections (Ho 06-9001 and Ho 06-9002) had maximum dry matter yields greater than 34 Mg/ha, whereas maximum yields of the sugarcane cultivar controls were less than 15 Mg/ha, highlighting the importance of selecting biomass grass crops for adaptation to their potential production environments. Moreover, top yields of the energycane and sugarcane entries at Tifton were in year 2, followed by a substantial decline in year 3 (Knoll et al., 2013), which is typical for these short-lived (usually 3–5 years) crops. In contrast, *Miscanthus* yields in our study increased each year from years 2–4 (Data S2), which was consistent with previous studies documenting that *Miscanthus* typically reaches a yield plateau during years 2–5 and can remain highly productive for more than 10 years (Heaton et al., 2004; Jones & Walsh, 2001; Lewandowski, Clifton-Brown, Scurlock, & Huisman, 2000). Thus, for subtropical environments, some *Miscanthus* germplasm, such as the highest-yielding genotypes we observed at ZJU, may have both short-term and long-term biomass yield

TABLE 9 Genetic correlations between dry biomass yield and 14 yield-component traits measured in year 3 on *Miscanthus sinensis* genotypes grown at five northern field trial locations (HU, NEF, CSU, UI, and KNU; 37.9–43.1° N) or one southern location (ZJU; 29.8° N)

	HU	NEF	CSU	UI	KNU	ZJU	Northern trial locations	All trial locations
Compressed circumference (cm)	0.96	0.93	0.95	0.91	1.00	0.96	1.04	1.06
Basal circumference (cm)	0.95	0.24	0.79	0.71	0.61	0.76	0.64	0.50
Compressed circumference/ basal circumference	0.43	0.64	0.57	0.59	0.93	0.84	0.71	0.96
Culm length (cm)	0.65	0.46	0.72	0.49	0.57	0.88	0.63	0.52
Culm node number	0.55	0.50	0.39	0.16	0.64	0.48	0.57	0.64
Internode length (cm)	0.01	-0.16	0.29	0.16	0.43	0.38	-0.11	-0.41
Culm dry weight (g)	0.71	0.66	0.85	0.38			0.72	
Culm volume (cm ³)	0.61	0.48	0.68	0.33	0.51	0.85	0.62	0.58
Culm density (g cm ⁻³)	0.36	0.38	-0.87	0.32			0.51	
Diameter of basal internode (mm)	0.54	0.47	0.36	0.07	0.52	0.83	0.54	0.67
Diameter of topmost internode (mm)	0.44	0.37	0.59	0.39	0.17	0.66	0.48	0.29
Total number of culms	0.39	0.42	0.39	0.55	0.29	0.46	0.23	0.08
Proportion of reproductive culms	0.02	0.12	0.23	0.56		0.52	-0.19	ZH
Culms per footprint (# cm ⁻²)	-0.64	-0.04	-0.53	-0.08	-0.13	-0.18	-0.50	-0.53

Note. All traits were Box-Cox transformed. Genetic correlation was estimated as the genetic covariance of two traits divided by the square root of the product of the genetic variance of each trait (Equation 5).

HU = Hokkaido University; NEF = New Energy Farms; CSU = Colorado State University; UI = University of Illinois at Urbana-Champaign; KNU = Kangwon National University; ZJU = Zhejiang University. ZH=not calculated due to zero heritability.

advantages over sugarcane. Moreover, the energycanes Ho 06-9001 and Ho 06-9002 had a high proportion of ancestry from *Saccharum spontaneum*, a wild species with thin stems (Tew & Cobill, 2008), which suggests that a similar breeding strategy of combining commercial sugarcane genotypes with *M. sinensis* that are highly productive in and adapted to subtropical environments may result greater gains still.

Atypical *M. sinensis* genotypes that performed well at trial sites that were far north or far south of their collection sites were also observed, and these likely represent a valuable breeding opportunity. Though the *M. sinensis* SE China/tropical and Sichuan basin were the highest-yielding groups at ZJU, few genotypes of these southern groups performed well at the northern trial sites. However, rare individuals from the SE China/tropical and Sichuan basin groups were among the highest-yielding entries at some of the northern trial sites, pointing the way toward a useful breeding strategy. If high yield potential from the SE China/tropical and Sichuan basin groups can be combined with greater winter hardiness from more northerly adapted *Miscanthus*, it may be possible to make large gains in yield potential for northern production environments. Similarly, rare genotypes from northern *M. sinensis* groups that performed well at ZJU may have

advantageous alleles that are rare or absent from southern groups. Thus, we might expect to obtain useful transgressive segregants by crossing individuals of differing provenance but each with complementary genes for high yield potential in a particular environment (Rieseberg, Archer, & Wayne, 1999).

The results of this study and previous studies indicate that interspecific progeny of *M. sinensis* and *M. sacchariflorus* were frequently, but not always, high yielding and vigorous, likely due to heterosis and/or transgressive segregation; this advantage has been conferred regardless of whether the progeny were diploid, triploid or tetraploid. In the current study, diploid *M. × giganteus* F₁ genotypes from 29° to 37° N in China were the highest-yielding entries at the northern trial locations, with yields at NEF and HU that were ~1.5- and ~2-fold greater than the high-yielding control, *M. × giganteus* '1993-1780' (Table 3, Figure 1). The best performing diploid *M. × giganteus*, PMS-430 and PMS-279, were collected from even lower latitudes (29–30° N) than the putative origin of *M. × giganteus* '1993-1780' (~35.4° N; Table S2), with the *M. sinensis* portion of their genome originating from the SE China/tropical group (Clark et al., 2014); we hypothesize that sufficient winter hardiness for the northern trial locations was

inherited primarily from the *M. sacchariflorus* parents. Yan et al. (2012) compared 31 *M. sinensis* and 48 *M. sacchariflorus* Chinese populations at Xilinhot, a cold-winter site in north China (43.9° N), and observed that for a given latitude of origin, overwintering survival of *M. sacchariflorus* accessions was substantially greater than for *M. sinensis* accessions. In contrast to the high yields of PMS-430 and PMS-279, the diploid *M. ×giganteus* ‘Purpurascens’ and the ornamental *M. sinensis* descended from it (Clark et al., 2015) were low-yielding compared to most *M. sinensis* (Table 3, Figure 1). Clifton-Brown et al. (2001) also found that diploid *M. ×giganteus* selected from a cross and grown at five locations in Europe typically, though not in all cases, had a yield advantage over selected *M. sinensis* genotypes collected in Japan. Uwatoko, Tamura, Yamashita, and Gau (2016) observed that seven new triploid *M. ×giganteus* genotypes collected from the wild in Japan had yields that were similar to the high-yielding control ‘1993-1780’, when grown in a field trial in Koshi, Japan (32.9° N). Matumura, Hasegawa, and Saijoh (1987), studying a cross between diploid *M. sinensis* and tetraploid *M. sacchariflorus*, observed that one triploid progeny had biomass yields intermediate to its parents but its tetraploid sibling yielded about twice as much as its highest-yielding parent. Thus, the development and testing of new *M. ×giganteus* genotypes are expected to be an important breeding strategy for obtaining new higher-yielding cultivars of *Miscanthus*.

4.2 | Yield-component traits for predicting *M. sinensis* yields

Compressed circumference, which serves as an easily measured proxy for culm diameter multiplied by the square root of the number of culms (genetic correlation of 0.85 across all sites in year 3), was a strong predictor of yield in the current study (genetic correlations ≥ 0.91 for each trial location; Table 9) and the best predictor of yield among all the yield-component traits we evaluated. Both yield and compressed circumference had a larger environmental component than many other traits (Table 7), but the high genetic correlation between them indicated that the phenotypic variation attributable to genotype followed a very similar pattern between yield and compressed circumference. Culm length, which had a moderate to high genetic correlation with yield (0.46–0.88; Table 9), is also easily measured, and together with compressed circumference provides a three-dimensional model of overall plant size. Gifford, Chae, Swaminathan, Moose, and Juvik (2015) also observed a strong genetic correlation between compressed circumference and yield (0.88) and a moderate genetic correlation between plant height and yield (0.54) in an F_1 population of *M. sinensis* ‘Grosse Fontaine’ \times *M. sinensis* ‘Undine’ evaluated at Urbana, Illinois. Similarly, Slavov et al. (2014) observed a moderate genetic correlation between

plant height and yield (0.65) in a panel of 138 *M. sinensis* genotypes phenotyped near Aberystwyth, UK. Previously published phenotypic correlations between height and yield for *Miscanthus* have also been mostly moderate, though less frequently high (Anzoua, Suzuki, Fujita, Toma, & Yamada, 2015; Clifton-Brown et al., 2001; Jezowski, 2008; Nie et al., 2016; Yan et al., 2012; Zhao et al., 2013) or low (Nie et al., 2016). Thus, compressed circumference and culm length (or height) are expected to be consistently good predictors of yield in *M. sinensis* populations, while being considerably less expensive to measure.

We also found that culm dry weight was a potentially useful predictor of yield, with genetic correlations ranging from 0.38 to 0.85 (Table 9). Similarly, Lim et al. (2014) observed a high phenotypic correlation between culm dry weight and yield (0.84) for 42 *M. sinensis* genotypes collected from South Korea, Kyushu, Japan, and southeastern Russia when evaluated in a field trial at Suwon, South Korea.

Genetic tradeoffs between total number of culms per plant, plant footprint, and culm diameter, volume and dry weight, were observed among the *M. sinensis* genotypes in this study. Genotypes with larger footprints tended to have fewer and sometimes thicker culms (Data S4). Also, genotypes with many culms typically had thinner and lighter culms. However, these tradeoffs were partial and varied in magnitude, sometimes substantially, by trial location. Such variability in partitioning tradeoffs is consistent with previous studies, which have reported highly diverse estimates for correlations between yield and number of shoots, and between yield and stem diameter for *M. sinensis* (Clifton-Brown et al., 2001; Gifford et al., 2015; Jezowski, 2008; Nie et al., 2016; Slavov et al., 2014; Yan et al., 2012). Moreover, exceptions to these tradeoffs may be important, as the broadly adapted and high-yielding S Japan group had the greatest average number of culms among the *M. sinensis* groups at all trial locations, but also the largest or nearly largest basal circumference at all locations tested (Data S2); diameter of basal internode was variable over locations but relatively high at HU, NEF, and ZJU. Thus a desirable ideotype for a biomass cultivar of *M. sinensis* may be a plant that has a large compressed circumference obtained via many thick and heavy culms, a large footprint, and long (>3 m) culms.

5 | CONCLUSIONS

Large genotype-by-environment interactions between the southern trial site, ZJU, and the northern trial sites indicates the need to breed *M. sinensis* separately for southern and northern production zones. However, good concordance of *M. sinensis* genotypic performance among northern trial locations, even those on different continents, will facilitate breeding for northern production zones. To establish a

new *M. sinensis* breeding program for high biomass yield, we recommend conducting field trials that are focused on genotypes belonging to the genetic groups that are expected to perform best in the target environment; these genetic groups are: S Japan for all environments, Sichuan and SE China/tropical for hardiness zones 8 or warmer, and Korea/N China and Yangtze-Qinling for hardiness zones 7 or colder. Our climate models suggest that additional germplasm collections should be performed in mountainous regions of China south of 32° N for hardiness zones 7 or colder, and in the Ryukyu Islands, Taiwan, and the Philippines for hardiness zones 8 or warmer.

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SUPPORTING INFORMATION

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