Development and characterization of a Napier grass (Cenchrus purpureus Schumach) mapping population for flowering-time- and biomass-related traits reveal individuals with exceptional potential and hybrid vigor

Marco Sinche1 | Baskaran Kannan1 | Dev Paudel1 | Carlos Corsato1 | Yolanda Lopez1 | Jianping Wang1,2,3 | Fredy Altpeter1,2,3

Abstract
Napier grass is a tropical perennial C4 grass with superior biomass yield and quality. It is an important forage crop and a promising feedstock for lignocellulosic biofuel production. However, precise phenotyping and genotyping data to support the molecular breeding of Napier grass are scarce. A Napier grass F1 mapping population (segregating pseudo-F2 population) was generated by hybridizing genetically distant parents (N190 × N122) with contrasting flowering-time- and biomass-related traits. True F1 hybrids were confirmed with a simple sequence repeat marker, vegetatively propagated, and phenotyped in replicate field plots for 2 years (2 harvests per year) in Citra, FL, USA (29.40N). Near-normal distributions were observed for flowering date, plant height, number of tillers, stem diameter, and leaf width, confirming the quantitative nature of these traits. The annual dry biomass yield of F1 hybrids varied between 21.0 and 41.1 t ha−1. The highest-yielding F1 hybrids showed remarkable hybrid vigor exceeding the annual biomass production of the highest-yielding parental accession (26.9 t ha−1) by 52% and the biomass yield of the control “Merkeron” cultivar (29.2 t ha−1) by 41% over 2 years. F1 hybrids with delayed flowering time along with significantly increased biomass yield were also identified. Late-flowering accessions allowed maximum biomass harvest before formation and dispersal of seeds, reducing invasion risk. The developed mapping population will be an excellent resource for identifying quantitative trait loci and candidate genes for flowering- and biomass-related traits in Napier grass that will accelerate the improvement of this non-domesticated bioenergy crop.

KEYWORDS
biomass yield, C4, elephant grass, flowering time, hybrid vigor, invasiveness, Napier grass, Pennisetum purpureum, perennial biomass crop, tropical
1 | INTRODUCTION

Napier grass (*Cenchrus purpureus* Schumach., syn. *Pennisetum purpureum* Schumach.), also known as elephant grass, is a tall, perennial 
C$_4$ grass originating from East Africa where it is a major forage crop due to its high yield, excellent forage quality, and stress tolerance (Orodho, 2006). Napier grass is a sexually reproducing allotetraploid species ($2n = 4x = 28$, genome A'A'BB); meiosis is regular with up to 14 bivalents (Hanna, 1981; Jauhar, 1981). Napier grass has been identified as a potential bioenergy crop (Sanderson & Adler, 2008) with superior biomass yields in the southeastern U.S. (Woodard & Sollenberger, 2012) and other tropical and subtropical regions of the world (Negawo et al., 2017). Lignocellulosic biomass grasses require fewer inputs and have higher energy yields and lower greenhouse gas emissions than annual biofuel crops like corn (Adler et al., 2007). Thus, biofuel produced from non-food crops like high-yielding lignocellulosic grasses is expected to reduce the use of fossil fuels (Ho et al., 2011). However, bioenergy crops also have traits that can contribute to invasiveness such as rapid growth and production of wind-dispersed seeds (López et al., 2014; Sakai et al., 2001; Whitney & Gabler, 2008). As such, Napier grass has been listed as a Category I invasive species in the U.S. state of Florida (FLEPPC, 2011). Eradication of Napier grass using herbicides or by tilling has proven challenging (Cutts et al., 2011; Grey et al., 2020). Selection from existing germplasm collections has resulted in the identification of accessions with resistance to stunt disease and head smut (Kawube et al., 2014; Omayio et al., 2015).

Several studies have been conducted to evaluate the potential of Napier grass as a feedstock for biofuel production (Mochizuki et al., 2014; Pérez-Boada et al., 2014; Yasuda et al., 2012). Biomass yield is a complex trait that is determined by multiple traits with a range of heritability values such as plant height, tiller number, and stem thickness. A better understanding of the heritability of these traits will enable more targeted selection.

Generating a mapping population from parental accessions with contrasting traits and phenotyping the progeny can help to identify molecular markers associated with traits of interest or quantitative trait loci (QTL) to accelerate breeding cycles through marker-assisted selection. Mapping populations have been successfully used in sorghum to identify quantitative trait loci (QTLs) for leaf width, length, and yield; stem diameter; plant height; and tiller number (Hart et al., 2001; Lu et al., 2011; Murray et al., 2008; Rami et al., 1998). QTLs for plant height, flowering time, leaf length, and width have been found in maize (Austin & Lee, 1996; Raymond et al., 2004); and QTLs related to leaf yield, stem yield, plant height, and flowering time have been identified in the diploid *Miscanthus sinensis* (Atienza et al., 2003; Jensen et al., 2008). In Napier grass, microsatellite markers have been used to genotype 16 naturalized populations and accessions bred for biofuel production (López et al., 2014). Other studies have analyzed the genetic diversity of Napier grass accessions using simple sequence repeats (SSRs), amplified fragment length polymorphisms (AFLPs), single nucleotide polymorphisms, and insertion–deletion markers from pearl millet, maize, and sorghum (Kandel et al., 2016; Kawube et al., 2015; Wanjala et al., 2013). Recently, genotyping-by-sequencing was used to study the genetic diversity of 105 Napier grass accessions stored at the International Livestock Research Institute (Muktar et al., 2019). However, biparental mapping population studies which are critical for identifying QTLs are lacking in Napier grass.

The objectives of the present study were as follows: (1) to select and hybridize two Napier grass accessions with contrasting flowering-time- and biomass-related traits to generate a mapping population; (2) to identify true F$_1$ hybrids and carry out phenotyping for flowering-time- and biomass-related traits to determine the broad-sense heritability, distribution pattern, and variability of each trait; and (3) to generate late-flowering hybrids that attain a high biomass yield before flowering and have reduced invasion risk (as seed formation and dispersal are prevented by harvesting) and identify superior hybrids for cultivar development.
2 | MATERIALS AND METHODS

2.1 | Parental accessions

Parents used to generate the mapping population were identified by phenotype screening of the Napier grass germplasm collection at the University of Florida (Gainesville, FL, USA) and U.S. Department of Agriculture, Agricultural Research Service (USDA-ARS) in Tifton, GA, USA. Two accessions with contrasting biomass-related traits, N190 (PS 300086) and N122 (81-23A × N16), were selected; these accessions clustered into two separate major groups in a genetic distance analysis performed with AFLP markers (Harris-Shultz et al., 2010). Accession N190 produces significantly more biomass than N122, shows very late flowering, and has a reduced number of tillers with larger stem diameter; and accession N122 shows prolific tillering with thin stalks that flower early.

2.2 | Generation of F1 hybrids and establishment of the nursery

The selected accessions were vegetatively propagated and maintained in a temperature-controlled greenhouse at the University of Florida. In fall 2010, the flowering of the two parental lines was synchronized by modifying the photoperiod inside the greenhouse. An automated blackout curtain controlled by an astronomic clock that shortened the day length by 1 min daily starting at 12:00 h was used to induce flowering in N190, while N122 flowered under natural day length. Emerging inflorescences were covered with glassine pollination bags to control pollination (Figure 1a). The pollen from N122 was collected in the morning as soon as the anthers dehisced and immediately dusted onto N190 inflorescences. Self-pollination of N190 was prevented by covering emerging inflorescences with glassine pollination bags and by selecting inflorescences in which stigmas but no anthers had emerged. This procedure was repeated for each pollination over a period of 2–4 days as additional stigmas emerged. The pollination bags were kept on the inflorescences until the seed was harvested to prevent seed shattering.

In March 2011, seeds were germinated in trays containing potting mix (Fafard #2). When the seedlings reached a height of approximately 10 cm, they were transferred to plastic pots with a diameter of 15 cm and maintained in a greenhouse until they were approximately 30 cm tall with 2 or 3 tillers. The parental lines were clonally propagated from stem cuttings and planted in similar pots as a control. On May 18, 188 F1 hybrids and 20 clones from each parent were transplanted to the field at the Plant Science Research and Education Unit (PSREU; Citra, FL) as individual plants for evaluation and production of propagules (Figure 1b). These non-replicate single plant plots were arranged in a row of 20 plants, with a distance of 0.9 m between plants and 1.5 m between rows. All plants received 210, 40, and 160 kg ha$^{-1}$ of N, P, and K, respectively, in three splits on June 8, July 6, and August 3, 2011. A lateral irrigation system was used for a weekly irrigation of 25 mm when there was no rainfall. No pesticides were applied.

2.3 | Phenotyping and harvesting of F1 hybrids

At 6 months after planting, plant height, number of tillers, stem diameter, and leaf width were measured on all F1 hybrids and parental accessions. Plant height was measured from the ground to the youngest node of the tallest stem of the plant. The number of tillers corresponded to the total number of mature tillers per plant. Stem diameter was the average diameter of three mature tillers in the middle of the internode at a height of approximately 1 m. As the stems
were elliptical, the smallest diameter was measured for each stem. Leaf width was measured at the middle of the leaf for three fully expanded leaves. Flowering date was recorded weekly starting with the emergence of flag leaves in the last week of September until harvest. In December 2011, the plants were harvested to a 10 cm stubble height and the entire aboveground biomass was weighed. Three F1 hybrids from the original 188 did not produce viable propagules for vegetative propagation and were therefore excluded from subsequent analyses.

2.4 Establishment of vegetative progeny of F1 hybrids in replicate row plots

In December 2011, 185 F1 hybrids were vegetatively propagated and planted at PSREU (latitude: 29.40N, longitude: 82.17W). Each block contained one replicate of each genotype in randomized plots. There were six blocks each containing 40 rows, with each row containing five (1.8 m long) single row plots (Figure 1c). Rows were separated by a spacing of 1.8 m and blocks by 3.6 m. Each block was surrounded by border rows. Mature stalks were cut into 1.5 m long segments. In each plot, two stalks of the corresponding genotype were placed horizontally side-by-side in opposite directions in furrows that were 7–10 cm deep; they were then cut at the middle with a machete to suppress apical dominance and stimulate bud sprouting. The parental lines, N122 and N190, were propagated in a similar manner with two replicates in each block. “Merkeron,” a commonly used Napier grass cultivar, was also included in the experiment as a control. Two F1 hybrids of the 185 F1 propagated hybrids did not germinate; thus, a total of 183 F1 hybrids, two parental accessions, and the control cultivar “Merkeron” were evaluated in replicate plots.

During the four growing periods (i.e., January–August and August–December in 2012 and 2013), each block received 210, 40, and 160 kg ha−1 of N, P, and K, respectively, along with micronutrients (1% B, 0.06% Cu, 0.06% Fe, 0.15% Mn, and 0.14% Zn) in two split applications. If there was no rainfall, the plots were irrigated weekly with 25 mm of water. On September 24, 2012, 30 g ha−1 of imidacloprid (Admire®; Bayer Crop Science) was applied to suppress armored scale (Duplichionaspis diversgens).

2.5 Phenotyping and harvesting of F1 hybrids in replicate row plots

Plant height, stem diameter, leaf width, and total number of tillers and flowers per plot were recorded in July 2012 prior to harvest. Plants were harvested in August 2012 to a stubble height of 6–10 cm and the fresh biomass weight was measured (Figure 1d). To determine the dry weight of the biomass, a subsample of two mature tillers from each plot were cut and the fresh weight was measured, and the material was oven-dried at 60°C until a constant weight was reached. The ratio of dry weight to fresh weight of the subsample was used to calculate the dry matter (DM) yield of each plot. Plants were regrown and biomass-yield-related measurements were repeated prior to harvest as described above. Flowering date was recorded weekly, and the emerging flowers were counted and cut to prevent seed production and dispersal. Harvest started after the first freeze event (defined as an average air temperature at 2 m of <0°C for 15 min), which was on November 26, 2012. Data generated by the Florida Automated Weather Network for Citra (https://fawn.ifas.ufl.edu) were used to monitor rainfall and temperature during the growing season. Biomass-related traits were measured, the number of flowers was counted, and flowering date was recorded in the same manner in 2013. Summer harvest started on June 24, 2013 while the winter harvest occurred on January 23, 2014, before the first predicted freeze event.

2.6 Genotyping of F1 hybrids

Young and healthy leaf tissue was harvested from each genotype of the mapping population. DNA was extracted from the tissue as previously described (Dellaporta et al., 1983). DNA quality was verified by electrophoresis on a 2% agarose gel. An SSR marker (ppsr245: forward primer, CATGCACACATTGACATGA; reverse primer, CAATGGATCTTCCAATCGCT) that was polymorphic between the two parental accessions was used to genotype the F1 hybrids. The PCR was performed in a 10 μl volume containing 1 μl of 10× PCR buffer, 0.8 μl of MgCl2 (25 mM), 0.8 μl of dNTP (2 mM), 0.3 μl of Taq polymerase, 1.4 μl each of forward and reverse primers (2 mM), 1 μl of DNA template (10 ng/μl), and 4 μl of double-distilled water. The PCR program was as follows: initial denaturation at 95°C for 1 min; 10 cycles of 95°C for 30 s, 56°C for 30 s, and 72°C for 60 s; 30 cycles of 95°C for 30 s, 55°C for 30 s, and 72°C for 8 s; and final extension at 72°C for 7 min. PCR products were separated by non-denaturing polyacrylamide gel electrophoresis on a 6% polyacrylamide gel at 150 V for 2 h and imaged by silver staining.

2.7 Statistical analysis

Phenotypic trait measurements and fresh biomass data of a single plant of each F1 hybrid were used to calculate descriptive statistics and correlation coefficients between phenotypic traits and biomass weight. The two harvests per year
for replicate field plots were summed to calculate the annual biomass yield of each accession. For all other traits, the average of two measurements per year was used to calculate the best linear unbiased predictor (BLUP).

Variance components and genetic parameters were estimated using linear mixed models in ASReml-R v.4.0 (Butler et al., 2017) implemented in R. A model with year and block as fixed effects and genotype as a random effect was established by fitting the data to the following equation to obtain a single genotypic value across years:

\[ y = X_1u + X_2i + X_3\beta(i) + Z_1g(i) + e \]

where \( y \) is the vector of phenotypic value, \( X \) is the incidence matrix for fixed effects, \( Z \) is the incidence matrix for random effects, \( u \) is the overall mean, \( i \) is the fixed vector of year effects, \( \beta(i) \) is the fixed vector of block effects within each year, \( g(i) \) is the random vector of genotype effects in each year with \( g(i) \sim MVN(0, G \otimes I) \), and \( e \) is the random vector of error with \( e \sim MVN(0, R \otimes I) \); \( R \) represents the variance and covariance matrices of residuals, \( G \) represents the variance and covariance matrices of genotypes across years, \( I \) is an identity matrix of the proper size, and \( \otimes \) is the Kronecker product.

For individual years, the following model was used:

\[ y = X_1u + X_2\beta + Z_1g + e \]

where \( y \) is the vector of phenotypic value, \( X \) is the incidence matrix for fixed effects, \( Z \) is the incidence matrix for random effects, \( u \) is the overall mean, \( \beta \) is the fixed vector of block effects, \( g \) is the random vector of genotype effects with \( g \sim MVN(0, G \otimes I) \), and \( e \) is the random vector of error with \( e \sim MVN(0, R \otimes I) \); \( R \) represents the variance and covariance matrices of residuals, \( G \) represents the variance and covariance matrices of genotypes across years, and \( \otimes \) is the Kronecker product.

Broad-sense heritability (\( H^2 \)) was calculated based on the estimated variance components for each analysis using the following equation:

\[ H^2 = \frac{\sigma_g^2}{\sigma_g^2 + \sigma_e^2} \]

where \( \sigma_g^2 \) is the genetic variance and \( \sigma_e^2 \) is the error variance.

Genotypic BLUP values for each genotype were used to generate GGE biplots using the GGBiplots package in R (Dumble, 2017). Predicted genetic values were used to rank genotypes and determine correlations between traits using the Pearson method in R. Principal component analysis (PCA) was carried out using the \texttt{prcomp} function in R based on a correlation matrix that used the genotypic values from the model. Coincidence percentage in the ranking of top-performing lines was estimated for dry biomass using three selection intensities (10%, 20%, and 30% of the top-performing lines). All plots were visualized using the ggplot2 package in R (Wickham, 2011) unless otherwise noted.

3 | RESULTS

3.1 | Amplification of microsatellites

The polymorphic SSR marker ppssr245 was used to distinguish between the two parental accessions and confirm that the F1 mapping population comprised true hybrids (Figure S1). A total of 183 F1 plants were confirmed as true hybrids of N190 and N122 as they showed four ampli- con bands (two of different sizes from each of the parental lines).

3.2 | Evaluation of F1 hybrids in the nursery

The results for 188 F1 hybrids obtained from the cross between the contrasting parental accessions N190 and N122 and evaluated in a nursery of a non-replicate single plant plot are shown in Table S1. The average fresh biomass weight of the most productive parent, N190, was 13.73 kg plant\(^{-1}\). The maximum weight among the hybrids was 34.6 kg plant\(^{-1}\) (hybrid 51#1), which was 152% higher than N190, while the mean weight was 14.7 kg plant\(^{-1}\). The average plant height of the hybrids was 4.9 m, which was between the mean heights of N190 (4.8 m) and N122 (4.9 m). Similarly, the mean stem diameter, leaf width, and flowering date of the hybrids were approximately equal to the average of the combined parental values. The hybrids produced three times more tillers than N190 and two times more than N122. None of the hybrids flowered before the early-flowering parent N122, which flowered on September 20, 2011. Several hybrids and the late-flowering parent N190 did not flower before the harvest and first predicted freeze event (end of November 2011). All biomass-related traits showed a highly significant \((p < 0.001)\) positive correlation with biomass yield (Table S2). The number of tillers per plant showed the highest correlation with biomass yield \((r = 0.57)\), followed by stem diameter and plant height, while leaf width showed the lowest correlation \((r = 0.33)\).

3.3 | Evaluation of F1 hybrids in replicate field plots

Phenotypic data of the 183 F1 hybrids, two parental accessions, and the control cultivar “Merkeron” from replicate row
plots used to calculate BLUPs across 2 years are summarized in Table 1. Individual year BLUPs are shown in Table S3. The early-flowering parent N122 flowered on October 12, 2012 (Figure S2) and October 10, 2013, with an average biomass yield of 25.6 ± 2.4 t ha−1 across 2 years (Table 1). The late-flowering parent N190 had an average biomass yield of 26.9 ± 4.2 t ha−1 across 2 years and did not flower during the 2 years of evaluation (Figure S2). Most of the hybrids flowered between November 5 and November 26 and had biomass yields ranging from 21.0 ± 1.5 (65#13) to 41.1 ± 2.3 (63#16) t ha−1, with a mean for all accessions of 30.1 t ha−1 across 2 years (Table 1).

More than 50% of the hybrids were more productive than the parents in both years of evaluation (Figure 2a,b). The hybrids started to flower on October 25 in 2012 but several genotypes did not flower until December 6, with the harvest occurring prior to the first predicted freeze event (Figure S2a). Only 10% of hybrids flowered on November 30 or later. The earliest flowering date in 2013 was October 26 (34#16) and the latest was December 3 (34#12); half of the hybrids started flowering between November 12 and 17 (Figure S2b). In 2012, the last freeze occurred on February 13. As there were no additional freezes, the plants were able to ratoon from belowground nodes, but most did not reach the maturity required to produce flowers under the inductive photoperiod in the spring. Hybrids that produced flowers in the spring showed early flowering in the fall except for accessions 64#12, 65#19, 4#14, and 63#20; these flowered later than November 20 but produced flowers in the spring. No flowering was observed in spring 2013, likely because the last freeze occurred on March 28.

The maximum number of flowers per plot in F1 hybrids was 119 (36#17; Figure 2b). The early-flowering parent N122 produced 328 flowers in a 1.8 m row plot throughout the season, corresponding to almost four flowers per tiller. In contrast, the standard cultivar “Merkeron” produced 0.8 flowers per tiller, while N190 did not flower until harvest before the first predicted freeze event. Some of the high-yielding hybrids such as 63#16 (41.1 ± 2.3 t ha−1) produced only 0.7 flowers per tiller, whereas others such as 35#6 (40.8 ± 3.8 t ha−1) produced 0.8 flowers per tiller. One-third of the hybrids had a higher biomass yield than “Merkeron” and produced fewer flowers (Figure 2b).

The most productive hybrid was 63#16, which yielded 41.1 ± 2.3 t ha−1 across 2 years, with an average height of 2 m, average stem diameter of 12.2 mm, and an average of 79 tillers per 1.8-m row plot. The hybrid with the lowest biomass yield (21.0 ± 1.5 t ha−1) was 65#13, which had an average height of 2.7 m, stem diameter of 11.3 mm, and an average of 50 tillers. Both accessions were slightly taller than the average, and the stems of 63#6 were slightly thicker than the average (Table 1). The biggest difference between the two accessions was in the tiller number: 63#16 had 14 more tillers, whereas 65#13 had 15 fewer tillers than the mean of the F1 hybrids (Table 1). Over the 2 years of evaluation, biomass yield showed the highest correlation with the number of tillers (r = 0.64), followed by plant height (r = 0.47; Figure 3). There was no significant correlation between stem diameter or flowering date and biomass yield in this study (Figure 3). Plant height and tiller number showed significant positive correlations with biomass yield in both growing periods (Figure S3), but the number of flowers showed a significant positive correlation in 2013 only. Leaf width showed a significant positive correlation with plant biomass in 2012 (Figure S3). Biomass yield was positively correlated with leaf width for the first harvest in summer 2012 but not in fall 2012 (data not shown). Therefore, leaf width measurements were not continued in 2013.

### Table 1: Phenotypic performance of 183 F1 hybrids of a cross (N190 × N122), the parental accessions, and a control cultivar in Citra, Florida

|                          | Plant height (m) | Number of tillers per 1.8 m row plot | Stem diameter (mm) | Dry biomass yield (t ha−1) | Days to flowering | Number of flowers per 1.8 m row plot |
|--------------------------|------------------|--------------------------------------|--------------------|---------------------------|------------------|--------------------------------------|
| Min                      | 2.6              | 47                                   | 9.5                | 20.9                      | 227              | 5                                    |
| Max                      | 2.9              | 86                                   | 13.8               | 41.1                      | 257              | 119                                  |
| Mean                     | 2.7              | 65                                   | 11.4               | 30.1                      | 240              | 33                                   |
| SEb                      | 0.04             | 4.67                                 | 0.50               | 2.10                      | 3.6              | 16.78                                |
| H2c                      | 0.20             | 0.30                                 | 0.29               | 0.29                      | 0.26             | 0.31                                 |
| N122                     | 2.5              | 87                                   | 9.3                | 25.6                      | 210              | 328                                  |
| N190                     | 2.7              | 45                                   | 13.9               | 26.9                      | 256              | 6                                    |
| Merkeron                 | 2.7              | 60                                   | 18.8               | 29.2                      | 240              | 47                                   |

aN122 and N190 were the parental accessions; Merkeron was a control cultivar.

bStandard error of the mean.

bBroad-sense heritability.
In the PCA, the first two components (PC1 and PC2) accounted for 98% of the variation in F1 hybrid traits including days to flowering and number of flowers, as well as dry biomass yield and contributing traits during the 2 years of evaluation (Figure 4). PC1 alone explained 92% of the variation and PC2 explained 6%. Flower number followed by days to flowering contributed most to PC1, whereas tiller number contributed most to PC2. The same was observed in 2013 (Figure S4). However, in 2012, flower number contributed most to PC1 and days to flowering contributed most to PC2. In sharp contrast to the very late-flowering parent N190, the early-flowering parent N122 showed profuse flowering and was positioned far away from other accessions in the PCA plot.

Consistent broad-sense heritability ($H^2$) estimates were recorded in both 2012 and 2013 for flower number (0.66 and 0.55, respectively) and plant height (0.35 and 0.29, respectively; Figure S5). In 2012, heritability estimates were 0.70 for leaf width, 0.67 for stem diameter, 0.24 for tiller number, 0.66 for flower number, and 0.30 for days to flowering. In 2013, heritability estimates increased to 0.67 and 0.44 for days to flowering and tiller number, respectively, and decreased to 0.23 for stem diameter (Figure 5). Across the 2 years, the broad-sense heritability estimate was 0.29 for biomass yield; for biomass and flowering traits, the estimates ranged from 0.21 for plant height to 0.31 for flower number (Figure S5).

The frequency distribution of F1 hybrids for biomass and related traits across 2 years is shown in Figure 6. The distribution of mean annual plant height of the hybrids was slightly skewed to the left (Figure 6b). All but one of the hybrids (36#9) were taller than the parent N122, and one (6#18) was significantly taller than the parent N190. All of the hybrids had stem diameters ranging from 9.5 to 13.4 mm (Figure 4c); none had a stem diameter smaller
than that of N122 or larger than that of N190. The distribution for tiller number was slightly skewed to the right (Figure 4d). All of the hybrids had more tillers than the low-tillering parent N190 and fewer tillers than N122. More than 90% of the hybrids had an average tiller number between 54 and 84 per 1.8 m row plot. The distribution of number of flowers was skewed to the right (Figure 4e), and 95% of the hybrids produced between 6 and 47 flowers per plot. The maximum number of flowers was 119 (hybrid 36#17), which was one-third of the number produced by the early-flowering parent N122.

Growth rate was calculated as the ratio of total accumulated DM to total number of days of growth. During summer 2012, the maximum growth rate (aboveground biomass) was 60.0 g DM day$^{-1}$ for accession 63#16, and the average growth rate for the 183 F1 hybrids was 38.3 g DM day$^{-1}$ (data not shown). In fall 2012, the maximum growth rate was 54.6 g DM day$^{-1}$ (accession 35#6) and the average growth rate was 37.0 g DM day$^{-1}$. Similarly, in summer 2013, the maximum and average growth rates were 77.68 (63#16) and 49.17 g DM day$^{-1}$, respectively; and in fall 2013, the rates were 41.06 (63#11) and 23.75 g DM day$^{-1}$, respectively (data not shown).

The top 10% of hybrids for biomass accumulation are listed in Table 2. These hybrids had a higher biomass yield on average over the 2 years of evaluation than the control cultivar “Merkeron” and both parents. The persistence of these hybrids (including 35#6, 63#16, 35#5, 64#7, 35#4, and 64#17) despite two harvests per year was demonstrated by their consistent ranking in the top 10% for biomass accumulation in both years. Some of the hybrids (65#1, 35#1, 6#13, 4#19, 6#18, and 64#19) ranked among the top-yielding biomass producers in 2012, but their yields declined more than those of other accessions in 2013 (Table S4). The coefficient of coincidence graph shows the concurrence of the top selected hybrids in each year and those in the 2 years combined (Figure 7). At 30% selection intensity, the coefficient of coincidence for biomass yield traits was >75% for top-ranking F1 hybrids in both the first and second years and combined-year top-ranking hybrids. However, at 10% selection intensity, 45% and 55% of the top-ranking hybrids in 2012 and 2013, respectively, showed concurrence with combined-year top-ranking hybrids.
Temperature and rainfall during the study

The average weekly minimum and maximum temperatures at PSREU, Citra, FL were recorded from May 18, 2011, when the plants were transferred to the field until January 8, 2014, when the fourth harvest in replicate plots concluded (Figure S6a). The average weekly maximum temperature from May to December 2011 was 35.7°C and the minimum was 7.8°C. Similarly, in 2012, the maximum and minimum temperatures were 34.0 and 2.9°C, respectively, with temperatures below 0°C on January 3, 4, 5, 14, 15, and 16; February 12 and 13; and November 25 and 26. In 2013, the average weekly maximum temperature was 33.8°C and the minimum was 5.7°C; the temperature was below 0°C on January 23, February 1, 2, 17, and 18; and March 3, 4, 15, and 28. In 2014, the temperature was below 0°C on January 7 and 8. Figure S6b shows the weekly rainfall pattern for the experimental plots from May 2011 to January 2014. The total rainfall from May to December 2011 was 690.4 mm, and the total rainfall in 2012 and 2013 was 1382.3 and 2096.8 mm, respectively. The longest drought period was between October 7 and December 6, 2012; the total rainfall during this 60-day period was 0.04 mm.

3.4 | DISCUSSION

Napier grass is a promising non-domesticated bioenergy feedstock and one of the most important tropical forage crops, but phenotype and genotype data to support molecular breeding efforts for this plant are scarce (Negawo et al., 2017). A Napier grass mapping population segregating for flowering time, biomass yield, and yield-related components was developed and characterized in this study. Napier grass accessions N122 and N190 were selected as parents based on their contrasting phenotypes and genetic distance. These accessions were shown to cluster into two different major groups based on AFLP markers (Harris-Shultz et al., 2010), suggesting that they belong to different heterotic groups. The true hybrid nature of the progeny was confirmed based on an SSR marker. Most F1 hybrids had biomass yields exceeding 20 t ha⁻¹, which is the average expected annual yield for north-central Florida (Woodard & Sollenberger, 2012). The results of the present study provide evidence that heterosis contributes to increased biomass production in the F1 hybrids. The average dry biomass yield of the F1 hybrids in replicate plots (30.1 t ha⁻¹) was higher than that of each of the parents over a 2-year period. More than half of the F1 hybrids (n = 111) produced more biomass than “Merkeron,” a well-established Napier grass cultivar included in the experiment as a control. The two highest-yielding F1 hybrids (63#16 and
35#6) had an annual dry biomass yield greater than 40 t ha\(^{-1}\), which was 57% and 55% higher, respectively, than the average productivity of parental accessions and 41% and 40% higher, respectively, than the yield of Merkeron over 2 years (Table 2).

Selecting the phenotypically contrasting and genetically distant accessions N122 and N190 as parents allowed us to analyze the segregation of flowering time, number of flowers, plant height, stem diameter, number of tillers, and leaf width in the F1 progeny, and their correlation with biomass yield as well as the potential for heterosis. Significant heterosis in traits such as plant height, stem diameter, leaf blade width, number of tillers, and DM yield has been observed in Napier grass hybrids (Menezes et al., 2015). Flowering date, plant height, stem diameter, tiller number, and leaf width segregated with normal or near-normal distributions in the F1 hybrids (Figure 6). Similarly, plant height, stem diameter, tiller number, and leaf width were normally distributed in sorghum and maize mapping populations (Lu et al., 2011; Pereira & Lee, 1995). The continuous distribution of these traits in Napier grass confirms that they are quantitative in nature and suggests that they are controlled by multiple alleles with small effects. Flowering at the optimum time increases fecundity and contributes to adequate development of the seeds (Mazer, 1987). Early flowering was found to be an advantageous trait of an invasive grass (Eragrostis curvula or “African lovegrass” (Han et al., 2012). Thus, early-flowering Napier grass likely has enhanced potential for invasiveness as it flowers at a time when environmental conditions are favorable for the production and dispersal of viable seeds (Bonin et al., 2017). Floral transition is regulated by the interaction of internal factors and environmental signals including photoperiod, temperature, light quality, cold temperature, and drought (Albani & Coupland, 2010; Levy & Dean, 1998). The transition from vegetative to reproductive growth diverts photosynthates away from biomass accumulation and restricts the length of the effective growing season by triggering the onset of senescence (Wingler et al., 2010). Therefore, late flowering should be a key trait for selection in a Napier grass breeding program. It would be useful to evaluate the feasibility of combining high biomass yield and late flowering to improve the biosafety of high-yielding cultivars.

The flowering date of the F1 hybrids was highly variable (ranging from October 25 to later than December 6), with a near-normal distribution and high heritability. Thus, this F1 hybrid population can be used to map QTLs associated with flowering time in Napier grass. Mapping populations have been successfully used in other species to identify QTLs and candidate genes. For example, 20 genes related to activation (e.g., Ehd2/RIDI/OsIDI, Ehd3, and Ghdl8) or suppression (e.g., DTH8, Hdl1, OsCOL4, OsGI, and OsLFL1) of flowering or to the regulation of this process (e.g., OsMADS15, OsMADS50, and OsMADS51) were identified in rice, a facultative short-day plant like Napier grass (Hao et al., 2009; Wei et al., 2010; Xiang et al., 2013). This information will be useful when searching for molecular markers for early or late flowering. We also found high heritabilities for flowering date (0.67 in 2012) and number of flowers (0.66 in 2012 and 0.55 in 2013), suggesting that the observed variations in these traits in the F1 hybrids of this mapping population can be applied to the selection of Napier grass with improved flowering time. Flowering time and number of flowers are highly heritable in other grasses including maize (Buckler et al., 2009), sorghum (Mace et al., 2013), and switchgrass (Casler, 2020; Van Esbroeck et al., 1998). In Napier grass, the number of flowers showed a significant positive correlation with biomass yield but a negative correlation with days to flowering across 2 years at the Citra location. High-tillering accessions produced more flowers and had a higher biomass yield. Several of the F1 hybrids that had a significantly higher yield than the control cultivar “Merkeron” flowered on November 22 or later (Figure 2a). These late-flowering genotypes could be used for genetic improvement through hybridization with other late-flowering accessions. Genotypes combining elevated biomass yield and delayed flowering have also been described for miscanthus (Clifton-Brown et al., 2001; Jensen et al., 2011, 2013) and switchgrass (Casler, 2020).

The top 10% of highest-yielding Napier grass accessions produced on average 50 tillers in 2012 and 91 in 2013. Differences in the production of belowground nodes and factors controlling bud outgrowth may have contributed to more tillering following the two harvests in the first year. Tillering showed the highest correlation \(r = 0.64\) with biomass accumulation in 2 years of observation, followed by plant height \(r = 0.47\). The correlation coefficients for these traits were highest in year 2, suggesting that differences in tillering are critical for the ratooning that supports sustained high biomass yields of this perennial crop (Figure S3). The high-tillering parental line N122 and low-tillering parental line N190 showed contrasting yield trends with subsequent ratoons. Similarly, biomass yield in switchgrass was positively correlated with tiller number per plant with correlation coefficients of 0.60–0.68, and it was suggested that selecting for increased tillering was the most effective strategy for increasing biomass yield (Das et al., 2004). A combined assessment of tiller number and stem diameter—which was determined by measuring the circumference of the compressed stem bundle at the middle height of the plant—showed the highest correlation with biomass yield in M. sinensis (Clark et al., 2019).
Delayed flowering extends the growing season, which may increase the yield of crops. However, it may diminish the recycling of nutrients from aboveground to belowground parts, thus compromising ratooning and reducing the output/input energy ratio (Karp & Shield, 2008). Early senescence of Miscanthus (a perennial grass for bioenergy) shortened the vegetative growth period, which reduced biomass yield (Robson et al., 2013). On the other hand, delayed senescence led to higher nutrient offtake, which limited nutrient cycling and nutrient use efficiency. Multiple harvests in a year are desired for a continuous feedstock supply and improved forage quality; structural carbohydrate components increase, whereas crude protein and ash decrease with maturity (Chiluwal et al., 2019). With two harvests per year, acid-insoluble lignin and carbohydrates were found to be higher while extractives were lower for winter harvests compared to biomass harvested in the summer (Dien et al., 2020). Persistence under multiple seasonal cuts is a major advantage of Napier grass over other similar species. However, multiple harvests per year lead to a yield reduction compared to a single harvest due to the depletion of N and P in the harvested biomass (Chiluwal et al., 2019; Dien et al., 2020; Na et al., 2015).

Our data indicate that genotypic differences in tiller production can boost crop productivity with multiple cuts per year. Parental accession N122 showed an increase in the number of tillers from 47 in year 2 to 124 in the ratoon crop, in contrast to N190 in which the number increased from 43 to 53. F1 hybrids had 48 tillers on average in year 1, which increased to 81 in year 2, similar to the control cultivar “Merkeron” (which showed an increase from 43 to 81). However, several of the F1 hybrids in the top 10% of biomass production more than doubled their tiller number in 2013 compared to 2012, suggesting increased persistence under 2 harvests per year. However, some of the hybrids that ranked among the highest biomass producers in 2012 showed only a modest increase in the number of tillers in 2013, which led to a decrease in performance ranking.

Leaf width showed a significant positive correlation with plant biomass only in the first growing season (from January to August 2012), consistent with the fact that plants with wider leaves produce more biomass (Zhang et al., 2015). However, this correlation was not significant in the following growing season, probably because there were more tillers competing for light within the canopy. Plant growth rate is proportional to the amount of photosynthetically active radiation (PAR) intercepted by a canopy (Madakadze et al., 1998); the fraction of intercepted PAR is related to the extinction coefficient (k), which is influenced by factors such as total leaf area index, average leaf inclination angle, distribution of foliage, or sun angle (Maddonni & Otegui, 1996).

Moderate heritabilities were observed for plant height, number of tillers, stem diameter, and dry biomass yield in our study. Similar heritabilities for these traits were reported in maize (Idris & Abuali, 2011; Sabiel, 2014). The broad-sense heritability estimates of biomass across 2 years was 0.29, which was similar to the heritability estimates of the contributing traits (ranging between 0.22 for plant height and 0.30 for tiller number). In individual years, heritability estimates approaching 0.7 were observed for stem diameter, leaf width, flower number, and days to flowering. Lower heritability estimates in both years were likely caused by the contrasting yield trends of both parents with subsequent ratoons and their quantitative transmission to the progeny. In the ratoon crop, heritability estimates for tiller number increased to 0.44, likely because of the contribution of this trait to persistence. The results of the PCA also demonstrated that tiller number, flower number, and days to flowering contributed most to the observed phenotypic variation across accessions. This suggests that the selection of hybrids that combine high tiller number, late flowering, and reduced flower number is an achievable target.

This study identified Napier grass hybrids combining late flowering with exceptional biomass production, which enhances the biosafety of this promising feedstock. The late-flowering hybrids reach peak biomass yields before flowering and can be harvested before seeds are formed, markedly reducing the potential for unintended seed dispersal and the associated invasiveness. The developed mapping population has been used to construct a Napier grass high-density linkage map through genotyping-by-sequencing (Paudel et al., 2018), and can serve along with the identified superior hybrids as an excellent resource for identifying QTLs and candidate genes for molecular breeding.

ACKNOWLEDGEMENTS
The information, data, and work presented herein were funded in part by USDA National Institute of Food and Agriculture (grant no. 2010-34135-21019). The authors thank William Anderson at USDA-ARS (Tifton, GA, USA) for providing Napier grass accession N122; and Sun Gro Horticulture (Apopka, FL) for donating Fafard #2 potting mix.

CONFLICT OF INTEREST
The authors declare that they have no conflicts of interest.

AUTHORS CONTRIBUTION
FA conceived and designed the experiments and generated the Napier grass F1 hybrids. Field experiments were carried out by MS, BK, and CC. Molecular analysis was done by YL, DP, and JW. Statistical analysis and manuscript preparation were done by MS, BK, DP, JW, and FA. All authors contributed for discussion and finalizing of the manuscript.
DATA AVAILABILITY STATEMENT
The data that support the findings of this study are available from the corresponding author upon reasonable request.

ORCID
Fredy Altpeter  https://orcid.org/0000-0002-0894-4976

REFERENCES
Adler, P. R., Del Grosso, S. J., & Parton, W. J. (2007). Life-cycle assessment of net greenhouse-gas flux for bioenergy cropping systems. Ecological Applications, 17(3), 675–691. https://doi.org/10.1890/05-2018

Albani, M. C., & Coupland, G. (2010). Comparative analysis of flowering in annual and perennial plants. Current Topics in Developmental Biology, 91, 323–348.

Atienza, S. G., Satovic, Z., Petersen, K. K., Dolstra, O., & Martín, A. (2003). Identification of QTLs influencing combustion quality in Miscanthus sinensis Anderss. I. Total height, flag-leaf height and stem diameter. Theoretical and Applied Genetics, 107, 857–863. https://doi.org/10.1007/s00122-003-1218-z

Austin, D. F., & Lee, M. (1996). Genetic resolution and verification of systems.

Babu, C., Sundaramoorthi, J., Vijayakumar, G., & Ram, S. G. (2009). Comparing establishment and productivity of fertile Pennisetum purpureum Schum. in prevention of Eucalyptus deglupta with tillage and herbicides. Ecological Applications, 9(3), 675–691. https://doi.org/10.1007/s10753-009-0424-y

Bonin, C. L., Mutegi, E., Snow, A. A., Miriti, M., Chang, H., & Carvalho, M. M., Mozzer, O. L., Silva, J. B., & Ferreira, J. G. (2012). Napiergrass has dual use as biofuel feedstock and animal fodder. Agronomy Journal, 11(4), 1752–1759. https://doi.org/10.2134/agronj2018.09.0601

Clark, L. V., Dwiyanti, M. S., Anthouza, K. G., Brummer, J. E., Ghimire, B. K., Glowacka, K., Hall, M., Heo, K., Jin, X., Lipka, A. E., Peng, J., Yamada, T., Yoo, J. H., Yu, C. Y., Zhao, H., Long, S. P., & Sacks, E. J. (2019). Biomass yield in a genetically diverse Miscanthus sinensis germplasm panel evaluated at five locations revealed individuals with exceptional potential. GCB Bioenergy, 11(10), 1125–1145. https://doi.org/10.1111/gcbb.12606

Clifton-Brown, J. C., Lewandowski, I., Andersson, B., Basch, G., Christian, D. G., Kjeldsen, J. B., Jørgensen, U., Mortensen, J. V., Riche, A. B., Schwarz, K. U., Tayebi, K., & Teixeira, F. (2001). Performance of 15 Miscanthus genotypes at five sites in Europe. Agronomy Journal, 93(5), 1013–1019. https://doi.org/10.2134/ agronj2001.9351013x

Cook, B. G., Pengelly, B. C., Brown, S. D., Donnelly, J. L., Eagles, D. A., Franco, M. A., Hanson, J., Mullen, B. F., Partridge, I. J., & Peters, M. (2005). Tropical Forages: An interactive selection tool. Web Tool. CSIRO, DPI&F, CIAT, ILRI.

Cutts, G. S., Webster, T. M., Grey, T. L., Vencill, W. K., Lee, R. D., Tubbs, R. S., & Anderson, W. F. (2011). Herbicide effect on napiergrass (Pennisetum purpureum) control. Weed Science, 59(2), 255–262. https://doi.org/10.1614/ws-d-10-00130.1

Daher, R. F., de Moraes, C. F., Cruz, D. C., Pereira, A. V., & Xavier, D. F. (1997). Seleção de caracteres morfológicos discriminantes em capim-elefante (Pennisetum purpureum Schum.). Revista Brasileira de Zootecnia, 26(2), 247–254.

Das, M. K., Fuentes, R. G., & Taliaferro, C. M. (2004). Genetic variability and trait relationships in switchgrass. Crop Science, 44(2), 443–448. https://doi.org/10.2135/cropsci2004.4430

del Lama, R., Daher, R. F., Gonçalves, L., Rossi, D. A., do Amaral Júnior, A. T., Pereira, M. G., & Lédo, F. (2011). RAPD and ISSR markers in the evaluation of genetic divergence among accessions of elephant grass. Genetics and Molecular Research : GMR, 10(3), 1304–1313. https://doi.org/10.4238/vol10-3gmr1107

Dellaporta, S. L., Wood, J., & Hicks, J. B. (1983). A plant DNA mini-preparation: Version II. Plant Molecular Biology Reporter, 1(4), 19–21. https://doi.org/10.1007/BF02712670

Dien, B. S., Anderson, W. F., Cheng, M. H., Knoll, J. E., Lamb, M., O’Bryan, P. J., Singh, V., Sorensen, R. B., Strickland, T. C., & Slininger, P. J. (2020). Field productivities of napiergrass for production of sugars and ethanol. ACS Sustainable Chemistry and Engineering, 8(4), 2052–2060. https://doi.org/10.1021/acssuschemeng.9b06637

Dumble, S. (2017). GGEBiplots: GGE Biplots with ’ggplot2’. R package version 0.1.1.

FLEPPC. (2011). List of invasive plant species. Florida Exotic Pest Plant Council.

Grey, T. L., Webster, T. M., Li, X., Anderson, W., & Cutts, G. S. (2015). Evaluation of control of napiergrass (Pennisetum purpureum) with tillage and herbicides. Invasive Plant Science and Management, 8(4), 393–400. https://doi.org/10.1614/ipsm-d-15-00012.1

Han, Y., Buckley, Y. M., & Firn, J. (2012). An invasive grass shows colonization advantages over native grasses under conditions of low resource availability. Plant Ecology, 213(7), 1117–1130. https://doi.org/10.1007/s11258-012-0707-0

Hanna, W. W. (1981). Method of reproduction in napiergrass and in the 3X and 6X allotriodybrids with pearl millet. Crop Science,
Kandel, R., Singh, H. P., Singh, B. P., Harris-Shultz, K. R., & Anderson, W. F. (2016). Assessment of genetic diversity in napier grass (Pennisetum purpureum Schum.) using microsatellite, single-nucleotide polymorphism and insertion-deletion markers from pearl millet (Pennisetum glaucum [L.] R. Br.). Plant Molecular Biology Reporter, 34, 265–272. https://doi.org/10.1007/s11105-015-0918-2

Karp, A., & Shield, I. (2008). Bioenergy from plants and the sustainable yield challenge. New Phytologist, 179(1), 15–32. https://doi.org/10.1111/j.1469-8137.2008.02432.x

Kawube, G., Alicai, T., Otini, M., Mukwaya, A., Kabirizi, J., & Talwana, H. (2014). Resistance of Napier grass clones to Napier grass Stunt Disease. African Crop Science Journal, 22(3), 229–236.

Kawube, G., Alicai, T., Wanjala, B. W., Njihira, M., Awalla, J., & Skilton, R. (2015). Genetic diversity in napier grass (Pennisetum purpureum) assessed by SSR markers. Journal of Agricultural Science, 7(7), 147–155. https://doi.org/10.5539/jas.v7n7p147

Langeland, K. A. (2008). Identification and biology of nonnative plants in Florida’s natural areas. IFAS Communication Services, University of Florida.

Levy, Y. Y., & Dean, C. (1998). The transition to flowering. The Plant Cell, 10(12), 1973–1989. https://doi.org/10.1105/tpc.10.12.1973

López, Y., Seib, J., Woodard, K., Chamusco, K., Sollenberger, L., Gallo, M., Flory, S. L., & Chase, C. (2014). Genetic diversity of biofuel and naturalized napiergrass (Pennisetum purpureum). Invasive Plant Science and Management, 7(2), 229–236. https://doi.org/10.1614/IPS-M-D-13-00085.1

Lu, X. P., Yun, J. F., Gao, C. P., Acharya, S., Xiao-ping, L., Jin-feng, Y., Cui-ping, G., & Acharya, S. (2011). Quantitative trait loci analysis of economically important traits in Sorghum bicolor × S. sudanense hybrid. Canadian Journal of Plant Science, 91(1), 81–90. https://doi.org/10.4141/CJPS09112

Mace, E. S., Hunt, C. H., & Jordan, D. R. (2013). Supermodels: Sorghum and maize provide mutual insight into the genetics of flowering time. Theoretical and Applied Genetics, 126(5), 1377–1395. https://doi.org/10.1007/s00122-013-2059-z

Madarakde, I. C., Coulman, B. E., Peterson, P., Stewart, K. A., Samson, R., & Smith, D. L. (1998). Leaf area development, light interception, and yield among switchgrass populations in a short-season area. Crop Science, 38(3), 827–834. https://doi.org/10.2135/cropssci1998.0011183X003800030035x

Maddonni, G. A., & Otegui, M. E. (1996). Leaf area, light interception, and crop development in maize. Field Crops Research, 48(1), 81–87. https://doi.org/10.1016/0378-4290(96)00035-4

Mazer, S. J. (1987). The quantitative genetics of life history and fitness components in Raphanus raphanistrum L. (Brassicaceae): Ecological and evolutionary consequences of seed-weight variation. The American Naturalist, 130(6), 891–914. https://doi.org/10.1086/284754

Menezes, B. R. S., Daher, R. F., Gravina, G. D. A., Pereira, A. V., Sousa, L. B., Rodrigues, E. V., Silva, V. B., Gottardo, R. D., Schneider, L. S. A., & Novo, A. A. C. (2015). Estimates of heterosis parameters in elephant grass (Pennisetum purpureum Schumach.) for bioenergy production. Chilean Journal of Agricultural Research, 75(4), 395–401. https://doi.org/10.4067/S0718-58392015005000003

Mochizuki, J., Yanagida, J. F., Kumar, D., Takara, D., & Murthy, G. S. (2014). Life cycle assessment of ethanol production from tropical banana grass (Pennisetum purpureum) using green and dry processing technologies in Hawaii. Journal of Renewable and Sustainable Energy, 6(4), 1–19. https://doi.org/10.1063/1.4893673

Muktar, M. S., Teshome, A., Hanson, J., Negawo, A. T., Habte, E., Domelevo Entfellner, J. B., Lee, K. W., & Jones, C. S. (2019). Genotyping by sequencing provides new insights into the diversity of Napier grass (Cenchrus purpureus) and reveals variation in genome-wide LD patterns between collections. Scientific Reports, 9(1), 1–15. https://doi.org/10.1038/s41598-019-43406-0
Zhang, L., Richards, R. A., Condon, A. G., Liu, D. C., & Rebetzke, G. J. (2015). Recurrent selection for wider seedling leaves increases early biomass and leaf area in wheat (*Triticum aestivum* L.). *Journal of Experimental Botany*, 66(5), 1215–1226. https://doi.org/10.1093/jxb/eru468

**SUPPORTING INFORMATION**

Additional supporting information may be found online in the Supporting Information section.

**How to cite this article:** Sinche, M., Kannan, B., Paudel, D., Corsato, C., Lopez, Y., Wang, J., & Altpeter, F. (2021). Development and characterization of a Napier grass (*Cenchrus purpureus* Schumach) mapping population for flowering-time- and biomass-related traits reveal individuals with exceptional potential and hybrid vigor. *GCB Bioenergy*, 00, 1–15. https://doi.org/10.1111/gcbb.12876