Estimating the Genetic Parameters of Flowering Time-Related Traits in a Miscanthus sinensis Population Tested with a Staggered-Start Design

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Received: 27 April 2021 / Accepted: 31 August 2021 / Published online: 5 October 2021 © The Author(s), under exclusive licence to Springer Science+Business Media, LLC, part of Springer Nature 2021

Abstract
The cultivation of Miscanthus has attracted growing interest despite its yield instability. Therefore, understanding what causes such instability is of primary interest for breeding. Our objectives were to estimate the genetic parameters—genetic variance and genetic heritability—and genetic correlations for flowering time-related traits in a biparental Miscanthus sinensis diploid population, and divide the year effect into age and growing season effects using a staggered-start design. The population was established with single plants organized with this design and consisted of two genotype groups established twice in a same field, in 2014 and 2015, with a total of 159 genotypes and 82 common genotypes between the groups. Soil conditions being identical between both stands, the growing season conditions corresponded to climatic conditions. All plants were extensively phenotyped for different panicle and anther emergence traits in 2018 and 2019. All traits were delayed by 3 weeks in 2019 compared to 2018, which was explained by climatic conditions that occurred before the floral transition, mainly a 3 °C decrease in temperatures. When dividing the year effect, the genotype × growing season interaction was much higher than the genotype × age interaction. This increased the genotype × growing season interaction variance compared to the genotype × age interaction variance: the growing season effect decreased the genetic parameters for all flowering time-related traits, up to 20% for broad-sense heritability. Interestingly, most traits responded similarly to this effect. Therefore, Miscanthus sinensis breeding for flowering time must be conducted under contrasted climatic conditions to select more stable genotypes.

Keywords Biomass crop · Heritability · Earliness · Year effect · Climatic condition effect

Introduction
Biomass is expected to play a major role in the energy transition, which involves switching to a safe and sustainable low-carbon economy, in order to address the growing challenges due to depleting fossil resources and climate change [1]. In their review, Gabrielle et al. [1] reported that a small proportion of European renewable energy is derived from dedicated bioenergy crops. Most of them are perennials grown to generate electricity and heating, with the most frequent species being Miscanthus, willow, reed canary grass, and poplar. In addition, some biomass crops are exploited in response to environmental pollution [2].

Miscanthus is a perennial C4 rhizomatous grass originating from eastern and southern Asia [3]. Notably, Miscanthus × giganteus was introduced in the 1930s in Europe from Japan by the Danish nurseryman, Aksel Olsen [4], and is known as a natural interspecific hybrid between Miscanthus sacchariflorus and Miscanthus sinensis [5]. Area cultivated with Miscanthus relies on M. × giganteus and has thus been used since 1983 [6]. It was rapidly identified as providing promising lignocellulosic biomass due to its high biomass yield per area [3], low nitrogen needs [7], and capability to recycle its nitrogen [8]. However, M. × giganteus is sterile and presents genetic uniformity. This crop currently relies on a single clone, which may cause risks that have been recognized in case of disease, pest, or climate conditions.
pressure [9]. As early as 1997, Greef et al. [10] reported that genetic diversity was very low within a pool of 31 European \textit{M. \times giganteus} accessions [11], concluded that 27 American and 6 European accessions were derived from a single clone by vegetative propagation. In contrast, \textit{M. sinensis} exhibits huge genetic variability, with major genetic groups originating from Asia [3, 12, 13], which offers numerous opportunities to enlarge the varietal offer and it is the reason why it is important to study its genetics.

To produce biomass, crops require the longest possible vegetative growth period because the shift from vegetative to reproductive growth prevents or stops biomass accumulation. This shift is of particular interest on marginal land where biomass yields are very often much lower than on good agricultural land, and where Wagner et al. [14] found that economic sustainability is limited by the biomass yield. \textit{M. sinensis}, several previous studies using different \textit{Miscanthus} germplasm found that late-flowering was associated with higher biomass production. Zub et al. [15] compared \textit{M. sinensis}, \textit{M. sacchariflorus}, and \textit{M. \times giganteus} and showed that the latest flowering genotypes produced more biomass than the earliest flowering ones. Clifton-Brown and Lewandowski [16] observed that some non-hybrid genotypes of \textit{M. sinensis} had shorter growing seasons and lower plant heights due to their earlier flowering, and their biomass yields were generally lower than those of later-flowering hybrids. Regarding the flowering diversity of \textit{Miscanthus}, Jensen et al. [17] compared 244 genotypes of two \textit{Miscanthus} species (\textit{M. sinensis}, \textit{M. sacchariflorus}) and 8 inter-specific hybrids including \textit{M. \times giganteus}. They found that \textit{M. sinensis} clones were the earliest ones to flower, but showed the greatest diversity in terms of flowering onset, which highlights the importance of this species for breeding new varieties. It also shows the need for new late-flowering \textit{M. sinensis} to increase their biomass production.

The genetic parameters, genetic variance, and heritability of traits related to flowering time in \textit{Miscanthus} are essential to breed such new \textit{M. sinensis} varieties. Slavov et al. [18] observed the date on which the first flag leaf emerged in a population of 138 \textit{M. sinensis} plants and found that this trait was highly heritable (broad-sense heritability = 0.83). Gif-ford et al. [19] observed the day of the year of the heading date and 50\% anthesis for two successive years in a population of 221 progenies from a cross between two \textit{M. sinensis}, and found that these traits were also highly heritable, with a broad-sense heritability of 0.80 and 0.67 for the heading date and 0.77 and 0.70 for the 50\% anthesis. Dong et al. [20] estimated the broad-sense heritability of two diploid F1 \textit{M. sinensis} populations for the date on which flowering first began (with opened florets, elongated stamens, and pollen shedding) and found high heritability levels (0.76 and 0.85).

In all these studies, the flowering-related traits were limited to a single trait except in the work of the last authors who studied several traits, but only one related to flowering, \textit{i.e.} the date on which flowering first began.

So far, all studies of genetic parameters have been addressed based on stands of \textit{Miscanthus} planted in a single year and measured across multiple subsequent seasons. In these “single start” designs, the growing season condition and age effects are confounded within the year effect. In their staggered-start design, Segura et al. [21] used the year effect to designate the growing condition effect while Tejera et al. [22] used “stand conditions”. In both studies, the age effects designated temporal effects. The growing season condition corresponds itself to the climatic and soil conditions. In the present paper, we studied a biparental diploid \textit{M. sinensis} population using a staggered-start design [23] established twice in a same field, in 2014 and 2015, and extensively phenotyped in 2018 and 2019 for flowering time-related traits. Soil conditions being identical between both stands, the growing season condition corresponded to climatic conditions. The stands were 3 and 4 years old in 2018, and then 4 and 5 in 2019. The power of such a design in which the population was established twice makes it possible to divide the “year” effect into “age” effect and “growing season” effects [21, 22]. Therefore, we paid attention to drivers of the flowering time-related traits, particularly to those of their delay in 2019 compared to 2018. What caused the genetic parameters to change?

Materials and Methods

Trail Conditions and Experimental Design

The field trial was established in 2014 at the INRAE unit in Estrées-Mons (49° 72′ N, 3° 00′ E) in northern France, relying on the frame of the Biomass For the Future (BFF) project. It was repeated twice in 2014 and in 2015 using a staggered-start design [23]. The field soil type was characterized as deep loam (Ortic luvisol, FAO, classification). The trial received no nitrogen input and weeds were regularly manually removed. Meteorological data were recorded daily at a short distance from the trial (about 1 km) and downloaded from the CLIMATIC database (INRAE AGROCLIM, 2020).

Population and Experimental Design

The population consisted of the F1 progeny of 159 individuals generated from an intraspecific cross between two diploid \textit{M. sinensis} varieties, “Silberspinne” and “Malepartus”. Plantlets were initially produced from the seeds and grown in a greenhouse in order to provide clonal replicates of each genotype using in vitro propagation method [24]. The population was established with single plants organized in a staggered-start design [23].
An initial stand or group of genotypes (coded G1) that contained a total of 111 genotypes and included the two parents was established in 2014 using an initial incomplete block design [25]. A second group of 130 genotypes (coded G2) included the two parents as well and was established in 2015 in the same field using a second incomplete block design. Eighty-two genotypes were common to both groups and established twice in 2014 and 2015 to allow the partition of the year effect into age and growing season effects (Fig. 1). Soil conditions being identical between both stands, the growing season conditions corresponded to climatic conditions. Each established group comprised 5 blocks of 90 individuals each (6 rows of 15 plants each). The two parents were grown in each block of the two stands, while the G1 remaining genotypes were repeated into four blocks on average and the G2 genotypes into at least three blocks. The two groups of genotypes, which were adjacent in the field, were separated using 75 plants (15 × 5) and surrounded by 180 border plants, to guarantee similar competition effects. Individuals were randomly planted using the Excel package «BICA» which was based on VBA programming language and developed for the randomization of incomplete blocks (JC Bastien, personal communication). A density of 1 plant/m² was applied at regular intervals of 1 m × 1 m between the plants.

**Phenotyping of Traits Related to Flowering**

The phenotypic observation of the individuals within the population for flowering-related traits was conducted in 2018 and 2019. The plants were 3 and 4 years old in 2018, and then 4 and 5 in 2019, for the first and second stands, respectively. The following traits were recorded on each plant and targeted stems belonging to the higher part of the plant canopy (above 80%): the start of panicle emergence (SPE), which corresponds to the date on which the first panicle was visible at least 1 cm above the plant stem; anther appearance (AA), which corresponds to the date on which anthers appeared and began to shed pollen—this observation was carried out on the first panicle that emerged in the plant; the mid-point panicle emergence (MPE), which corresponds to the date on which 50% of the panicles had emerged in the plant; and finally, the complete panicle emergence (CPE), which corresponds to the date on which over 80% of the panicles of the entire plant had emerged. In addition, one variable was calculated: the interval between heading and

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**Fig. 1** Description of the trial at the INRAE unit in Estrées-Mons in northern France: over time (left-hand side) and space (right-hand side). The population was established based on a staggered-start design: the first stand corresponded to a group of 111 genotypes (coded as G1) and was established in 2014, while the second stand corresponded to a group of 130 genotypes (coded as G2) and was established in 2015. Eighty-two genotypes were established in both 2014 and 2015, which allowed the year effect to be divided into age and growing condition effects. In the present study, growing conditions corresponded to climatic conditions. On the right, green plants represent the population of individuals, while brown plants represent border plants.

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flowering (IHF) that was defined as the difference between the heading and flowering dates observed on the first panicle that emerged and flowered. To reduce bias and increase accuracy in the data, phenotypic observations of the traits were made three times a week, starting with the first plant that showed panicle emergence. The first panicle to appear on the plant canopy above 80% was marked and used for further determination of the date of another appearance to facilitate determination and avoid sampling bias. In addition, other traits were observed in the population: the emergence date (ED), the senescence date (SD) that corresponded to the date on which the first stem cohort had become completely senescent, and the date of the first winter frost (FD). Based on the widely used BBCH scale or “Biologische Bundesanstalt, Bundesforschungsanstalt, Chemische Industrie” scale [24], it can be noted that ED, SPE, MPE, and SD corresponded to 09, 51, 45, and 99 stages, respectively.

To further calculate climatic indicators related to the climatic condition effect, extensive observations were conducted on Malepartus, one of the two population parents, in order to determine the date of the floral transition. This clone was established in 2014 in a specific trial not far from the population trial, at a plant density of 2 plants/m² (see Leroy et al., submitted to BioEnergy research). The sampling was carried out in 2018 on three plots of about 75 m² each. The floral transition corresponded to the differentiation of the terminal vegetative apex into a panicle and was observed by dissecting 30 apices per day on average from May 24 onwards. The floral transition was determined as the date of the first floral transition appearance and was only for the first stand, in 2018. It corresponded to the “double ridge stage” of winter wheat [25].

Then, the floral transition was estimated for Malepartus, in 2018 for the second stand and in 2019 for both stands. For that purpose, the base temperature was refined for Malepartus, and the estimations were based on methods developed in maize, a relative of Miscanthus. In maize, primordia rate and duration responded to temperature in approximately the same way as for leaf appearance and were constant across temperature regimes, at least for the appearance of the first 12 leaves [26]. According to Hesketh and Warrington [27], the use of a degree-day model must predict the appearance rate of new organs, such as leaf primordia, tassel, and ears, as a function of air temperature or degree days. These results relating to constant degree-day durations were applied here in Miscanthus to predict floral transition (referring to leaf primordia results in maize) and flowering (referring to tassel and ear results in maize). Testing several potential base temperatures ranging from 6 to 8.5 °C, we found that the sum of temperatures calculated from emergence to flowering for the Malepartus clone was almost equal in 2018 and 2019 when the base temperature used was of 8 °C. This meant that the base temperature was close to 8 °C for Malepartus and could be applied for further calculations. Therefore, growing-degree days (GDD) were estimated using 8 °C as a base temperature for Malepartus. Using the floral transition date determined from the apices observations in Malepartus in 2018, the ratio between the GDD for the emergence-to-floral transition period and the GDD for the emergence-to-flowering period was calculated for Malepartus and reached 0.17. This ratio was then applied for the estimations in 2018 of the second stand and in 2019 of both stands.

**Determination of Plant Growth Periods**

Five periods of the plant cycle were taken into account to calculate the climatic indicators related to the climatic condition effect (Table 1). The first period corresponded to the pre-emergence phase (coded period 1) and the calculations started at senescence, which occurs in the autumn of the previous year, and precedes shoot emergence. The second period (period 2) started with shoot emergence and ended with floral transition. The third period (Period 3) corresponded to the interval between floral transition and anthesis. The fourth period (Period 4) was from anthesis to senescence. Lastly, the fifth period (Period 5) covered the duration from senescence to the date of the first winter frost, the plant cycle being stopped by the first frosts. As

| Period | Description | Year | Beginning | Ending | Duration |
|--------|-------------|------|-----------|--------|----------|
| 1      | Pre-emergence from the senescence of the year \((n-1)\) to emergence year \(n\) | 2018 | 324 | 97 | 138 |
|        |             | 2019 | 289 | 81 | 157 |
| 2      | Emergence to beginning of floral transition | 2018 | 98 | 134 | 36 |
|        |             | 2019 | 82 | 147 | 65 |
| 3      | Beginning of floral transition to beginning of anthesis | 2018 | 135 | 204 | 69 |
|        |             | 2019 | 148 | 228 | 80 |
| 4      | Beginning of anthesis to senescence | 2018 | 205 | 288 | 83 |
|        |             | 2019 | 229 | 327 | 98 |
| 5      | Senescence to the date of frost day of the year | 2018 | 289 | 346 | 57 |
|        |             | 2019 | 328 | 337 | 9 |
previously, shoot emergence and senescence corresponded to 09 and 99 stages of the BBCH scale, respectively [24]. The floral transition was equivalent to “double ridge stage” of winter wheat [25].

**Climatic Indicator Description Related to Each Period**

In order to explain the climatic condition effect, the following meteorological indicators were determined: air minimum temperature (MinT), air maximum temperature (MaxT), air mean temperature (MeanT), soil minimum temperature (MinTs), precipitation (Prec), cumulated precipitation (Prec_S), mean humidity (MeanH), vapour pressure deficit (VPD), maximum VPD (VPD_M), Penman evapotranspiration (ETPP), photosynthetically active radiation (PAR), and cumulated growing degree-days (CGDD). The summary of the meteorological indicators per growing period over the 2 years is shown in Table 2.

**Statistical Analyses**

Two different analyses for unbalanced datasets were performed, according to the “age” effect and the “climatic condition” effect models (Fig. 2). One hundred fifty-nine genotypes were included in each of these analyses, with 82 in common between the G1 and G2 genotype groups.

An initial round of analyses of linear mixed models consisted of a full statistical model which took into account block and spatial effects using the “breedR” package implemented in the R software [28]. It accounted for the environment heterogeneity within each trial among the blocks and diagnosed spatially distributed patterns of remaining residual variations using variograms. All variance components of the progeny were estimated by using the remlf90 function of the package, based on restricted maximum likelihood estimations. The equation of model 1 was as follows:

\[
Y_{ijkl} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + \delta_l + \epsilon_{ijkl}
\]

where \(Y_{ijkl}\) represents the phenotypic value measured on plant k of genotype i at age j in block l; \(\mu\) is the overall mean; \(\alpha_i\) is the random effect of genotype i; \(\beta_j\) is the fixed effect of age j; \((\alpha\beta)_{ij}\) is the random interaction between genotype i and age j; \(\delta_l\) is the effect of block l; and \(\epsilon_{ijkl}\) is the random residual for plant k of genotype i at age j in block l. An autoregressive spatial component was also considered in the model based on x and y coordinates in each plot in order to partition the residual \(\epsilon_{ijkl}\) into a spatially dependent parameter, \(\theta_{ikl}\), for plant k of genotype i in block l, and an independent remaining residual [29].

This full model was compared with two sub-models for each trait: a sub-model with no decomposition of the residual term into spatially dependent and independent effects and another sub-model with no block effect but with the decomposition of the residual term into spatially dependent and independent effects. The performance of the three models was compared on their Akaike information criterion, for which a lower value corresponded to a better performance. For all traits, the last sub-model, which dropped the block effect but took into account the spatial effect, was found to have a slightly better performance than the full model or than the sub-model which dropped the spatial effect but took into account the block effect. Accordingly, it was retained

| Weather indicator | Period 1 | Period 2 | Period 3 | Period 4 | Period 5 |
|-------------------|----------|----------|----------|----------|----------|
| Prec_S/mm         | 312.5    | 327.5    | 26.0     | 54.5     | 136.5    | 112.5    | 126.5    | 208.5    | 116.5    | 21.5     |
| Prec/mm           | 2.25     | 2.09     | 1.08     | 0.97     | 1.64     | 1.22     | 1.18     | 2.11     | 2.38     | 2.39     |
| MeanH/mm          | 88.5     | 87.5     | 75.4     | 72.1     | 72.0     | 72.1     | 73.3     | 81.4     | 89.8     | 91.4     |
| MinTs/℃           | 4.1      | 5.6      | 11.9     | 9.5      | 18.1     | 18.1     | 16.7     | 13.1     | 7.3      | 6.6      |
| MinT/℃            | 2.3      | 3.5      | 8.2      | 5.2      | 12.2     | 12.2     | 11.6     | 9.0      | 4.8      | 4.2      |
| MaxT/℃            | 7.8      | 9.6      | 18.3     | 15.3     | 23.8     | 24.0     | 23.1     | 17.4     | 10.4     | 9.1      |
| MeanT/℃           | 4.8      | 6.3      | 13.1     | 10.1     | 17.9     | 18.0     | 16.9     | 12.9     | 7.4      | 6.5      |
| VPD/%             | 0.1      | 0.13     | 0.41     | 0.37     | 0.61     | 0.63     | 0.57     | 0.33     | 0.11     | 0.08     |
| VPD_M/%           | 0.27     | 0.37     | 1.16     | 1.16     | 1.66     | 1.74     | 1.64     | 0.96     | 0.31     | 0.23     |
| ETPP/mm           | 0.63     | 0.57     | 2.88     | 2.81     | 4.27     | 4.42     | 2.84     | 1.78     | 0.38     | 0.23     |
| PAR/d⋅cm⁻²         | 230.4    | 217.5    | 747.9    | 720.2    | 989.0    | 932.6    | 678.6    | 438.5    | 163.2    | 120.6    |
| CGDD/d⋅℃          | -        | -        | 209.2    | 203.1    | 963.5    | 957.6    | 590.6    | 453.1    | -        | -        |

*Prec_M* mean precipitation; *Prec_S* accumulated precipitation; *MeanH* mean humidity; *MinTs* soil minimum temperature; *MinT* minimum temperature; *MaxT* maximum temperature; *MeanT* mean temperature; *VPD* vapour pressure deficit; *VPD_M* maximum VPD; *ETPP* Penman evapotranspiration; *PAR* photosynthetically active radiation; *CGDD* cumulated growing degree-days
The performance of the full model including the interaction term was compared with the corresponding sub-model with no interaction by using the Akaike information criterion. Based on the AIC, the analysis showed that a model with no interaction by using the Akaike information criterion term was compared with the corresponding sub-model when the genotypes were 1 year older. In case modeling (b), only 4-year-old plants were considered: they corresponded to G1 genotypes which grew in 2018 and G2 genotypes in 2019. In this case, plants of the same age grew in two different climate conditions, related to each year considered.

Fig. 2 The staggered-start design which was composed of two groups of genotypes, G1 established in 2014 and G2 in 2015, was analyzed using an initial model which accounted for the age effect modeling per climatic condition related to a given year (a) and a second model which accounted for the climatic condition effect modeling at 4-year-old plants (b). In case (a), the year 2018 was considered for G1 and G2, with the age effect that was taken into account by 4-year-old plants with G1 and 3-year-old plants with G2. Plants of different ages grew in the same climatic condition during a same year (2018) which makes it possible to take into account the age effect in the same climatic conditions. The age effect was also analyzed in 2019 in the same manner when the genotypes were 1 year older. In case modeling (b), only 4-year-old plants were considered: they corresponded to G1 genotypes which grew in 2018 and G2 genotypes in 2019. In this case, plants of the same age grew in two different climate conditions, related to each year considered.

According to these two models, genotypic broad-sense heritability was estimated as follows:

- For the age effect,
  \[ H^2_{\alpha} = \frac{\sigma^2_{\alpha}}{\sigma^2_{\alpha} + \sigma^2_{\alpha\beta} + \sigma^2_{\theta} + \sigma^2_{\varepsilon}} \]  
  \[ (3) \]
  where \( \sigma^2_{\alpha} \) is the variance attributed to the genotype, \( \sigma^2_{\alpha\beta} \) is the variance of the genotype \( \times \) age interaction, \( \sigma^2_{\theta} \) is the spatial variance and \( \sigma^2_{\varepsilon} \) is the residual variance.

- For the climatic condition effect,
  \[ H^2_{\gamma} = \frac{\sigma^2_{\gamma}}{\sigma^2_{\alpha} + \sigma^2_{\alpha\gamma} + \sigma^2_{\theta} + \sigma^2_{\varepsilon}} \]  
  \[ (4) \]
Here, each term is similar to the previous formula, except for $\sigma^2_{a\gamma}$, which is the variance of the genotype × climatic condition interaction.

According to the two previous models, progeny-mean heritability \cite{30, 31} was also estimated for the age and climatic condition effect models, respectively as:

$$H^2_{Pi} = \frac{\sigma^2_a}{\sigma^2_a + \frac{\sigma^2_{a\gamma}}{J} + \frac{(\sigma^2_{a\gamma} + \sigma^2_{e\gamma})}{JK}}$$ \hspace{1cm} (5)

$$H^2_{Pi} = \frac{\sigma^2_{a\gamma}}{\sigma^2_a + \frac{\sigma^2_{a\gamma}}{L} + \frac{(\sigma^2_{a\gamma} + \sigma^2_{e\gamma})}{LK}}$$ \hspace{1cm} (6)

where $J$ are the two ages considered, $L$ are the two climatic conditions (of each year), and $K$ is the mean number of plant repetitions per genotype.

Due to the unbalanced feature of the design, as all genotypes were not common between G1 and G2 (i.e. not established twice in 2014 and 2015), most previous models were applied twice as a second round of analyses was restricted to the 82 common genotypes between G1 and G2. It allowed a more precise comparison of the climatic condition and age interaction effects, thus avoiding biases due to non-common genotypes between the comparisons.

Genetic and phenotypic correlations were then assessed for each trait, considering a given age or a given year. Genetic correlations were estimated as described by Howe et al. \cite{32}. To calculate phenotypic correlations, Pearson correlation coefficients were computed using R package “stats” and visualized using R package “corrplot” \cite{33}.

A principal component analysis (PCA) was performed on the climatic indicators determined in Table 2 using the R “FactoMineR” package. This PCA was followed by a clustering that was applied to the coordinates of the individuals in the two first components of the PCA. These individuals corresponded to combinations between the periods and the 2 years. Within a period, the number of clusters was defined when the 2 years of a given period were separated. Then, the climatic features of the separated combinations were pointed out.

### Results

**A Delay of About 20 Days Was Observed in 2019 Compared to 2018 for All Flowering Time-Related Traits**

When comparing the 2 years of observation of a same stand age, the flowering time was significantly delayed—by around 20 days on average—in 2019 in comparison to 2018 (Table 3). In 2018, the median DOY values for the start of spike emergence (SPE), anther appearance (AA), mid-point of spike emergence (MPE), and completion of spike emergence (CPE) were 212, 218, 218, and 222, respectively. In 2019, the median DOY values for SPE, AA, MPE, and CPE were all delayed to 233, 238, 239, and 243, respectively. After discarding extreme odd values (corresponding to a 95% interval from 2.5 to 97.5% DOY quantiles), the range of the population was rather stable in both years for each flowering trait. It was around 30 days excepting for CPE observed in the first group of established genotypes (G1).

### The Climatic Condition Effect Induced Lower Genetic Parameters than the Age Effect

The genetic and environmental variance traits were calculated for the flowering time-related traits using the two models that were distinguished by the age and climatic condition effects, and by the corresponding interaction effects with the

| Trait     | Genotype group | 2.5% quantile/day | Median/day | 97.5% quantile/day | Mean/day | S.D | 95% quantile interval/day |
|-----------|----------------|-------------------|------------|--------------------|----------|-----|----------------------------|
| SPE       | G1             | 197               | 218        | 213                | 213      | 8.1 | 32                         |
|           | G2             | 197               | 214        | 211                | 212      | 6.6 | 30                         |
| AA        | G1             | 201               | 224        | 218                | 218      | 8.7 | 33                         |
|           | G2             | 203               | 220        | 218                | 218      | 7.5 | 31                         |
| MPE       | G1             | 206               | 230        | 218                | 219      | 7.7 | 30                         |
|           | G2             | 206               | 222        | 217                | 218      | 6.6 | 28                         |
| CPE       | G1             | 211               | 233        | 222                | 223      | 7.6 | 27                         |
|           | G2             | 208               | 230        | 222                | 222      | 6.7 | 29                         |
| IHFG1     | G1             | 2.0               | 1.0        | 5.0                | 4.0      | 2.8 | -                          |
|           | G2             | 2.0               | 1.3        | 5.0                | 5.0      | 3.1 | -                          |

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Table 3 Day of year (DOY) quantities, mean and standard deviation calculated for all flowering time-related traits and genotype groups in both years. Range of the population determined by the interval between 2.5 and 97.5%
genotype (models 1 and 2). These traits included SPE, AA, MPE, CPE, and IHF (Fig. 3).

Considering the climatic condition effect model, an initial dataset was used, which corresponded to G1 tested in 2018 and G2 in 2019 (Fig. 3a): all these plants presented the same plant age of 4 years old, but grew in different years, i.e. different climatic conditions, which made it possible to test the climatic condition effect on the variance components. A second dataset was also used as it corresponded to the previous one which was restricted to the common genotypes between G1 and G2 (Fig. 3b). The results showed that genetic variance most influenced each flowering trait (Fig. 3a, b). The variance corresponding to the interaction between genotype and climatic condition was substantial, while the residual variance was lower than the two previous ones. Therefore, the broad-sense heritability estimations for all flowering time-related traits were moderate and ranged from 0.55 to 0.62, whereas the progeny-mean heritability estimations were higher than expected, and ranged from 0.77 to 0.82. The second dataset, which was restricted to the common genotypes between G1 and G2, provided similar trends. Therefore, estimates of heritability were relatively similar for balanced designs using these common genotypes compared to the unbalanced dataset (i.e. the first dataset): broad-sense heritability estimations for all flowering time-related traits were moderate and ranged from 0.53 to 0.61 while the progeny-mean heritability estimations were high and varied from 0.79 to 0.85.

Regarding the age effect model, two datasets were observed: G1 (established in 2014) and G2 (established in 2015) that were observed either in 2018 (dataset corresponding to Fig. 3c) or in 2019 (dataset corresponding to Fig. 3e). G1 and G2 corresponded to the plant groups at different ages but grew during the same year. In 2018, when the plant age was 4 for G1 and 3 for G2, all flowering time-related traits showed large genetic variances but displayed small variances for the interaction of the genotype with age, as well as for the spatial effect (Fig. 3c). Due to these small environmental component variances, the broad-sense heritability estimations for all flowering time-related traits were higher than those observed with the climatic condition effect model and ranged from 0.73 to 0.77, while the progeny-mean heritability estimations were very high and varied from 0.9 to 0.92. As for the climatic condition effect model, the same trend was observed when the analysis was restricted to the common genotypes as the same estimates of genetic parameters were obtained (Fig. 3d).

In 2019, older plant ages were analyzed and corresponded to plant age 5 for G1 and plant age 4 for G2 (Fig. 3e). In comparison to 2018, there was a noticeable shift in the total variance and genetic variance towards lower values while the genetic variance remained the largest contribution to the total variance. Larger interaction effects between genotype and age were also noticeable in 2019, in particular for SPE and AA. Consequently, broad-sense heritability was lower in 2019 than in 2018 and varied from 0.61 to 0.69, and so were progeny-mean heritability estimations that ranged from 0.81 to 0.83 for all flowering time-related traits.

When comparing Fig. 3a with c in order to better understand the difference between the climatic condition and age effects on flowering time-related traits, the results showed that the total variance of each flowering trait was almost similar under the effects of climatic condition and plant age (observed in 2018). For all flowering time-related traits, the interaction variance between genotype and climatic condition (with model 2) was much higher than the interaction variance between genotype and plant age (with model 1): the climatic condition increased these interaction variances from three to sevenfold according to the trait. The interactions between genotype and climatic condition (on the x-axis, Fig. 4) were indeed higher than interactions between genotype and age (on the y-axis). For most traits, the range of the effects was indeed greater on the x-axis than on the y-axis. In contrast, the spatial and residual variances were rather stable between the climatic condition effect model and the age effect modelled in 2018. To avoid biases in these comparisons, the datasets were restricted to the common genotypes between G1 and G2 (Fig. 3b, d) and provided very similar results to those obtained from the whole datasets by comparing Fig. 3a to e. When comparing the climatic condition effect model to the age effect modelled in 2019 (Fig. 3a, e), the trend was the same, although the total variance of the age effect modelled in 2019 was lower and the increase due to the climatic condition was of a lesser extent: nevertheless, the interaction variance between the genotype and the climatic condition for all flowering time-related traits was still higher than the interaction variance between the genotype and the age for all flowering time-related traits modelled in 2019, but increased from two to fourfold.

### High Positive Genetic Correlations Where Observed Among Most Flowering Time-Related Traits When the Plant Age or Climatic Condition Effects Were Modelled

Genotypic and phenotypic correlations among the flowering time-related traits were estimated using climatic condition and age effect models (reported above and below the diagonal of Fig. 5, respectively). The results showed that the phenotypic correlation coefficients for all flowering time-related traits in all data sets were lower than the corresponding genotypic correlation coefficients, but all values coincided. Therefore, the present study focused on comparing correlations at the genetic level. The same traits as before were studied (SPE, AA, MPE, CPE, and IHF).
Fig. 3 Estimations of genetic and environmental variances and heritability for flowering time-related traits considering age effect (model 1) or climatic condition effect (model 2) models. The flowering time-related traits included the start of panicle emergence (SPE), anther appearance (AA), mid-point panicle emergence (MPE), complete panicle emergence (CPE), and the interval between anther appearance and the start of panicle emergence (IHF). Five datasets were modelled: the climatic condition effect model using the whole dataset (on a) or common genotypes between G1 and G2 (b), the age effect model observed in 2018 using the whole dataset (c) or common genotypes between G1 and G2 (d), and finally, the age effect model observed in 2019 using the whole dataset (e). Vgeno stands for genotypic variance, \( V_{g\times a} \) for interaction variance between genotype and plant age, \( V_{g\times c} \) for interaction variance between genotype and climatic condition, Vspat for spatial variance and Vres for residual variance.
In 2018, the estimates found under the age effect modelling process showed strong and positive genetic correlations among SPE, AA, MPE, and CPE (Fig. 5). For instance, CPE showed the highest correlation (0.99) with MPE among these higher correlated flowering time-related traits, and the smallest correlation coefficient (0.93) was observed for AA with SPE, MPE, and CPE. IHF showed a positive and significant correlation (0.45) with AA and a positive but not significant correlation with other flowering time-related traits (0.08 to 0.18).

Similarly, in 2019, the age effect model of the population corresponded to older plants; the genetic correlation estimates remained strong among SPE, AA, MPE, and CPE. IHF showed a shift toward negative and weak values with SPE, MPE, and CPE. A positive (but not significant) correlation with AA can also be noted. In addition, the genetic correlation estimates relating to the climatic condition model at plant age 4 also performed a very similar result as the age effect model of the population.

Comparing the genetic (above the diagonal of Fig. 5) and phenotypic (below the diagonal of Fig. 5) correlations of all traits, the result provided very similar information, where the genetic correlation coefficient coincided with the phenotypic correlation coefficient, and the genetic correlation coefficient a little larger than the phenotypic correlation coefficient.

**Climatic Conditions that Occurred Before the Floral Transition Contributed to the Differences Between the 2 Years**

Due to the previously highlighted high climatic condition effect, a principal component analysis (PCA) was conducted to identify the climatic indicators that contribute to the climatic condition effect for the flowering time-related traits observed in the F1 population. The first two principal components (PCs) accounted for a large proportion of the total variation (93.0%), with PC1 explaining 84.2% and PC2 explaining 8.8% (Fig. 6).

The PC1 first component showed that 8 climatic indicators had high positive loadings (> 0.9), on the right-hand position of Fig. 6a: indicators related to temperatures (MinT, MeanT, and MaxT for the air and MinTs for the soil), vapour pressure deficit (VPD and maximal VPD_M), Penman evapotranspiration (ETPP), and photosynthetically active radiation (PAR). Moreover, these indicators were highly correlated with each other. PC1 also indicated that MeanH had a high negative loading (< −0.9), on the left-hand position of Fig. 6a. PC2 was explained by a single variable that was related to the sum of precipitation calculated for each period and each year (Prec_S).

The clustering of the coordinates on the first two PCA components of the individuals (i.e. periods according to year) clearly divided them into seven distinct groups (Fig. 6b). Most groups included both years for the periods, excepting two periods: period 2 which corresponds to the shoot emergence to floral transition phase (determined using the Malepartus clone) and period 4 which corresponds to the flowering to senescence phase. Interestingly, period 2 gathered climatic events which preceded flowering: it can be noted that both years received little precipitation while the temperature was greater in 2018 compared to 2019. During period 2, all indicators related to temperature were higher for 2018 compared to 2019 which points to warmer climatic conditions in 2018: maximum temperature of 18.3 °C against 15.3° and minimum temperature of 8.2 °C against 5.2 °C. The duration of period 2 in 2018 was 35 days, which...
was 30 days less than the same period in 2019, but it was

Fig. 5 Phenotypic and genotypic correlation estimates among flowering time-related traits for the age effect model in 2018. The flowering time-related traits (SPE, AA, MPE, CPE, and IHF) were the same as in Fig. 3. Above the diagonal were the estimations of genotypic correlation, and below were the estimations of phenotypic correlation. Significant correlations are given at the 0.05 probability level and cells with values <0.18 correspond to non-significant correlations.

Fig. 6 The principal components analysis (PCA) plot of the climatic indicators according to the first and second principal components, PC1 and PC2, respectively (a). Climatic indicators were related to each period of the plant cycle in a given growing season (2018 and 2019). Plot illustrating the clustering of the coordinates on the first two PCA components of the individuals, i.e. 5 periods according to 2 years (b).
rather constant in both years when estimated in cumulative growing degree-days (209.2 for 2018 and 203.1 for 2019).

Discussion

In the present study, the flowering-related traits were extensively phenotyped within an F1 diploid population of *M. sinensis* for 2 years (2018 and 2019) and included the start of spike emergence (SPE), anther appearance (AA), midpoint of spike emergence (MPE), and completion of spike emergence (CPE) as well as the interval between the start of spike emergence and anther appearance (IHF). All flowering time-related traits differed significantly within the population according to the genotypes and years. Interestingly, the flowering time was significantly delayed—by around 20 days in 2019 compared to in 2018 (Table 3). A climatic condition effect on the genetic parameters was also observed but traits responded similarly to this effect. Therefore, the discussion focused on three points: (1) the flowering time delay may be explained by climatic conditions that occurred before the floral transition, (2) the genotype × climatic condition variances were greater than the genotype × age variances for all flowering time-related traits, which implied a decrease in heritability, and (3) although the existence of these previously significant effects of the climatic condition, most flowering time-related traits of the population behaved similarly to changes in climatic conditions.

The Delay in the Flowering Time Was Explained by Climatic Conditions That Occurred Before the Floral Transition

Two periods were highlighted based on an extensive analysis of climatic indicators according to different periods of the plant cycle: a principal component analysis followed by a clustering of the first two component coordinates of the periods for both years showed that period 2 and period 4 were subjected to different climatic conditions in 2018 and 2019. Period 2, which corresponds to the shoot emergence to floral transition period, is interesting because it concerned climatic events that occurred before flowering, which may have an impact on the flowering time. In particular, cooler temperatures during the period preceding floral transition (a 3 °C decrease in maximal and minimal temperatures) contributed to the 20-day delay for the flowering time between the 2 years. In this study, we also observed that all climatic indicators for the subsequent period from floral transition to flowering were very similar in both years, especially those related to the temperature that were almost identical. Therefore, differences in the temperatures observed during the emergence to floral transition period accounted for the delay in the flowering time in 2019. A significant delay was also observed between years (including both age and climatic condition effects) in the mean annual flowering day in *M. sinensis* [17]. Growing seasonal precipitation, degree days, and the temperature calculated for the whole climatic condition partially accounted for this delay. In our study, we highlighted that the period preceding flowering, and in particular the period preceding the floral transition stage, is critical and has an impact on the flowering of *Miscanthus*. Regarding cooler conditions, Clifton-Brown et al. [9] found that in a multi-environment experiment, *M. sinensis* flowered later in the climatic condition at lower-temperature experimental sites. Nunn et al. [34] compared the growth of 15 *Miscanthus* germplasm types, including *M. sinensis*, *M. sacchariflorus*, and *M. × giganteus*, in six European countries with different climatic conditions and found that the climatic condition was accelerated in the hottest experimental site, with earlier emergence, flowering, and senescence than at other experimental sites. Here, we noticed that a three-degrees decrease during the plant cycle preceding the floral transition can imply a substantial delay in the flowering time by about 20 days, since the cumulative growing degree-days of that period were constant between the 2 years. Regarding the cumulative growing degree-days, according to Deuter [25], *M. sinensis* is a plant that will flower upon an accumulation of a certain number of degree days, which was verified with our population. But our experiment did not allow for the testing of an additional effect of photoperiod on the flowering time in *M. sinensis*.

This highlights the importance of the emergence to floral transition period in explaining differences in earliness/lateness from 1 year to another. In particular, the differences in temperature indicators observed during that period contributed to the year effect on the flowering time in the *M. sinensis* population observed in the present study. This involved a substantial delay in the flowering time, moreover within the same site, as observed here.

The Genotype × Climatic Condition Variances Were Greater than the Genotype × Age Variances for All Flowering Time-Related Traits, Which Implied a Decrease in the Genetic Parameters

In the present study, we modelled the age effect (model 1) and the climatic condition effect (model 2) and the corresponding interaction effects on genotypes, using a staggered-start design. In both modelling processes, the spatial effects were adjusted and contributed to obtain more accurate estimates of the traits by decreasing the residual term. In all cases and for all flowering time-related traits, the genetic variance contributed to the largest part of the variance, which implied rather high heritability levels (broad-sense heritability above 0.55 and progeny-mean heritability above 0.77). Interestingly, the variance due to the
genotype × climatic condition interaction was much greater than that of the variance due to the genotype × age interaction (observed for the 4–3 year and the 5–4 year ages), thus highlighting the efficiency of the experimental staggered-start design in dividing the year effect into age and climatic condition effects. Therefore, the climatic condition not only implied a delay in the flowering time but also involved the differential behaviours of the genotypes, which led to higher genotype × climatic condition interaction variances and consequently lower genetic parameters (genetic variances and heritability) for all flowering time-related traits.

Such an environmental influence on phenotypic traits has also been reported in previous studies. Clark et al. [35] estimated the heritability of 569 M. sinensis genotypes and 9 M. × giganteus for 14 yield component traits at six experimental sites, respectively. They found that the heritability estimates for the same trait were significantly different across experimental sites due to different environmental conditions. But in the present study, we also highlighted such environmental effect in a single location. Hazard et al. [36] observed that perennial ryegrass established under outdoor conditions had lower heritability estimates than ryegrass grown in greenhouses, and they suggested that the different temperature conditions during the growth processes may result in heritability changes. Regarding the year effect, Gifford et al. [19] estimated the heritability of 13 phenotypic traits associated with biomass productivity for 221 M. sinensis and found an effect of the year (which includes both age and climatic condition effects). Segura et al. [21] observed that the heritability of most architecture-related traits showed highly significant age effects by using a staggered-start design on apple trees, which was interpreted as the result of significant changes in climatic conditions. When modelling the age effect on flowering time-related traits in our study, significant changes were observed between 2018 and 2019, which can be interpreted as a result of significant different climatic conditions as well, in particular during the plant cycle prior to the floral transition.

The results above highlighted the efficiency of the staggered-start design in dissecting the year effect into age and climatic condition effects in Miscanthus. Consequently, we could observe that the genotype × climatic condition interaction effects were much greater than the genotype × age interaction effects. Therefore, the climatic condition not only implied a delay in the flowering time but also implied the differential behaviour of the genotypes leading to higher genotype × climatic condition interaction variances, and consequently lower genetic parameters (genetic variances and heritability). Such a result would provide further evidence that climatic condition influences flowering time-related traits, resulting in an up to 20% decrease in broad-sense heritability according to the flowering trait. Therefore, the breeding of such traits has to be conducted under contrasted climatic conditions in order to take into account the interactions of the genotypes with changing climatic condition conditions.

**Most Flowering Time-Related Traits of the Population Behaved Similarly to Changing Climatic Condition Conditions**

In order to better understand the effects of climatic conditions and plant age on the flowering time-related traits in Miscanthus, our study further analyzed the genetic and phenotypic correlations among all flowering time-related traits (Fig. 5). The results showed that genetic and phenotypic correlations for flowering time-related traits provided very similar information, where genetic correlation coefficients coincided with phenotypic correlation coefficients, the former being slightly larger than the latter. This indicates that the different flowering time-related traits were mainly genetically determined. Genetic correlation is a component of phenotypic correlation, and the phenotypic correlation coefficient for two traits with high genetic correlation is largely determined by the genetic correlation coefficient, which is more reliable than phenotypic correlation because genetic correlation can eliminate the influence of the environment. In this study, there were positive and strong correlations between the flowering time-related traits of SPE, AA, MPE, and CPE under the climatic condition effect and age effect models. Interestingly, the differences in the correlation estimates were very small when comparing the climatic condition and age models except for the variate IHF, which corresponds to the interval between the start of panicle emergence (SPE) and that of anther appearance (AA): the lowest values were indeed obtained when the climatic condition was modelled in comparison to the age model, and this was due to smaller genotype × age interactions than genotype × climatic condition interactions. It seemed that all traits behaved in the same way within the population when subjected to climatic condition changes, excepting the interval between the start of panicle emergence and that of anther appearance. This interval tended to be shorter under warmer conditions such those observed in 2018, and showed large differences among genotypes. Such changes according to years, regrouping age and climatic condition effects, have been observed by Gifford et al. [19] who compared the genetic and phenotypic correlations of the 2 years for biomass yield, plant height, and plant circumference observed on the 221 progeny of Miscanthus sinensis.

Although changes in climatic conditions implied a significant delay in the flowering time and large variations in the genetic parameters of flowering-related traits, most flowering time-related traits of the population behaved similarly to varying climatic condition conditions. The
single exception was observed for the interval between the start of panicle emergence and that of anther appearance.

**Conclusion and Prospects**

In conclusion, the climatic conditions preceding the floral transition of *M. sinensis* led to changes in its flowering time, and temperature was one of the main variables that affected the flowering time observed on a diploid *M. sinensis* progeny. In our study, a staggered-start design was efficient in dividing the year effect into plant age and climatic condition effects and made it possible to discover that the genotype × climatic condition variance was much greater than the genotype × age variance for each flowering trait. This implied that the differential behaviour of genotypes led to genotype × climatic condition interactions, which resulted in altered genetic parameters (genetic variances and heritability). In addition, most of the flowering time-related traits observed in our population—start of panicle emergence, anther appearance, mid-point of emergence, and completion of emergence—behaved similarly to changing climatic conditions, excepting the interval between the panicle emergence and anther appearance. All the results above brought strong evidence of the effect of changing climatic conditions on flowering time-related traits in *M. sinensis*, moreover observed within a single location. This resulted in non-stable earliness/lateness of the genotypes, which may impact the stability of their biomass yield itself. Therefore, breeding for flowering time-related traits in *M. sinensis* must be carried out under contrasted climatic conditions in order to consider the interaction of the genotypes with changes in climatic conditions and select more stable genotypes for the flowering time. This is critical for targeting new stable varieties, especially on marginal land where yields are known to be not only lower but highly variable. This study provides new insights for the breeding of more stable genotypes in *M. sinensis*, which is an important consideration for the cultivation of *Miscanthus* in new areas, in particular on marginal land.

**Acknowledgements** The authors gratefully acknowledge financial support from the China Scholarship Council. The authors thank the staff at the INRAE experimental unit of Estrées-Mons, GCIE Picardie, and in particular Marie-Chantal Mansard, Marie Heumérez-Lévéque. We wish to thank Rebecca James who edited the English text.

**Author Contribution** Maryse Brancourt-Hulmel and Wei Hou contributed to the study conception and design. Material preparation and data collection were performed by Wei Hou, Emilie Mignot, and Stéphanie Arnoult. Wei Hou, Raphaël Raverdy, Catherine Giauffret, and Maryse Brancourt-Hulmel contributed to data analysis. The first draft of the manuscript was written by Wei Hou, and all authors commented the manuscript.

**Funding** The funding for this research was provided by the French National Research Agency (Agence Nationale de la Recherche, ANR), grant ANR-11-BTBR-0006-BFF in the frame of the program Investments for the Future.

**Availability of Data and Material (Data Transparency)** Availability of data through GnpIS platform of INRAE: https://urgi.versailles.inra.fr/Tools/GnpIS.

**Declarations**

**Ethics Approval and Consent to Participate** Not applicable.

**Consent for Publication** All authors agree to publish this article in BioEnergy Research.

**Competing Interests** The authors declare no competing interests.

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