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RESEARCH ARTICLE

Biomass yield in a genetically diverse *Miscanthus sacchariflorus* germplasm panel phenotyped at five locations in Asia, North America, and Europe

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Abstract

Miscanthus is a high-yielding bioenergy crop that is broadly adapted to temperate and tropical environments. Commercial cultivation of *Miscanthus* is predominantly limited to a single sterile triploid clone of *Miscanthus* × *giganteus*, a hybrid between *Miscanthus sacchariflorus* and *M. sinensis.* To expand the genetic base of *M.* × *giganteus*, the substantial diversity within its progenitor species should be used for cultivar improvement and diversification. Here, we phenotyped a diversity panel of 605 *M. sacchariflorus* from six previously described genetic groups and 27 *M.* × *giganteus* genotypes for dry biomass yield and 16 yield-component traits, in field trials grown over 3 years at one subtropical location (Zhuji, China)

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and four temperate locations (Foulum, Denmark; Sapporo, Japan; Urbana, Illinois; and Chuncheon, South Korea). There was considerable diversity in yield and yield-component traits among and within genetic groups of *M. sacchariflorus*, and across the five locations. Biomass yield of *M. sacchariflorus* ranged from 0.003 to 34.0 Mg ha^{-1} in year 3. Variation among the genetic groups was typically greater than within, so selection of genetic group should be an important first step for breeding with *M. sacchariflorus*. The Yangtze 2x genetic group (=ssp. *lutarioriparius*) of *M. sacchariflorus* had the tallest and thickest culms at all locations tested. Notably, the Yangtze 2x genetic group's exceptional culm length and yield potential were driven primarily by a large number of nodes (>29 nodes culm^{-1} average over all locations), which was consistent with the especially late flowering of this group. The S Japan 4x, the N China/Korea/Russia 4x, and the N China 2x genetic groups were also promising genetic resources for biomass yield, culm length, and culm thickness, especially for temperate environments. Culm length was the best indicator of yield potential in *M. sacchariflorus*. These results will inform breeders' selection of *M. sacchariflorus* genotypes for population improvement and adaptation to target production environments.

KEYWORDS

bioenergy, biomass yield, genetic diversity, *Miscanthus* × *giganteus*, *Miscanthus sacchariflorus*, multilocation field trials

1 | INTRODUCTION

Miscanthus is a perennial, rhizomatous, self-incompatible, C_4 grass with promising potential as a bioeconomy crop. Uses of *Miscanthus* biomass include combustion to produce electricity and heat, anaerobic digestion to produce methane fuel, conversion to liquid fuels, gasification, production of paper and fiber boards, renewable and compostable food service packaging, improving tensile strength of concrete, absorbent barriers, biodegradable plastic, animal bedding, mulch, human consumption of young shoots, dietary fiber for pet food, and cattle feed (Acikel, 2011; Allphin, 2020; Clifton-Brown & Lewandowski, 2002; Engel-Smith, 2017; Heaton et al., 2004; Johnson et al., 2005; Lane, 2017; Pude et al., 2005; Sacks et al., 2013; Xue et al., 2015). Its high yields of biomass coupled with high sustainability make it particularly valuable for proposed use of carbon negative bioenergy with carbon capture and storage (BECCS) projects (Cobo et al., 2022; IPCC, 2022).

Commercial cultivation of *Miscanthus* in North America and Europe is currently limited predominantly to a single, high-yielding, sterile, triploid clone of *M.* × *giganteus*, named "1993–1780" for the type specimen at Kew Royal Botanic Gardens Herbarium (also referred to as "Illinois") (Głowacka et al., 2015; Hodkinson & Renvoize, 2001). *M.* × *giganteus* "1993–1780" was imported from Yokohama Japan to Denmark in the 1930s and was subsequently distributed widely (Linde-Laursen, 1993). Although *M.* × *giganteus* "1993–1780" is high-yielding and broadly adapted, it is insufficiently winter hardy in USDA hardiness zone 5b (average annual minimum temperature of −26.1 to −23.3°C) and colder, yet in the southern coastal plain of the United States, it flowers too early to optimize biomass production (Bonin et al., 2014; Clifton-Brown & Lewandowski, 2002; Kalinina et al., 2017). Moreover, large-scale cultivation of a single clone places it at risk of large losses from pests and diseases. Thus, there is a great need for new biomass cultivars of *Miscanthus*, especially those that can extend the range of adaptation. *M.* × *giganteus* is an interspecific hybrid between *M. sacchariflorus* and *M. sinensis* (Clifton-Brown et al., 2019; Hodkinson et al., 2002; Kalinina et al., 2017). Most efforts to develop new biomass cultivars of *Miscanthus* focus on breeding new selections of *M.* × *giganteus* because the interspecific progeny are typically more vigorous than their parents (Kalinina et al., 2017; Xiang et al., 2020). *M.* × *giganteus* are typically diploid, triploid, or tetraploid, depending on the ploidy of the parents and their propensity to produce unreduced gametes, though we have also observed pentaploid or hexaploid progeny (Chae et al., 2013).

Miscanthus sacchariflorus and *M. sinensis*, the progenitor species of *M.* × *giganteus*, are vast genetic resources for improving this crop and their close relative, sugarcane (Clark et al., 2018; Sacks et al., 2013). Notably, *M.*

sacchariflorus has the most northerly distribution in the genus, extending from ~50° N along the eastern portion of the Amur River watershed in eastern Russia, through the Korean peninsula, Japan, and northeastern China to ~28° N along the Yangtze River watershed, encompassing hardiness zones 3–8 (−40 to −12.2°C average annual minimum air temperature) (Clark et al., 2018; Clifton-Brown et al., 2008; Hirayoshi et al., 1957; Lee, 1964). Thus, *M. sacchariflorus* is a source of genes for winter hardiness (Dong et al., 2019) and chilling-tolerant C_4 photosynthesis (Pignon et al., 2019). In contrast to *M. sinensis*, which has a tufted form and prefers aerobic soils often on hills, *M. sacchariflorus* has long rhizomes with a spreading habit and is typically found on moist soils, especially near the edges of rivers and lakes. Thus, in addition to heterosis, each of *M.×giganteus* parental species can provide complementary traits, such as winter hardiness, tall and thick stems and tolerance to flooding from *M. sacchariflorus*, and high numbers of stems per area (footprint) and adaptation to subtropical or tropical environments from *M. sinensis*. As Matumura et al. (1986, 1987) documented, optimization of number of stems per area in *M.×giganteus* progeny is critical to obtaining high biomass yields per area. Therefore, the key yield-component traits that *M. sacchariflorus* contributes to *M.×giganteus* may be more important than *M. sacchariflorus*' yield per se. Previously, we identified six genetic groups of *M. sacchariflorus*, including three diploid groups ($2n = 38$; Korea/NE China/Russia, N China, and Yangtze [ssp. *lutarioriparius*]) and three tetraploid groups ($4n = 76$; N Japan, S Japan, and N China/Korea/Russia) (Clark et al., 2018; Sacks et al., 2013), but comparisons among and within all of these groups for biomass traits have not been previously made. Notably, *M. sacchariflorus* has greater genetic diversity than *M. sinensis*, even though the former currently has a narrower geographic range than the latter (Clark et al., 2018; Sacks et al., 2013).

Understanding how different genotypes within and among the genetic groups of *M. sacchariflorus* are adapted to different potential production environments and vary in yield potential and yield stability would be beneficial for breeding improved cultivars of *M.×giganteus*. Field performance of *M. sacchariflorus* has been studied in Europe (Clifton-Brown et al., 2001; Robson et al., 2013), North America (Clark et al., 2019; Kaiser et al., 2015), and Asia (Lim et al., 2014; Yan et al., 2012). However, few *M. sacchariflorus* genotypes have been evaluated outside of Asia and most prior studies were conducted at only one location. Moreover, to the best of our knowledge, no prior study has compared in field trials all the *M. sacchariflorus* genetic groups. Lim et al. (2014) evaluated 22 *M. sacchariflorus* accessions from China at a single location in Korea and found that accessions which originated from low latitudes typically had greater biomass yield, height, and stem diameter than those from high latitudes. Yan et al. (2012) compared 62 populations of *M. sacchariflorus* at three locations in north, central, and southern China, and observed significant genotype × environment (G × E) interactions for biomass yield and many other traits, though only accessions that originated from China were studied.

In the current study, we report on yield performance for a diversity panel of 605 *M. sacchariflorus* genotypes grown over 4 years at five locations: Denmark, China, Japan, South Korea, and USA. The *M. sacchariflorus* panel represents all of the previously identified genetic groups (Clark et al., 2018; Sacks et al., 2013) and covers the species entire known geographic range, with accessions collected from eastern Russia, China, South Korea, and Japan. The objectives of this study were to (1) quantify the range of variation for yield and yield-component traits in *M. sacchariflorus* and determine how location of origin and genetic group affect performance, (2) quantify G × E effects and determine how well performance at one trial site predicts performance at other trial sites, and (3) determine which yield-component traits contribute most to biomass yield and how this varied among and within genetic groups. The information generated from this study will inform breeders' selection of *M. sacchariflorus* genotypes for population improvement and contribute to future breeding of *M.×giganteus* for improved yield and adaptation in target production environments.

2 | MATERIALS AND METHODS

2.1 | Plant materials and field trials

In total, 632 *Miscanthus* genotypes were studied, including 605 *M. sacchariflorus* and 27 *M.×giganteus* genotypes. The *M. sacchariflorus* genotypes were comprised of 351 diploid and 254 tetraploid genotypes belonging to six genetic groups including: N China 2x ($n = 58$), Korea/NE China/Russia 2x ($n = 241$), Yangtze 2x (ssp. *lutarioriparius*) ($n = 52$), S Japan 4x ($n = 77$), N Japan 4x ($n = 67$), and N China/Korea/Russia 4x ($n = 110$). The *M. sacchariflorus* accessions were collected from the wild across East Asia (Figure 1, Data S1). The 27 *M.×giganteus* genotypes also originated from the wild and were comprised of 15 tetraploids, five diploids, and seven triploids, including the triploid biomass cultivar *M.×giganteus* "1993–1780" (Figure 1; Data S1). The *Miscanthus* genotypes were vegetatively propagated using rhizomes by the collectors at their respective institutions and distributed for planting at each of the trial sites in 2015.

Ramets of the genotypes were established via rhizomes in a multilocation field trial at five locations: Foulum, Denmark by Aarhus University (AU); Sapporo, Japan by Hokkaido University (HU); Urbana, Illinois by the University of Illinois (UI); Chuncheon, South Korea by Kangwon National University (KNU); and Zhuji, China by Zhejiang University (ZJU) (Table 1; Figure 4). The trial sites AU, HU, UI, and KNU were located from 37.9 to 56.5° N and have temperate growing conditions, while ZJU (29.8° N) is subtropical. The field trials were planted in spring 2015 in a randomized complete block design with one to four replications per location, depending on availability of space at each site. Single plant plots were established at a spacing of 2.0 m × 2.0 m and with an estimated plant density of one plant per 0.64–2.89 m^2 depending on the alley spacing maintained at a given trial site (Table 1). The number of *M. sacchariflorus* genotypes established at each non-United States location ranged from 473 to 541, and at UI was 206, the lower number resulting from quarantine restrictions on plants imported to the United States (Table 1). At UI, the *M. sacchariflorus* genotypes were primarily from the Korea/NE China/Russia 2x and the S Japan 4x genetic groups. During the establishment year (year 1), irrigation was provided as needed at all locations. Irrigation was also provided at UI and KNU in year 3 and KNU in year 4 due to drought. Nitrogen fertilizer was applied to the field trials each spring in the following amounts: 100 kg ha^{-1} at HU and UI; 80 kg ha^{-1} at KNU; 50 kg ha^{-1} at AU; and 19 kg ha^{-1} at ZJU. Weeds were controlled mechanically and/or with herbicides as needed. Climate data were recorded at weather stations near each of the field trial locations (Figure S1).

2.2 | Phenotypic data collection and analyses

Phenotypic data were collected in years 2 (2016), 3 (2017), and 4 (2018); the 17 traits are defined in Table 2. At each location in years 2–4, biomass was harvested in late autumn after dormancy by cutting the culms ~20 cm above the ground. Dry biomass yield was measured by either weighing the entire oven-dried harvested plant material or by recording the fresh weight at harvest and oven-drying a subsample to correct the total mass for moisture content. In years 2 and 3, data were collected for 16 additional yield-component traits: compressed circumference (CC), basal circumference (BC), ratio of CC to BC (CC/BC), culm length, culm node number, internode length, culm dry weight (CmDW), culm volume (CmV), culm density (CmDW/V), culm diameter at basal internode, culm diameter at last internode, total number of culms, proportion of reproductive culms, culms per area, first heading date, and overwintering ability (Table 2). At KNU and ZJU, data for all traits except overwintering ability were also recorded in year 4. For *Miscanthus* field trials, we typically collected yield and yield-component data in years 2 and 3 only, with greater emphasis on year 3, as the plants had then reached maturity. Due to limited replication at some trial sites, we also took yield data in year 4 to improve estimates of accession values and facilitate future genome-wide association and genomic selection studies. However, for this study, we focus on year 3 results, where we have both yield and yield-component data for all trial sites.

For each trait, the Box-Cox procedure implemented in the R package MASS (Kafadar et al., 2002) was used to determine the appropriate transformation to mitigate the impact of potential violations of model assumptions of normality (Table S1). Using transformed data, four types of analyses of variance (ANOVAs) were conducted for each location and for multiple locations, each with fixed and completely random models. A linear model was fit for each location with genotype as fixed effects to estimate least square means (LS means) for each genotype and trait in every location and year combination using the R package lsmeans (Lenth, 2016). We also fit multilocation linear models with genotype within location as fixed effects to estimate the LS means of the genotypes across multiple locations for each trait. LS means were backtransformed to estimate their values in original measurement units. LS means of the genotypes for each trait in each location were compared to *M.* × *giganteus* "1993–1780" as a control (Figures 1 and 2).

Completely random model ANOVAs were conducted with SAS 9.4 procedure MIXED (SAS Institute, Inc.) to estimate variance components, which were subsequently used to calculate genotypic repeatabilities and proportion of total variance for each source. To estimate variance components within individual locations, a linear model was fit where γ is the transformed phenotypic value; μ is the overall mean; G, R, Y, GY and ε are the effect of the genotype, replicate, year, genotype × year, and the random error term, respectively (Equation 1).

$$\gamma_{ijk} = \mu + G_i + R_j + Y_k + GY_{ik} + \varepsilon_{ijk} \tag{1}$$

Multilocation random effect models were also fit for each trait to estimate the effects of genotype, location (L), year, replication within location, genotype × location, genotype × year, and genotype × location × year interaction for the four northern locations (AU, HU, UI, and KNU) and across all trial locations (Equation 2).

$$\gamma_{ijkl} = \mu + G_i + L_j + Y_k + RL_{lj} + GL_{ij} + GY_{ik} + GLY_{ijk} + \varepsilon_{ijkl} \tag{2}$$

The extent to which phenotypic variation was accounted for by difference among genotypes that were clonally replicated was estimated as the repeatability

TABLE 1 Field trial locations for phenotypic evaluations of *Miscanthus sacchariflorus* and *M.* × *giganteus*.

Site code	Location	Latitude (°N)	Longitude (°E)	Elevation (m)	Hardiness zone[a]	Soil type	Number of *M. sacchariflorus* genotypes	Number of *M.* × *giganteus* genotypes
AU	Foulum, Denmark	56.5	9.6	48	8a	Sandy loam soil	532	26
HU	Sapporo Japan	43.1	141.3	11	7b	Humic andosol	473	25
UI	Urbana, Illinois, USA	40.1	−88.2	223	5b	Drummer silty clay loam[b]	206	4
KNU	Chuncheon, South Korea	37.9	127.8	97	6b	40% Alfisols, 60% Inceptisols	541	26
ZJU	Zhuji, China	29.8	120.2	44	9a	Silty loam	529	24

Site code	Planting date	Number of replicates	Alley spacing between plots (cm)	Estimate plot area (m^2)	Length of growing season (days)[c]			Growing degree days (days °C)[d]		
					Y2[e]	Y3	Y4	Y2	Y3	Y4
AU	5-Aug-2015 to 14-Aug-2015	1	100	1.00	169	189		844	739	
HU	18-Jun-2015	4	80	1.44	212	239		1604	1558	
UI	28-Apr-2015	4	101.6	0.97	235	228		2209	1950	
KNU	15 and 28 May-2015	1	120	0.64	259	257	210	2396	2275	2147
ZJU	36-May-2015 to 5-June-2015	1	30	2.89	283	260	250	3258	3131	3098

Abbreviations: AU, Aarhus University; HU, Hokkaido University; KNU, Kangwon National University; UI, University of Illinois at Urbana-Champaign; ZJU, Zhejiang University.

[a] USDA hardiness zone estimate based on plantmaps.com.

[b] US Dept. of Agriculture—Natural Resources Conservation Service. (2015). Drummer Series. National Cooperative Soil Survey. Available at https://soilseries.sc.egov.usda.gov/OSD_Docs/D/DRUMMER.html.

[c] Length of growing season was calculated from planting date (year 1) or the first date of spring emergence (year 2–4) to the last dormancy date in autumn.

[d] To calculate the growing degree days, the daily average temperature was summed across all the days in the growing season. The daily average temperature was calculated as the mean of the daily maximum and minimum temperature between 10 and 30°C. If the daily maximum temperature was >30°C, then it was set to 30°C and if the daily minimum temperature was <10°C, then it was set to 10°C.

[e] Trials were planted from April to August 2015, so years (Y) 2, 3, and 4 were 2016, 2017, and 2018, respectively.

TABLE 2 Yield and yield-component traits measured in multilocation field trials of *Miscanthus sacchariflorus*.

Trait	Description
Dry biomass yield (g plant^{-1} or Mg ha^{-1})	Single plant plots on 2 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. To express yield in Mg ha^{-1}, the yield in grams was divided by the plot area
Compressed circumference (cm)	Circumference of plant compressed at the midpoint (halfway between the soil surface and the tip of the tallest panicle) such that adjacent tillers were in contact without air gaps
Basal circumference (cm)	Uncompressed circumference at the base of plant, including all tillers
Compressed circumference/ basal circumference (proportion)	Compressed circumference divided by basal circumference, to estimate the proportion of the plant's footprint filled by stems
Culm length (cm)	Measure of the tallest flowering culm from the soil surface to the tip of the panicle if present, else to the highest part of the highest leaf
Culm node number (count)	The number of nodes of the tallest culm of each plant
Internode length (cm)	Culm length divided by culm node number
Culm dry weight (g)	Dry weight of the tallest culm, after oven-drying at 60°C until no change in weight is observed
Culm volume (cm^3)	Calculated from the culm length, culm diameter at basal internode (DBI), and diameter at last internode (DLI), assuming the culm is shaped like a frustum: $\text{culm volume} = \frac{\pi \times \text{culm length}}{12} \times \left(\text{DBI}^2 + \text{DLI}^2 + (\text{DBI} \times \text{DLI})\right)$
Culm density (g cm^{-3})	Culm dry weight divided by culm volume
Culm diameter at basal internode (mm)	The outer internode diameter of the culm measured at the first basal internode, after removal of leaves
Culm diameter at last internode (mm)	The outer internode diameter of the culm measured at the last internode, after removal of leaves
Total number of culms (count)	The number of culms of each plant
Proportion of reproductive culms	Total number of culms divided by the number of culms that reached reproductive stages
Culms per area (# m^{-2})	The number of culms divided by the area of the plant footprint as estimated from the basal circumference
First heading date (calendar date)	Date of emergence of the first inflorescence of a plant ≥1 cm beyond the flag leaf sheath
Overwintering ability (proportion)	If the plant was alive in autumn of the prior year and alive in spring of the current year, then overwintering ability = 1. If the plant was alive in autumn of the prior year but dead in spring of the current year, then overwintering ability = 0. If plant was dead or missing in the prior autumn, then overwintering ability is missing data (N/A). Values averaged for entries

(T) (Equation 3), where σ^2_G, σ^2_{GL}, σ^2_{GY}, σ^2_{GLY}, and σ^2_ε are the variance components estimated for the genotype, genotype × location, genotype × year, genotype × location × year, and the residual; n_L is the number of locations, n_Y is the number of years, n_{LY} is the number of locations × years, and n_{LR} is the total number of replications across all locations. Variance components from the random effects model in Equations (1) and (2) were used to estimate the repeatability (Equation 3) within individual locations and across multiple locations for the 16 traits.

$$T = \frac{\sigma^2_G}{\sigma^2_G + \sigma^2_{GL}/n_L + \sigma^2_{GY}/n_Y + \sigma^2_{GLY}/n_{LY} + \sigma^2_\varepsilon/n_{LR}} \quad (3)$$

To further explore the importance of variation among and within genetic groups, random effects ANOVAs were conducted to estimate the proportion of total phenotypic variance of biomass yield contributed by the effects of location, replicates within location, year, year × location, genetic group (D), genetic group × location, genetic group × location group × year, genotype within genetic group, genotype within genetic group × location, genotype within genetic group × year, and genotype within genetic group × location × year (Equation 4).

$$y_{ijklm} = \mu + L_i + RL_{ij} + Y_k + YL_{ik} + D_l + DL_{li} + DY_{lk} + GD_{ml} + GDL_{mli} + GDY_{mlk} + GDLY_{mlik} + \varepsilon_{ijklm} \quad (4)$$

Pearson correlation coefficients between the yield-component traits and dry biomass yield were calculated for each location and year to identify the strength, significance, and direction of association among the traits. Additionally, we assessed the correlations between years 2 and 3 for each of the traits at each trial location, as well as the correlations between all pairs of locations in each year for all traits.

$$
\begin{aligned}
Y_{\mathrm{PS}} = {} & b_0 + b_1\mathrm{BIO1_P} + b_2\mathrm{BIO1_P^2} + b_3\mathrm{BIO1_P BIO1_S} + b_4\mathrm{BIO2_P} + b_5\mathrm{BIO2_P^2} + b_6\mathrm{BIO2_P BIO2_S} \\
& + b_7\mathrm{BIO9_P} + b_8\mathrm{BIO9_P^2} + b_9\mathrm{BIO9_P BIO9_S} + b_{10}\mathrm{BIO12_P} + b_{11}\mathrm{BIO12_P^2} + b_{12}\mathrm{BIO12_P BIO12_S} \\
& + b_{13}\mathrm{BIO14_P} + b_{14}\mathrm{BIO14_P^2} + b_{15}\mathrm{BIO14_P BIO14_S} + b_{16}\mathrm{BIO18_P} + b_{17}\mathrm{BIO18_P^2} + b_{18}\mathrm{BIO18_P BIO18_S} \\
& + b_{19}\mathrm{T\_SAND_P} + b_{20}\mathrm{T\_SAND_P^2} + b_{21}\mathrm{T\_SAND_P T\_SAND_S} + b_{22}\mathrm{T\_SILT_P} + b_{23}\mathrm{T\_SILT_P^2} \\
& + b_{24}\mathrm{T\_SILT_P T\_SILT_S} + b_{25}\mathrm{T\_REF\_BULK\_DENSITY_P} + b_{26}\mathrm{T\_REF\_BULK\_DENSITY_P^2} \\
& + b_{27}\mathrm{T\_REF\_BULK\_DENSITY_P T\_REF\_BULK\_DENSITY_S} + b_{27}\mathrm{Lat_P} \\
& + b_{28}\mathrm{Lat_P^2} + b_{29}\mathrm{Lat_P Lat_S} + \varepsilon_{\mathrm{PS}}
\end{aligned}
\tag{5}
$$

To predict the likely phenotypic values of any *M. sacchariflorus* genotype at specific target planting locations (e.g., UI and ZJU) based on the accession's location of origin, we used the universal response function described by Wang et al. (2010). Linear models were fitted for observed dry biomass yield, culm length, and heading date for year 3 using bioclimatic variables from WorldClim (Fick, 2017) and soil attributes from the Harmonized World Soil Database (Wieder et al., 2014). The R step function (Zhang, 2009) was implemented in both directions to initially select the bioclimatic and soil variables to include in the model, retaining only variables that were significant at $p \leq 0.05$. The significant bioclimatic variables selected for the biomass yield model included annual mean temperature (BIO1), mean diurnal temperature range (BIO2), mean temperature of driest quarter (BIO9), annual precipitation (BIO12), precipitation of driest month (BIO14), and precipitation of warmest quarter (BIO18). The soil attributes incorporated in the model included topsoil sand fraction (T_SAND), topsoil silt fraction (T_SILT), and reference topsoil bulk density (T_REF_BULK_DENSITY). Latitude was also included in the model. The variables used for the culm length and heading date models are detailed in Table S2. The universal response function is a quadratic function (Equation 5) where the dependent variable Y_{PS} is the transformed phenotypic value of the genotypes; b is the parameter to be estimated; BIO1, BIO2, BIO9, BIO12, BIO14, BIO18, T_SAND, T_SILT, and T_REF_BULK_DENSITY are the bioclimatic and soil variables in the 'P' provenance location and 'S' planting location, respectively; and ε_{PS} is the error term. The interaction between the variable in the planting and provenance locations is included in the model, but we excluded the variables at the planting location, as this resulted in prediction of extreme phenotypic values as previously noted by Clark et al. (2019), due to management differences among trials. The resulting model was used to predict the optimal provenance for target planting sites.

3 | RESULTS

3.1 | Yield performance

Miscanthus sacchariflorus genotypes had large and significant variation for biomass yield among and within the genetic groups and across the five locations, with yields ranging from 0.003 to 47.4 Mg ha^{-1} across years 2–4 of this study (Figures 1 and 3; Table 3; Data S1). Year 3 biomass yield of *M. sacchariflorus* genotypes ranged from 0.003 to 34.0 Mg ha^{-1} (Figure 1; Data S1). Notably, at the southern location, ZJU, the Yangtze 2x (ssp. *lutarioriparius*) genetic group outyielded (average of 20.1 Mg ha^{-1} in year 3) significantly and substantially all other *M. sacchariflorus* genetic groups and the *M.×giganteus* "1993–1780" control at that location (Figure 1). In the higher latitude locations (HU, UI, and KNU), average yields among the best-performing *M. sacchariflorus* genetic groups (S Japan 4x, N China/Korea/Russia 4x, N China 2x, and Yangtze 2x (ssp. *lutarioriparius*)) were similar and moderate (averages ranging from 2.7 to 13.4 Mg ha^{-1} in year 3) (Figure 1; Data S1). The lowest yielding genetic groups across all the trial locations were N Japan 4x and Korea/NE China/Russia 2x, which were collected in the most northerly latitudes (35–49° N) and tended to flower early, especially at UI, KNU, and ZJU, our lowest latitude trial sites (Figure 2). Additionally, in the higher latitude locations, many of the five triploid and 14 tetraploid *M.×giganteus* genotypes included in the study outyielded the *M. sacchariflorus* genotypes, likely due to hybrid vigor and a more optimal number of stems per area than either parent (Figure 1; Data S1). Moreover, a few tetraploid *M.×giganteus* genotypes outyielded the commercial triploid *M.×giganteus* control at HU where it yielded expectedly well (Figure 1; Data S1).

The top 10% high-yielding *M. sacchariflorus* genotypes in year 3 in the higher latitude locations ranged from 12.6 to 33.8 Mg ha^{-1} in HU, 11.3–17.8 Mg ha^{-1} in

TABLE 3 Results of analyses of variance, using a fixed or completely random model, for dry biomass yield (Mg ha^{-1}) among three northern locations (HU, UI, and KNU; 37.9–43.1° N), and among all locations (one southern [ZJU; 29.8° N] and three northern locations), over 3 years, six genetic groups, genotypes within genetic groups, and their interactions for 577 of *Miscanthus sacchariflorus* genotypes (Equation 4).

	Northern locations					**All locations**				
	Fixed		**Random**			**Fixed**		**Random**		
Source of variation	**df**	***p***	**Variance**	**Prop. total variance**	***p***	**df**	***p***	**Variance**	**Prop. total variance**	***p***
Loc	2	<0.0001	0.31	0.01	0.4625	3	<0.0001	0.00	0.00	
Year	2	<0.0001	2.51	0.08	0.3080	2	<0.0001	3.53	0.09	0.2247
Rep within Loc	6	<0.0001	0.06	0.00	0.1076	6	<0.0001	0.07	0.00	0.1007
Loc × Year	8	<0.0001	6.34	0.20	0.0912	11	<0.0001	4.00	0.10	0.0447
Group	5	<0.0001	5.99	0.19	0.0779	5	<0.0001	10.96	0.27	0.0901
Group × Loc	17	<0.0001	1.02	0.03	0.1422	23	<0.0001	6.22	0.15	0.0079
Group × Year	17	<0.0001	0.00	0.00		17	<0.0001	0.00	0.00	
Group × Loc × Year	53	<0.0001	2.06	0.06	0.0002	71	<0.0001	2.23	0.05	<0.0001
Genotype within Group	522	<0.0001	1.58	0.05	<0.0001	562	<0.0001	1.35	0.03	<0.0001
Genotype within Group × Loc	418	<0.0001	2.99	0.09	<0.0001	1015	<0.0001	4.45	0.11	<0.0001
Genotype within Group × Year	1037	<0.0001	0.00	0.00		1685	<0.0001	0.00	0.00	
Genotype within group × Loc × Year	754	<0.0001	0.85	0.03	0.0014	4153	<0.0001	0.46	0.01	0.0094
Residual		<0.0001	8.42	0.26	<0.0001		<0.0001	7.83	0.19	<0.0001

Note: The sources of variation include Loc, location; Rep, replication; Group, Genetic group; *p* = significance.

UI, and 8.4–18.3 Mg ha^{-1} in KNU, with the genotypes being primarily from the genetic groups Yangtze 2x (ssp. *lutarioriparius*), S Japan 4x, N China/Korea/Russia 4x, and N China 2x (Data S2). The best-performing genotypes at HU were from the Yangtze 2x (ssp. *lutarioriparius*) genetic group (Figure 1), including accessions JY105 (33.8 Mg ha^{-1}) and JY090 (24.2 Mg ha^{-1}). At ZJU, the top-yielding *M. sacchariflorus* genotypes ranged from 15.8 to 34.0 Mg ha^{-1} with genotypes primarily from the Yangtze 2x (ssp. *lutarioriparius*) group and rare high-yielding genotypes from N China/Korea/Russia 4x, S Japan 4x, and N China 2x groups (Data S2). The highest year 3 yield among all the *M. sacchariflorus* genotypes was 34.0 Mg ha^{-1} at ZJU for Yangtze 2x (ssp. *lutarioriparius*) accession JY059 (Figure 1; Data S2). The top yielding genotypes differed in each trial site with only two *M. sacchariflorus* genotypes, JY022 and JY072 from genetic group Yangtze 2x (ssp. *lutarioriparius*) being high-yielding in HU, KNU, and ZJU in year 3 (these were not planted at UI). In addition, two genotypes of *M.* × *giganteus*, JM2014-S-2 and JM2014-K-6, were high yielding in both northern (HU and KNU) and southern (ZJU) locations in year 3 (Data S2).

In comparison to *M. sacchariflorus*, year 3 yield of the *M.* × *giganteus* "1993–1780" control was typically higher at each location except AU (where all entries had low yield), with AU, HU, UI, KNU, and ZJU averaging 1.04, 22.8, 26.0,

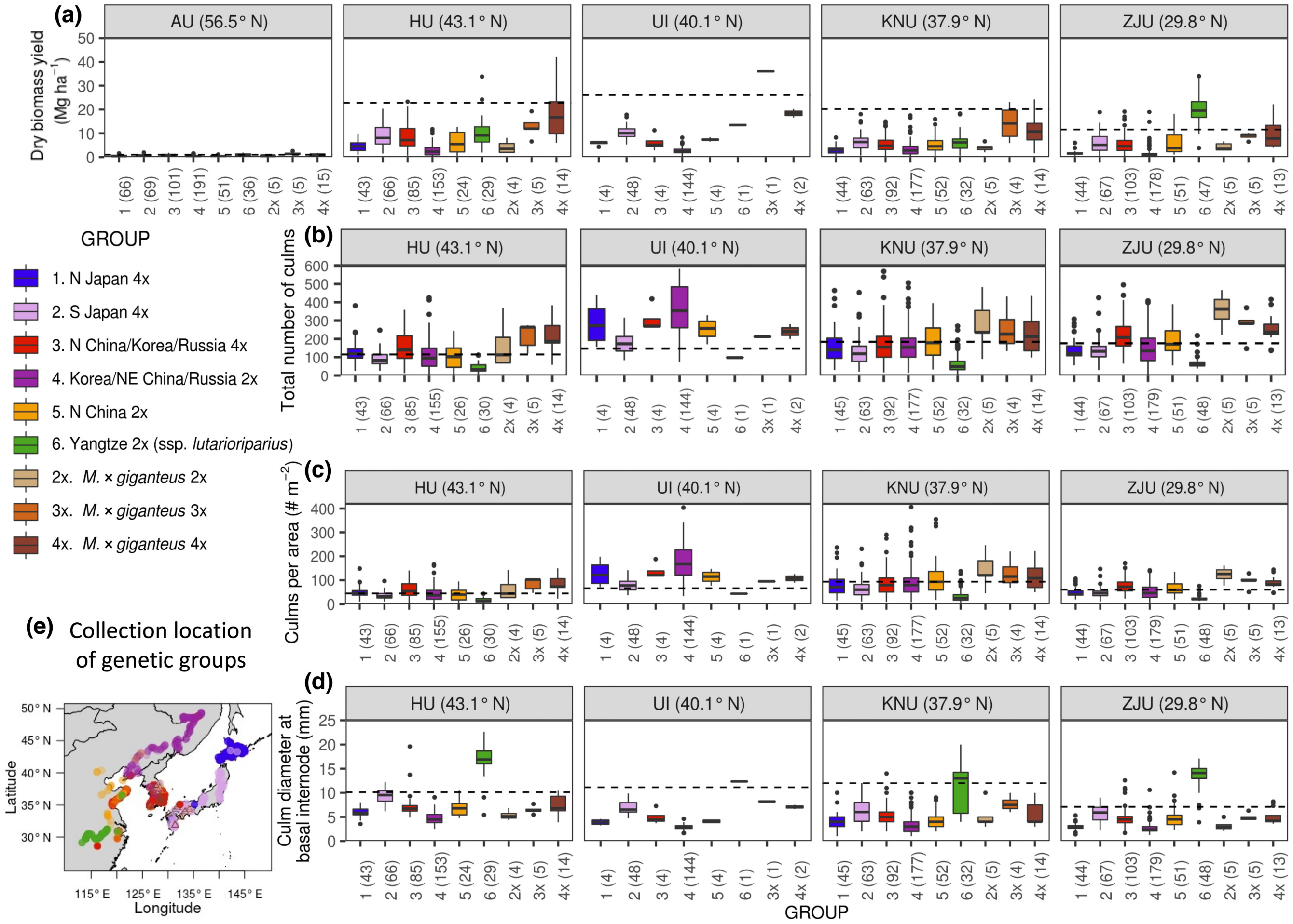

FIGURE 1 Box plots depicting variation in genotype least square means for (a) dry biomass yield, (b) total number of culms, (c) culms per area, and (d) culm diameter at basal internode, among six genetic groups of *Miscanthus sacchariflorus* and three ploidy groups of *M.* × *giganteus* in year 3 at five field trial locations (AU, HU, UI, KNU, and ZJU). The collection locations of the genotypes used in this study are shown in (e), and the colors represent the genetic groups of *M. sacchariflorus* and ploidy groupings *M.* × *giganteus*. The genetic groups of *M. sacchariflorus* were previously identified by discriminant analysis of principal components (Clark et al., 2018). The number of genotypes studied in each genetic/ploidy group is indicated in parentheses below the *x*-axis. The dashed horizontal line indicates the value of the control, triploid *M.* × *giganteus* '1993–1780' (however, it was not also included in the boxplot for *M.* × *giganteus* 3x). Boxplots have a middle box representing the interquartile range (25th–75th percentile in the data), the horizontal line within the box is the median, while the upper and lower whiskers represent data points that are (1.5 × interquartile range) above or below the interquartile range, and the points beyond that are outliers. AU, Aarhus University; HU, Hokkaido University; KNU, Kangwon National University; UI, University of Illinois at Urbana-Champaign; ZJU, Zhejiang University.

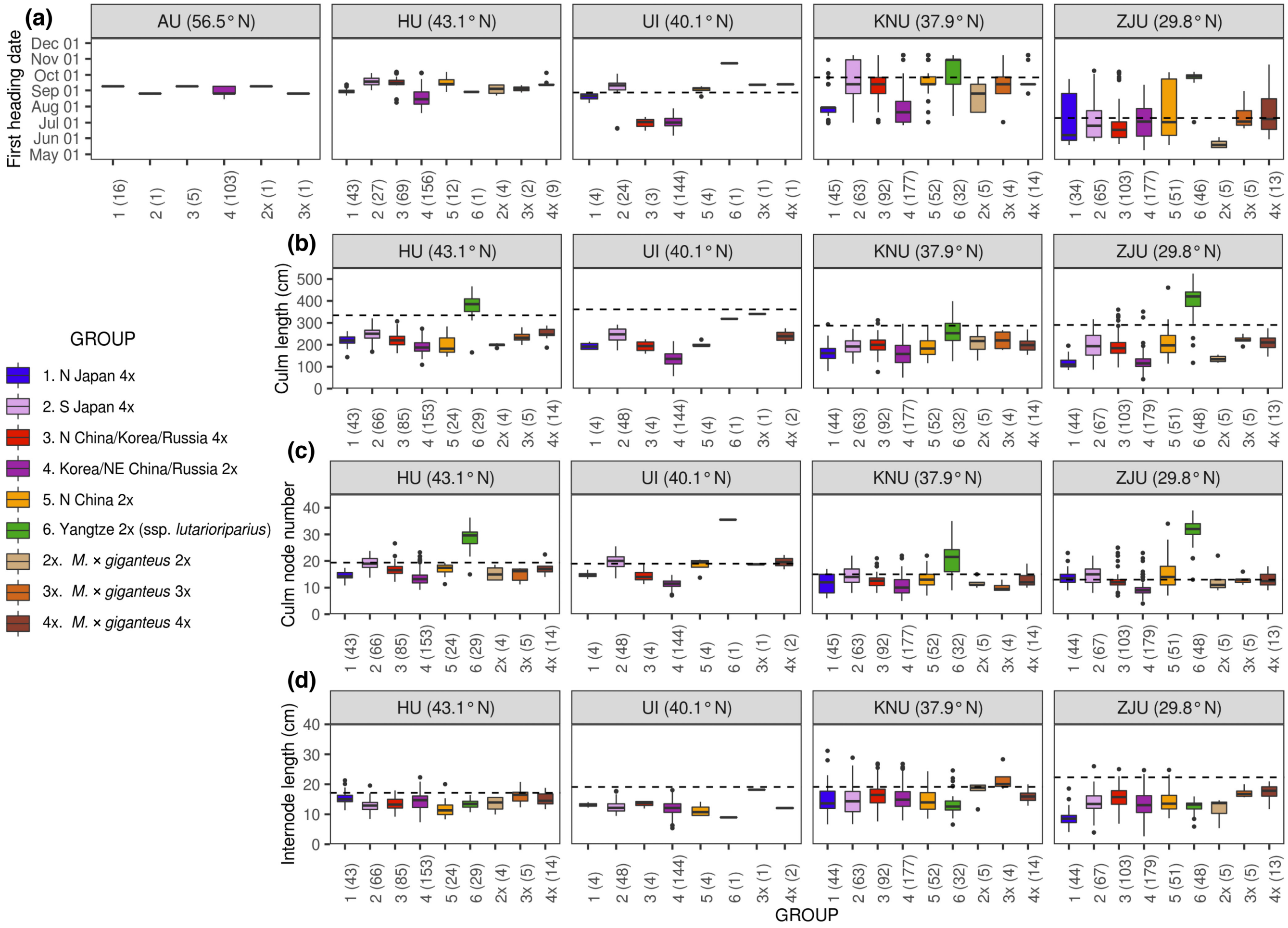

FIGURE 2 Boxplots depicting variation in genotype least square means for (a) first heading date, (b) culm length, (c) culm node number, and (d) internode length, among six genetic groups of *Miscanthus sacchariflorus* and three ploidy groups of *M.*×*giganteus* in year 3 at five field trial locations (AU, HU, UI, KNU, and ZJU). The genetic groups of *M. sacchariflorus* were previously identified by discriminant analysis of principal components (Clark et al., 2018). The number of genotypes studied in each genetic/ploidy group is indicated in parentheses below the *x*-axis. The dashed horizontal line indicates the value of the control, triploid *M.*×*giganteus* '1993–1780' (however, it was not also included in the boxplot for *M.*×*giganteus* 3*x*). Boxplots have a middle box representing the interquartile range (25th–75th percentile in the data), the horizontal line within the box is the median, while the upper and lower whiskers represent data points that are (1.5×interquartile range) above or below the interquartile range, and the points beyond that are outliers. AU, Aarhus University; HU, Hokkaido University; KNU, Kangwon National University; UI, University of Illinois at Urbana-Champaign; ZJU, Zhejiang University.

20.2, and 11.6 Mg ha^{-1}, respectively (Figure 1, horizontal dashed lines). However, there were some exceptionally high-yielding *M. sacchariflorus* genotypes at HU, KNU, and ZJU and nearly all of the Yangtze 2x (ssp. *lutarioriparius*) genetic group at ZJU outyielded the *M.*×*giganteus* "1993–1780" control in year 3 (Figure 1). Similar third-year biomass yields of *M.*×*giganteus* "1993–1780" in temperate regions have been reported, ranging from 15 to 37 Mg ha^{-1} in North America (Arundale et al., 2014; Clark et al., 2019; Kaiser et al., 2015) and 10–29 Mg ha^{-1} in Northern Europe (Dubis et al., 2019; Heaton et al., 2008). The lower yield performance of *M.*×*giganteus* "1993–1780" at the most northern location, AU, was likely due to late planting, a short growing season, low-growing degree days, and sandy soils with limited moisture retention capacity (Table 1), whereas in the southern location, ZJU, early flowering (in mid-July), limited its effective growing season (Figure 2).

Large and significant differences among locations were observed for biomass yield of *M. sacchariflorus* genotypes (Figure 1; Table 3). In particular, AU recorded the lowest *M. sacchariflorus* yields among trial sites each year, averaging 0.2, 0.6, 0.9 Mg ha^{-1} in years 2, 3, and 4, respectively (Figure 1; Data S1). In contrast to AU, biomass yields of *M. sacchariflorus* genotypes for the other higher latitude locations (HU, UI, and KNU) were similar and averaged 3.8, 5.6, and 7.5 Mg ha^{-1} in year 2, 3, and 4, respectively (Figure 1; Data S1). Due to the low yields at AU (Figure 1), for the remainder of this manuscript, estimates of performance at the higher latitude

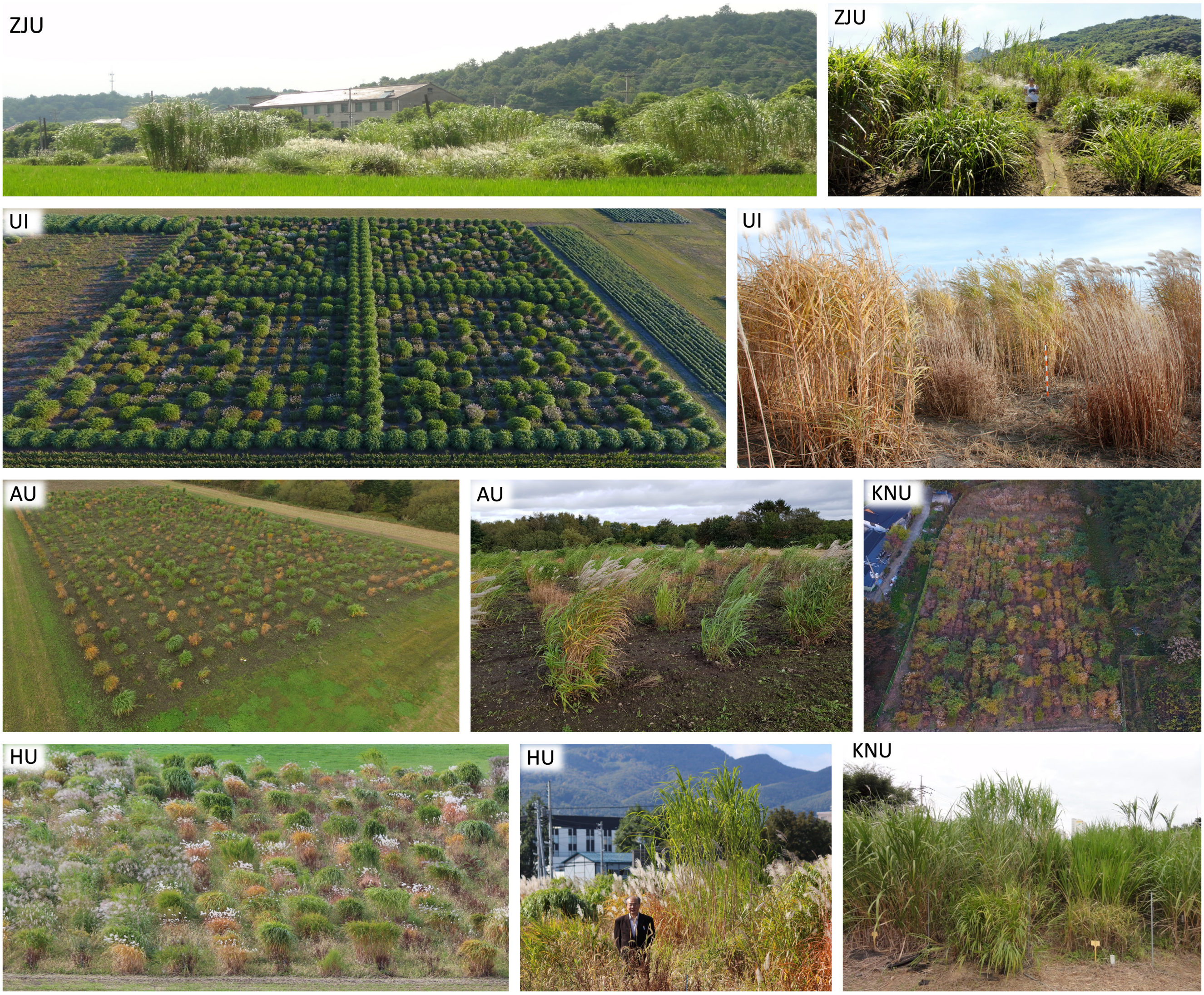

FIGURE 3 Photos of *Miscanthus* growing in the five trial sites showing both an overview of the field trial and a section of the field that captures the diversity in the genotypes. Note that the tallest plants at each location were from the Yangtze 2x (ssp. *lutarioriparius*) genetic group. AU, Aarhus University; HU, Hokkaido University; KNU, Kangwon National University; UI, University of Illinois at Urbana-Champaign; ZJU, Zhejiang University.

locations will include only HU, UI, and KNU, and we now refer to these as the northern locations. Biomass yields of *M. sacchariflorus* genotypes in the southern location, ZJU, averaged 4.1, 5.2, and 7.6 Mg ha^{-1} in years 2, 3, and 4, respectively (Figure 1; Data S1). ZJU had the longest growing season and largest number of growing degree days among the five trial locations, which likely contributed to the high yields recorded there (Table 1; Figure 1).

Analyses of variance components revealed that the largest sources of variation when southern and northern locations were evaluated together (i.e., all locations) were genetic group (27%), residual (19%), and genetic group × location (15%) (Table 3). In contrast, when just the northern locations were analyzed (i.e., excluding ZJU), the largest sources of variation were residual (26%), location × year (20%), and genetic group (19%), whereas genetic group × location accounted for only 3% of the total variation (Table 3). If proportions of total variance for group × location and group × location × year are summed, then this accounts for 9% in the northern locations and 20% in all locations. Thus, the interaction between genetic group and location was about two to five times smaller when just the northern locations were compared than when the southern location ZJU was included in the analysis, indicating that adaptation of different *M. sacchariflorus* genetic groups was greatly affected by large differences in latitude of trial sites. Genotype within genetic group accounted for 3% of the total variation in the analysis of all locations and similarly 5% of the total variation in the analysis of northern locations (Table 3). The interactions between genotype within genetic group and location summed to 12% of the total variation for either all locations or northern locations (Table 3). Thus, there was substantial variation both among and within genetic groups of *M. sacchariflorus*, but interactions with location were larger with genetic group than among genotypes within genetic groups when both northern and southern locations were included in the analysis.

3.2 | Yield-component traits

Large and significant differences among and within *M. sacchariflorus* genetic groups were observed for most yield-component traits at each of the trial locations (Figures 1 and 2; Figures S3–S18; Table S3). LS means for *M. sacchariflorus* genotypes in year 3 ranged from 3 to 900 for total number of culms per plant, 43–525 cm for culm length, 4–39 culm node numbers, and 1–23 mm for culm diameter at basal internode (Figures 1 and 2; Figures S3–S18; Data S1). The main effect of genetic group was the largest proportion of the total phenotypic variance for culm length (57%), culm node number (62%), culm volume (78%), culm diameter at basal internode (74%), and culm diameter at last internode (28%) in year 3 (Table S3). The Yangtze 2x (ssp. *lutarioriparius*) genetic group had the longest culms, the most nodes, and the thickest stems at all locations, which contributed to its superior yield at ZJU and to a lesser extent at HU (Figures 1–3). Indeed, culm length and thickness of the Yangtze 2x (ssp. *lutarioriparius*) genetic group were exceptional, averaging 398.5 cm and 13.7 mm, respectively, at ZJU (ranging 117.6–525.0 cm for culm length and 3.9–17.1 mm for culm diameter at basal internode) and 378.3 cm and 16.9 mm, respectively, at HU (ranging 164.9–466.7 cm for culm length and 5.4–22.6 culm diameter at basal internode). These values greatly exceeded that of the *M.* × *giganteus* "1993–1780" control even where it performed best in the north at HU (averaging 334.4 cm for culm length and 10.1 mm for culm diameter at basal internode) and UI (averaging 361.5 cm for culm length and 11.1 mm for culm diameter at basal internode) (Figures 1 and 2; Data S1). However, the Yangtze 2x (ssp. *lutarioriparius*) genotypes had fewer culms than all the other *M. sacchariflorus* genetic groups at each location (Figure 1). The genetic group S Japan 4x ranked second to Yangtze 2x (ssp. *lutarioriparius*) in culm volume across all growing locations, and genotypes from N China/Korea/Russia 4x and N China 2x groups were also relatively tall in the northern locations (Figure 2).

Heading date also varied greatly and significantly, especially among the genetic groups and locations, extending across 7 months from May to November each year (Figure 2; Figure S17; Table S3). Heading date had a significant negative correlation with latitude of the genotype's collection site (i.e., latitude of origin; $r = -0.396$, $p < 0.0001$), indicating that genotypes collected from northern latitudes tended to flower earlier, having fewer nodes and shorter culms (Figure 2; Figure S19). We observed the widest variability in heading dates within the northern-adapted genetic groups (N China 2x, Korea/NE China/Russia 2x, S Japan 4x, N Japan 4x, and N China/Korea/Russia 4x) when grown in more southern latitudes KNU (37.9° N) and ZJU (29.8° N) ation field trial at five location(Figure S18). In ZJU, genotypes of the southern adapted genetic group Yangtze 2x (ssp. *lutarioriparius*) mostly flowered in September, which was much later than other genetic groups at this site (Figure 2).

3.3 | Repeatabilities and correlations

Repeatabilities (Equation 4) between years 2 and 3 for biomass yield, culm length, culm node number, culm volume, culm diameter at the basal internode, and heading date were high to moderate at each location (≥0.58; Table 4). Moderate to low repeatabilities (0.20–0.69) were observed for internode length, BC, culm density, and ratio of CC to BC (Table 4). The moderate to high repeatability for most traits, particularly in the replicated sites, suggests that there is good potential for selection with moderate replication. Repeatability across years 2 and 3 was higher within individual locations than among multiple locations (Table 4). Additionally, we observed higher estimates of repeatability across all locations than in just the northern locations for all traits except for first heading date (Table 4).

Correlations between locations for yield were highest between UI and HU in year 3 (0.70) and 4 (0.75) and between UI and ZJU in years 2–4 (0.78, 0.60, and 0.73, respectively) (Table 5). The correlation between UI and KNU for yield was moderate in year 2 (0.66) but low in year 3 (0.42) and negligible in year 4 (0.04). AU had the lowest correlation in biomass yield to other locations, likely due to the low yield recorded at AU (Table 5).

Most yield component traits correlated significantly with biomass yield in year 3 ($p < 0.05$; Table 6). The most important yield-component traits for *M. sacchariflorus* were culm length, culm diameter at basal internode, and culm node number, with high correlations to yield for over all locations of 0.81, 0.77, and 0.74, respectively (Table 6). Similar to the estimates of repeatabilities, correlations estimated for across all locations were higher than estimates from just the northern locations for most traits (Table 6). For just the northern locations, correlations between culm length, culm diameter at basal internode, and culm node number with biomass yield were moderate to low at 0.60,

TABLE 4 Genotypic repeatabilities between year 2 and 3 for yield and yield-component traits of *Miscanthus sacchariflorus* for each of three northern field trial locations (HU, UI, and KNU; 37.9–43.1° N), one southern location (ZJU; 29.8° N), across all northern locations, and across all locations (Equation 3).

Trait	HU	UI	KNU	ZJU	Northern locations except AU	All locations except AU
Dry biomass yield (g plant^{-1})	0.84	0.97	0.66	0.94	0.63	0.69
Compressed circumference (cm)		0.74		0.85		
Basal circumference (cm)		0.69	0.45	0.67		
Compressed circumference/basal circumference (proportion)		0.67		0.18		
Culm length (cm)	0.74	0.96	0.70	0.96	0.58	0.69
Culm node number (count)	0.82	0.97	0.67	0.95	0.78	0.82
Internode length (cm)	0.66	0.68	0.32	0.66	0.24	0.32
Culm dry weight (g)	0.84	0.64				
Culm volume (cm^3)	0.88	0.96	0.79	0.95	0.71	0.79
Culm density (g cm^{-3})	0.20	0.00				
Culm diameter at basal internode (mm)	0.89	0.96	0.72	0.94	0.72	0.80
Culm diameter at last internode (mm)	0.45	0.92	0.66	0.80	0.44	0.54
Total number of culms (count)	0.79	0.78	0.52	0.81	0.27	0.33
Proportion of reproductive culms	0.52	0.74		0.6		
Culms per area (m^{-2})	0.79	0.78	0.53	0.81	0.27	0.33
First heading date	0.97	0.97	0.86	0.83	0.85	0.67

Note: The field trial locations were AU = Aarhus University; HU = Hokkaido University; UI = University of Illinois at Urbana-Champaign; KNU = Kangwon National University; ZJU = Zhejiang University.

Shaded areas represents the aid comparison of the numbers in each location and trait from low (deep blue) to high (red).

0.48, and 0.48, respectively (Table 6). With the exception of KNU, culm length had the highest positive correlation to biomass yield at each location in year 3, ranging from 0.59 at HU to 0.90 at ZJU (Table 6). Positive correlations (0.31–0.89) with biomass yield in year 3 in each location were observed for culm node number, culm dry weight, BC, and culm diameter at basal internode (Table 6). Heading date was also an important component trait of biomass yield, with a correlation of 0.67 over all locations and 0.48 at just the northern locations (Table 6). At each location, later heading dates were positively correlated with higher yields (0.24–0.79). In year 3, the total number of culms had a low positive correlation with biomass yield at HU (0.46), whereas at UI, it was low and negative (−0.46, likely biased by limited sampling of some genetic groups at this location), and at KNU and ZJU, it was negligible. Lower yielding genetic groups like the N Japan 4x and Korea/NE China/Russia 2x had numerous short and thin culms, whereas the higher yielding genetic groups like Yangtze 2x (ssp. *lutarioriparius*) and S. Japan 4x had fewer, but taller and more voluminous culms (Figures 1 and 2). However, there were exceptions. In particular, the N China/Korea/Russia 4x and N China 2x genetic groups had relatively high yields in the northern locations and numerous culms per plant. Internode length had negligible correlations with biomass yield in year 3 (Table 6).

There was a moderate to high correlation (0.62–0.85) between the culm length and culm node number in year 3 (Data S3). Conversely, we observed a low correlation (0.21–0.44) between culm length and internode length in year 3, indicating that the length of culms in *M. sacchariflorus* was primarily controlled by the number of nodes and not their elongation (Data S3). In all locations, except KNU, we observed moderate to high correlation (0.50–0.82) between heading date and culm volume, culm diameter at basal internode, and culm node number, indicating that late-flowering genotypes tended to be voluminous with more nodes than early flowering genotypes (Data S3).

4 | DISCUSSION

4.1 | Genetic diversity for yield and adaptation traits: Implications for breeding with *M. sacchariflorus*

A unique contribution of this study was the direct comparison, in common gardens at five field trial locations,

TABLE 5 Pearson's correlations among four northern field trial locations (AU, HU, UI, and KNU; 37.9–56.5° N), and one southern location (ZJU; 29.8° N) for dry biomass yield (Mg ha^{-1}) in years 2, 3, and 4 for *Miscanthus sacchariflorus* accessions.

	AU	HU	UI	KNU	ZJU
Year 2					
AU		0.23***	0.41***	0.23***	0.30***
HU	409		0.44***	0.29***	0.30***
UI	170	161		0.66***	0.78***
KNU	413	363	150		0.48***
ZJU	441	384	153	399	
Year 3					
AU		0.32***	0.44***	0.15**	0.22***
HU	385		0.70***	0.27***	0.45***
UI	168	154		0.42***	0.60***
KNU	412	348	150		0.25***
ZJU	444	369	156	401	
Year 4					
AU		0.42***	0.46***	0.08 ns	0.16***
HU	387		0.75***	0.13*	0.44***
UI	171	154		0.04 ns	0.73***
KNU	412	348	150		0.18***
ZJU	447	370	157	403	

Note: Correlations are shown above the diagonal, and the number of surviving genotypes in common between each pair of sites is shown below the diagonal.The field trial locations were AU = Aarhus University; HU = Hokkaido University; UI = University of Illinois at Urbana-Champaign; KNU = Kangwon National University; ZJU = Zhejiang University.

Abbreviation: ns, not significant.

*Significance at the 0.05 probability level; **Significance at the 0.01 probability level; ***Significance at the 0.001 probability level.

Shaded areas represents the aid comparison of the numbers in each location and trait from low (deep blue) to high (red).

of all six known genetic groups of *M. sacchariflorus*. The substantial variation for yield and yield-component traits observed among and within the genetic groups of *M. sacchariflorus* (Figure 1) indicates that genetic improvement of this species by selection on existing natural variation should be feasible. Variation among the genetic groups was typically greater than within, so selection of genetic group should be an important first step for breeding with *M. sacchariflorus*.

Notably, the Yangtze 2x (ssp. *lutarioriparius*) genetic group was observed to be an outstanding genetic resource with great potential for biomass improvement of *Miscanthus*. The Yangtze 2x (ssp. *lutarioriparius*) group was high yielding, with the tallest and thickest culms in both the northern and southern field trial locations (Figures 1 and 2; Data S1 and S2), indicating that it has the potential to contribute highly advantageous yield traits in a broad range of potential production environments. At ZJU especially, the Yangtze 2x (ssp. *lutarioriparius*) group had high yields, reaching 46.0 Mg ha^{-1} in year 4 and culms as tall as 5 m. Within the *Miscanthus* genus such long stems are rivaled only by accessions of *M. floridulus* and are similar to culm lengths typically observed in sugarcane (van der Weijde et al., 2013). Additionally, we know of no other *Miscanthus* germplasm with stems as thick as those we observed for the Yangtze 2x (ssp. *lutarioriparius*) group. *M. sacchariflorus* ssp. *lutarioriparius* has been previously reported to be vigorous and high yielding (~30 Mg ha^{-1}), with tall (3–7 m) and thick (10–25 mm) culms (Chen et al., 2005; Miao et al., 2021; Sacks et al., 2013; Zheng et al., 2019). However, to the best of our knowledge, the present study is the first to document that the Yangtze 2x (ssp. *lutarioriparius*) genetic group typically grows taller and thicker stems than all other *M. sacchariflorus* when cultivated together in the same northern or southern production environments, with the best-performing ssp. *lutarioriparius* genotypes far exceeding those of the other genetic groups. In addition to high biomass yield, prior studies have documented that ssp. *lutarioriparius* has excellent flooding tolerance (Xue et al., 2020), high water use efficiency, and high photosynthesis rates (Yan et al., 2015).

Given that the Yangtze 2x (ssp. *lutarioriparius*) group is endemic to the middle and lower reaches of the Yangtze River watershed (27° to 31° N), and thus well adapted

TABLE 6 Pearson's correlations between dry biomass yield and 15 yield-component traits in year 3 for *Miscanthus sacchariflorus* genotypes grown at four northern field trial locations (AU, HU, UI, and KNU; 37.9–56.5° N), one southern location (ZJU; 29.8° N).

Trait	AU	HU	UI	KNU	ZJU	Northern locations except AU	All locations except AU
Compressed circumference			0.24***		0.63***		
Basal circumference			0.64***	0.47***	0.46***		
Compressed circumference/ basal circumference			0.03 ns		0.49***		
Culm length		0.59***	0.86***	0.36***	0.90***	0.60***	0.81***
Culm node number		0.52***	0.84***	0.31***	0.86***	0.48***	0.74***
Internode length		0.00 ns	0.19**	0.03 ns	0.05 ns	0.12**	0.07 ns
Culm dry weight		0.48***	0.83***				
Culm volume		0.40***	0.81***	0.26***	0.86***	0.34***	0.72***
Culm density		0.31***	−0.59***				
Culm diameter at basal internode		0.53***	0.84***	0.35***	0.89***	0.48***	0.77***
Culm diameter at last internode		0.07 ns	0.68***	0.33***	0.67***	0.37***	0.64***
Total number of culms		0.46***	−0.46***	0.14**	−0.08 ns	0.14**	−0.03 ns
Proportion of reproductive culms		−0.51***	−0.18**		0.38***	−0.30***	−0.03 ns
Culms per area		0.46***	−0.46***	0.14**	−0.08 ns	0.12**	−0.04 ns
First heading date	0.24**	0.54***	0.79***	0.25***	0.61***	0.48***	0.67***

Note: The field trial locations were AU = Aarhus University; HU = Hokkaido University; UI = University of Illinois at Urbana-Champaign; KNU = Kangwon National University; ZJU = Zhejiang University.

Abbreviation: ns, not significant.

**Significance at the 0.01 probability level; ***Significance at the 0.001 probability level.

Shaded areas represents the aid comparison of the numbers in each location and trait from low (deep blue) to high (red).

to warm and humid subtropical conditions (Clark et al., 2018; Sun et al., 2010; Xue et al., 2020), its exceptional performance at our northern trial sites (HU, KNU, and UI; Table 1) was not expected. However, one potential limitation when breeding with ssp. *lutarioriparius* for temperate environments with cold winters was indicated by the modestly lower overwintering ability we observed for this group at AU, HU, and KNU relative to the other *M. sacchariflorus* groups (Table S5). At UI, the one accession of ssp. *lutarioriparius* tested in the current study overwintered, but those winters were relatively mild for central Illinois; however, in prior years, mature plantings at UI of this same clone and its hybrids with *M. sinensis* failed to overwinter (data not shown). Yan et al. (2012) reported low first-winter overwintering ability (<5%) of ssp. *lutarioriparius* at Xilinhot, Inner Mongolia (43.9° N; hardiness zone 4a, average annual minimum air temperature of −34.4 to −31.7°C), whereas in Qingyang, Gansu Province (35.7° N; hardiness zone 7b to 8a, average annual minimum air temperature of −15.0 to −9.4°C) overwintering and yield were high. Thus, for cold-temperate target production environments, it would be advantageous to conduct recurrent selection within the Yangtze 2x (ssp. *lutarioriparius*) group for overwintering ability and/or cross this group to a more winter hardy *M. sacchariflorus* genetic group, such as the Korea/NE China/Russia 2x group, followed by concurrent selection for yield traits and overwintering ability over multiple generations.

Zheng et al. (2019) previously documented high biomass potential of diploid *M.*×*giganteus* selections obtained by crossing *M. sacchariflorus* ssp. *lutarioriparius* with *M. sinensis* (32 Mg ha^{-1} on saline lands in the Yellow River Delta of China). However, diploids are fertile and therefore an invasive risk for fields where *Miscanthus* is non-native, such as in North America and Europe. Sterile triploid *M.*×*giganteus* is typically produced by crossing tetraploid *M. sacchariflorus* with diploid *M. sinensis*, as the latter species is nearly always diploid. Importantly for breeding, the Yangtze 2x (ssp. *lutarioriparius*) genetic group is predominantly diploid (Clark et al., 2018), which is a barrier to producing triploid *M.*×*giganteus*. However, natural tetraploids of ssp. *lutarioriparius* have been reported (Li et al., 2013; Sheng et al., 2016), and methods for artificially inducing tetraploids from diploid *Miscanthus*

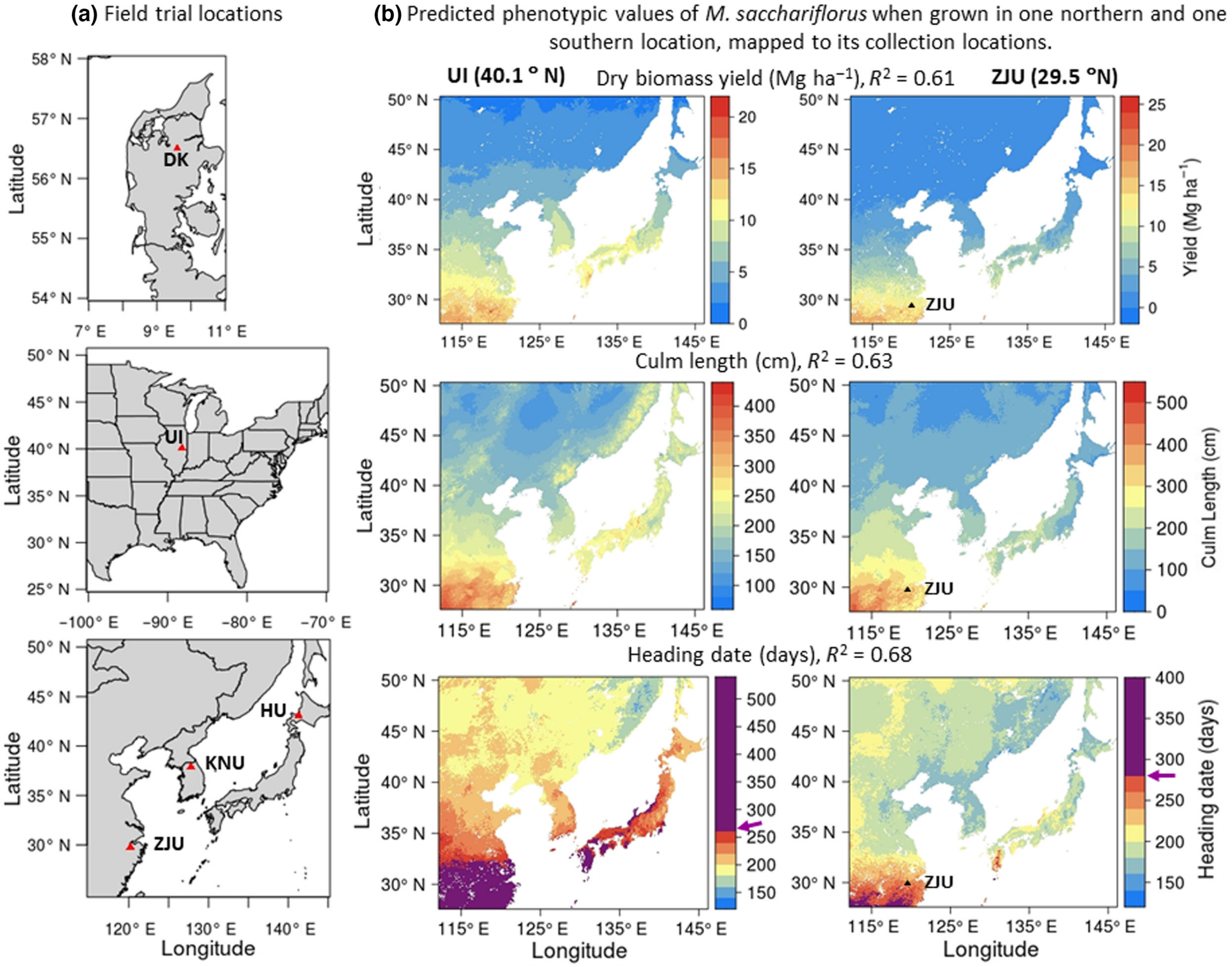

FIGURE 4 (a) Maps showing the field trial locations in Denmark, North America, and Asia. (b) Maps showing predicted dry biomass yield (Mg ha^{-1}), culm length (cm), and heading date (days) of *Miscanthus sacchariflorus* from throughout its native range when grown at a northern trial site, Urbana, Illinois (UI; 40.1° N) and (b) a southern trial site, Zhejiang University (ZJU; 29.5° N). Estimates were based on a universal response function, using soil and bioclimatic variables, the adjusted R^2 of the prediction models are indicated. For the southern trial site, ZJU, the tallest and highest yielding genotypes are predicted to originate from primarily the lower Yangtze River watershed, including Zhejiang, Fujian, Anhui, Jiangxi, Hubei, and Hunan provinces in China. For the northern trial site, UI, the highest yielding genotypes are predicted to originate from Miyazaki and Kagoshima prefectures in Japan, as well as Zhejiang, Fujian, and Jiangxi provinces in China. The purple arrows indicate the length of the growing season (days) in a given trial site, predicted heading dates beyond this (shown in purple) are of genotypes not likely to flower in a given trial location.

have been developed (Chae et al., 2013; Głowacka et al., 2009; Petersen et al., 2003). Thus, the Yangtze 2x (ssp. *lutarioriparius*) group should be useful for breeding improved, high-yielding *M.*×*giganteus*, albeit with some effort needed to fully use this genetic resource for breeding triploids.

Additionally, the S Japan 4x and the N China/Korea/Russia 4x genetic groups were also observed to be promising genetic resources for *Miscanthus* biomass improvement (Figures 1 and 2), and differences between them offer opportunities for breeding. In particular, in our field trials, the S Japan 4x group typically had fewer but thicker and longer stems than the N China/Korea/Russia 4x group, though both groups had relatively high yields (Figures 1 and 2). Both groups also included some highly promising outlier genotypes. The phenotypic differences between the two groups suggest that different combinations of stem traits can result in similarly high biomass yields. *M.*×*giganteus* accessions typically have intermediate numbers of culms per area relative to their parents, or numbers similar to the cespitose *M. sinensis* parent, which has many culms per area, and in contrast to the long-rhizome spreading habit of the *M. sacchariflorus* parent, which has few culms per area. Matumura et al. (1986,

1987) documented that optimized culm number per area contributes to high biomass yields in *M.×giganteus*, and more recent data from Zheng et al. (2019) further support this concept. The triploid *M.×giganteus* genotype most widely grown for biomass production originated from a S Japan 4x parent (Clark et al., 2018). To date, efforts in the United States to breed new triploid *M.×giganteus* have primarily used tetraploids from the S Japan 4x group as parents to combine with diploid *M. sinensis*. In addition to the phenotypic differences between the S Japan 4x and the N China/Korea/Russia 4x genetic groups, Clark et al. (2018) determined that they originated from independent polyploidization events. Thus, the N China/Korea/Russia 4x genetic group is a unique and promising germplasm resource for breeding *M.×giganteus*, and this group may interact with *M. sinensis* in different ways than the also valuable S Japan 4x group.

At the temperate northern trial sites, most *M. sacchariflorus* genotypes had inferior yields compared to *M.×giganteus* "1993–1780," as observed in prior studies (Dubis et al., 2019; Lim et al., 2014). However, we identified a few *M. sacchariflorus* genotypes, belonging primarily to the S Japan 4x and N China/Korea/Russia 4x genetic groups, that outperformed the *M.×giganteus* "1993–1780" control with high yields ranging from 23.3 to 33.8 Mg ha^{-1} in year 3 at HU and 21.2–47.4 Mg ha^{-1} in year 4 at HU and KNU (Data S2). These exceptional tetraploid genotypes from two of the best-performing genetic groups are expected to be especially good parents for improving biomass yield of *M.×giganteus*.

4.2 | Effect of environment and genotype × environment interactions

Although the *M. sacchariflorus* genotypes that originated at the lowest latitudes performed well in the northern trial locations, the most northern-originating accessions were not well adapted to the most southern trial location, ZJU (29.8° N). In particular, at ZJU, most of the accessions that originated from Russia typically flowered, went dormant, and regrew multiple times during the growing season, which likely accounted for the relatively low overwintering estimates, short culms, and low yield for the Korea/NE China/Russia 2x genetic group at this southern location (Figures 1 and 2; Table S5). All of the *M. sacchariflorus* genetic groups flowered later at the most northern trial locations, AU (56.5° N) and HU (43.1° N), than at the most southern trial location, ZJU (Figure 2), which was consistent with the conclusion of Jensen et al. (2012) that *M. sacchariflorus* is a quantitative short-day plant. For the low-latitude-adapted Yangtze 2x (ssp. *lutarioriparius*) genetic group, the effect of trial site latitude was extreme, with none of 36 genotypes having flowered at AU, and only one of 29 flowered at HU, but at the southern site ZJU, 46 out of 47 flowered in late September (Figure 2). Notably, the difference in flowering time between the most northern and most southern trial locations varied substantially by genetic group, indicating that adaptation to day length varied with location of origin. These differences in adaptation can profoundly affect the value of a genotype for biomass breeding. For example, the Korea/NE China/Russia 2x genetic group typically flowered in August at HU (43.1° N) but at UI (40.1° N) and ZJU (29.8° N), first flowering was in July, whereas first flowering for the S Japan 4x group was in September at HU and UI but typically in July at ZJU (Figure 2). There was also more variability within genetic groups for flowering time at ZJU than at the northern trial locations, suggesting that selection within northern-originating genetic groups (≥40° N) could quickly result in improved adaptation to lower latitude environments (e.g., ~30° latitude).

Similar patterns of response to photoperiod have been observed for other quantitative short-day, perennial, C4 grasses. In a controlled environment study of an *M. sinensis* mini-core panel, Dong et al. (2021) observed that accessions originating from high latitudes flowered under 15 h days, but short days (12.5 or 10 h) resulted in short, stocky plants that did not flower, whereas for accessions from low latitudes, shorter day lengths typically resulted in earlier flowering on tall plants. Nearly all the *M. sinensis* tested by Dong et al. (2021) grew the longest culms under the 15 h treatment, but the nearer to the equator an accession originated, the less of a difference in culm length between the short-day treatments and the 15 h day treatment. In switchgrass (*Panicum virgatum*) and big bluestem (*Andropogon gerardii*), short days (<12.5 h) can promote flowering and limit plant height but in populations that originated from high latitudes, the reduction in height and yield is typically great and dormancy can be induced prior to flowering even when temperatures are conducive to growth (Benedict, 1940; Castro et al., 2011; Mcmillan, 1959). Notably, extension of day length with low-intensity light prevented or reversed this dormancy in switchgrass (van Esbroeck et al., 2004). Results from our study suggest that short day lengths can also induce dormancy in *M. sacchariflorus*, but additional studies in controlled environments will help resolve this question unambiguously.

4.3 | Bioclimatic modeling identified regions where high-yielding *M. sacchariflorus* genotypes are most likely to be found

Linear modelling of soil and bioclimatic variables predicted that the best sources of high-yielding, tall, and late-flowering genotypes of *M. sacchariflorus* for the UI trial site (40.1° N) would be southeastern China (Zhejiang, Fujian, and Jiangxi provinces), southern Japan (Miyazaki and Kagoshima prefectures), and south Korea (~27–37° N) (Figure 4). The model also predicted genotypes with relatively tall culms for high-latitude, temperate-growing regions can be collected in Hokkaido prefecture in Japan, South Hamgyong province in North Korea, and the Primorsky Krai region in southeastern Russia. Predictions of heading date that exceeded the length of the growing season in the target growing region indicated that genotypes collected in these regions would likely not flower in the planting location (Table 1; Figure 4). Additionally, our models predicted that the tallest and highest yielding genotypes for both temperate and subtropical (~30° latitude) growing regions would originate from the lower Yangtze River watershed in central China (including Zhejiang, Fujian, Anhui, Jiangxi, Hubei, and Hunan provinces in China; Figure 4), which reflects the broad adaptability of the Yangtze 2x (ssp. *lutarioriparius*) genetic group. Thus, for *M. sacchariflorus*, late flowering and even no flowering can be adaptive and result in high yield-potential in high latitude production environments, but early flowering is non-adaptive in long-season low latitude production locations and is disadvantageous for yield in all production environments.

4.4 | High biomass yield of *M. sacchariflorus* was conferred primarily by tall, thick, and voluminous culms

In this study, heading date, culm length, culm diameter, and culm volume were the primary drivers of biomass yield for *M. sacchariflorus* (Table 6). Prior studies have also identified flowering time, culm length (or plant height), and stem thickness as important contributors to *Miscanthus* biomass yield (Clark et al., 2019; Clifton-Brown et al., 2001; Feng et al., 2015; Jezowski, 2008; Lim et al., 2014; Yan et al., 2012; Zub et al., 2011). However, a novel insight from the current study was that variation among *M. sacchariflorus* genotypes for plant height and consequently yield was conferred primarily by the number of nodes and not internode length. Thus, given that *M. sacchariflorus* is a quantitative short-day plant, breeding *M. sacchariflorus* or *M.×giganteus* for greatest height and yield-potential will likely require careful selection for optimal photoperiod response in the latitudes of target production regions. Whether flowering time and dormancy time in *M. sacchariflorus* and *M.×giganteus* can be fully decoupled, or whether there is an inherent tradeoff between height or yield and overwintering ability in high-latitude cold-temperate *Miscanthus* production environments, are questions worthy of further inquiry. The *M. sacchariflorus* genetic groups with the tallest and thickest culms typically had few culms per area, which hindered their ability to yield optimally. Therefore, to maximize biomass yield, *Miscanthus* breeding should focus on developing hybrids of *M.×giganteus* that incorporate the high culm length, culm diameter, and winter hardiness of *M. sacchariflorus* with the high number of culms per area from *M. sinensis*.

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CONFLICT OF INTEREST

The authors declare no competing interests.

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