

# Nonlinear climatic regulation of flowering phenology in sugarcane wild relatives revealed through mixed-effects and survival modelling

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## Research Article

**Keywords:** Distributed lag nonlinear model, Generalized additive model, Linear mixed-effects model, Survival analysis

**Posted Date:** August 26th, 2026

**DOI:** <https://doi.org/10.21203/rs.3.rs-10648620/v1>

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**Additional Declarations:** No competing interests reported.

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# Abstract

Flowering phenology is a key adaptive trait influencing plant reproduction and responses to climate change, yet the climatic regulation of flowering in tropical perennial grasses remains poorly understood. We investigated the influence of pre-flowering climatic conditions on flowering initiation in 251 sugarcane wild and allied genotypes evaluated over multiple years, representing 1,330 genotype–year observations. Flowering records were integrated with climatic variables during the 30-day pre-flowering period, including growing degree days (GDD30D), cumulative rainfall (RF30D), relative humidity (RH30D), and solar radiation (SRAD30D). Linear mixed-effects models identified relative humidity as the strongest predictor of flowering initiation ( $P < 0.001$ ), followed by rainfall and solar radiation, whereas GDD showed no significant linear effect. Fixed climatic variables explained 68.2% of the variation, increasing to 88.6% after accounting for genotype and year effects. Interannual environmental variation was approximately 64-fold greater than genotypic variation, highlighting the dominant influence of climatic variability. Generalized additive models revealed pronounced nonlinear responses, while mixed-effects Cox models showed that flowering probability increased with GDD and rainfall but declined with increasing relative humidity and solar radiation. These findings demonstrate that flowering phenology in sugarcane wild relatives is governed by nonlinear climatic regulation, providing new insights into climate–phenology relationships and supporting climate-resilient germplasm conservation and breeding.

## Introduction

Flowering phenology is a fundamental adaptive trait that governs reproductive success, species persistence, and ecosystem functioning by synchronizing reproductive development with favourable environmental conditions. The timing of flowering determines opportunities for pollination, fertilization, and seed production while influencing plant interactions with pollinators, competitors, and other components of the ecosystem (Forrest and Miller-Rushing 2010; Cleland et al. 2007). Because flowering is highly responsive to environmental conditions, phenological shifts have become one of the most sensitive biological indicators of ongoing climate change (Parmesan and Yohe 2003; Menzel et al. 2006). Rising temperatures, altered rainfall patterns, changing atmospheric humidity, and variations in solar radiation affect physiological and developmental processes that regulate flowering initiation, duration, and reproductive success (Piao et al. 2019). Consequently, understanding the climatic regulation of flowering phenology has become increasingly important for predicting plant responses to future climate scenarios and developing effective strategies for biodiversity conservation, germplasm management, and crop improvement.

Temperature has traditionally been regarded as the principal environmental driver of flowering through the accumulation of thermal time or growing degree days (GDD), which regulate the transition from vegetative to reproductive growth (Chuine 2010). However, increasing evidence demonstrates that flowering is regulated by multiple interacting climatic variables rather than temperature alone. Rainfall, atmospheric humidity, and solar radiation influence plant water status, carbon assimilation, hormonal signalling, and floral development, particularly in tropical and subtropical ecosystems where climatic

variability is pronounced (Richardson et al. 2013; Polgar and Primack 2011). Furthermore, flowering responses frequently exhibit nonlinear relationships, threshold effects, and delayed responses to climatic conditions experienced several days or weeks before floral initiation (Wolkovich et al. 2012; Piao et al. 2019). These findings indicate that flowering phenology should be considered a complex ecological process regulated by interacting climatic drivers operating across multiple temporal scales rather than by a single environmental cue.

Flowering phenology is especially important in sugarcane (*Saccharum spp.*), where synchronized flowering is essential for successful hybridization and efficient utilization of genetic resources. Modern commercial sugarcane cultivars possess a relatively narrow genetic base and rely extensively on wild relatives for the introduction of genes conferring disease resistance, abiotic stress tolerance, ratooning ability, biomass production, and climate resilience (Pavani et al. 2023). Wild and allied genera, including *Saccharum spontaneum*, *Erianthus*, *Miscanthus*, *Narenga*, and *Sclerostachya*, represent valuable reservoirs of adaptive genetic diversity that are indispensable for broadening the breeding pool. However, flowering in these taxa often varies considerably among years because of environmental fluctuations, resulting in poor synchronization among potential parents and reducing the efficiency of crossing programmes. Therefore, reliable prediction of flowering behaviour is critical for germplasm conservation, flowering management, and climate-resilient sugarcane breeding.

Although photoperiod and temperature are widely recognized as major environmental cues controlling flowering in sugarcane, increasing evidence suggests that rainfall, atmospheric humidity, and solar radiation also play significant roles in floral induction, panicle emergence, pollen fertility, and reproductive success (Pavani et al. 2023). Climatic conditions influence not only the initiation of flowering but also the physiological processes that determine floral development and successful reproduction. Consequently, flowering in sugarcane should be viewed as a multidimensional ecological response arising from interactions among several climatic variables rather than as a process controlled by temperature or photoperiod alone.

Despite the importance of flowering phenology, most previous studies in sugarcane have relied on correlation analyses or conventional regression models that evaluate individual climatic variables independently and assume linear relationships. While these approaches have improved understanding of broad environmental influences, they are limited in their ability to account for nonlinear climatic responses, delayed effects of environmental conditions, repeated observations across years, and hierarchical variation among genotypes. Flowering is inherently a time-to-event process influenced simultaneously by genotype, annual climatic variation, and interactions among multiple environmental drivers. Consequently, statistical approaches capable of incorporating random effects, nonlinear relationships, and survival processes provide a more realistic framework for analysing flowering phenology than traditional linear models.

Recent advances in ecological statistics have provided powerful tools for investigating complex phenological responses. Linear mixed-effects models partition variation attributable to climatic

predictors while accounting for repeated observations across genotypes and years (Bolker et al. 2009; Zuur et al. 2009). Best linear unbiased predictions (BLUPs) estimate genotype-specific flowering tendencies after minimizing environmental noise, enabling identification of consistently early- and late-flowering genotypes. Generalized additive models (GAMs) characterize nonlinear climatic responses without imposing restrictive functional assumptions (Wood 2017). Mixed-effects Cox proportional hazards models analyse flowering as a time-to-event process by estimating flowering probability under changing climatic conditions, whereas distributed lag nonlinear models (DLNMs) quantify delayed effects of climatic variables occurring days or weeks before flowering (Gasparrini et al. 2010). The integration of these complementary analytical approaches provides a comprehensive framework for disentangling climatic regulation of flowering phenology while simultaneously separating environmental and genetic sources of variation.

In this study, we analysed flowering phenology using a unique multi-year dataset comprising 251 sugarcane wild and allied genotypes representing 1,330 genotype-year observations. Flowering initiation records were integrated with climatic conditions during the 30-day period preceding flowering, including mean daily growing degree days (GDD30D), cumulative rainfall (RF30D), relative humidity (RH30D), and solar radiation (SRAD30D). The objectives were to (i) quantify the relative contributions of climatic variables to flowering initiation using linear mixed-effects models, (ii) estimate genotype-specific flowering tendencies using BLUPs, (iii) characterize nonlinear climatic responses using generalized additive models, (iv) evaluate flowering probability using mixed-effects Cox proportional hazards models, and (v) assess delayed climatic effects using distributed lag nonlinear models. We hypothesized that flowering initiation is regulated by multiple interacting climatic variables rather than temperature alone, that climatic responses exhibit nonlinear and lagged behaviour, and that interannual environmental variability exerts a stronger influence on flowering phenology than genotypic differences. The study demonstrates that flowering phenology in sugarcane wild relatives is predominantly governed by interacting climatic drivers, with annual environmental variation outweighing genetic effects, and establishes a quantitative framework for predicting flowering responses under changing climates to support climate-resilient germplasm conservation and sugarcane breeding.

## Materials and Methods

The study was conducted at the ICAR–Sugarcane Breeding Institute (SBI), Coimbatore, Tamil Nadu, India (11°00' N, 76°57' E; 427 m above sea level). The region has a tropical semi-arid climate with distinct southwest (June–September) and northeast (October–December) monsoon seasons, a mean annual temperature of 23–32°C, and annual rainfall of 650–700 mm. The study included 251 sugarcane wild and allied genotypes maintained in the National Active Germplasm Repository, representing *Saccharum spontaneum*, *S. robustum*, *Erianthus*, *Miscanthus*, *Narenga*, *Sclerostachya*, and related genera. Flowering observations were recorded annually from 2015 to 2024, resulting in 1,330 genotype–year observations (Supplementary table 1). Flowering initiation was defined as the first visible emergence of the panicle from the flag leaf sheath. Flowering dates were converted to day of year (DOY) to standardize

observations across years, and flowering duration was calculated as the interval between flowering initiation and completion.

## Climatic data

Daily meteorological data were obtained from the NASA Prediction of Worldwide Energy Resources (NASA POWER) Data Access Viewer (<https://power.larc.nasa.gov/>) (Stackhouse et al. 2023). Weather data for the study location (11.00° N, 76.97° E) were extracted for 2015–2024.

Climatic variables were summarized over the 30-day period preceding flowering initiation, representing the critical phase for floral induction. The variables included growing degree days (GDD30D), cumulative rainfall (RF30D), mean relative humidity (RH30D), and mean solar radiation (SRAD30D). Growing degree days were calculated from daily maximum and minimum temperatures using a base temperature of 18°C, the standard developmental threshold for sugarcane.

## Statistical analysis

Descriptive statistics were calculated to summarize climatic variability during the pre-flowering period. The effects of climatic variables on flowering initiation were analysed using linear mixed-effects models (LMMs), with climatic variables as fixed effects and genotype and year as random effects. Model performance was evaluated using Akaike Information Criterion (AIC), Bayesian Information Criterion (BIC), and marginal and conditional  $R^2$ . Genotype-specific flowering tendencies were estimated using Best Linear Unbiased Predictions (BLUPs). Nonlinear climatic responses were evaluated using generalized additive models (GAMs) with penalized regression splines. Flowering probability was analysed using mixed-effects Cox proportional hazards models, and hazard ratios (HR) with 95% confidence intervals were estimated. Delayed climatic effects during the 30-day pre-flowering period were assessed using distributed lag nonlinear models (DLNMs). All analyses were performed in R version 4.5.0 (R Core Team, 2025). Linear mixed-effects models were fitted using lme4 (Bates et al. 2015), GAMs using mgcv (Wood 2017), survival analyses using survival and coxme, DLNMs using dlnm (Gasparrini et al. 2010), and figures were prepared using ggplot2.

## Results

### Flowering phenology and interannual climatic variability

Flowering phenology varied considerably among years across the study period (2015–2024), reflecting substantial interannual variation in environmental conditions (Fig. 1). A total of 1,330 genotype–year observations representing 251 sugarcane wild and allied genotypes were analysed (Supplementary table 1). Flowering initiation occurred over a broad seasonal window extending from early April to early August, indicating pronounced differences in flowering behaviour among years. Likewise, flowering duration exhibited noticeable variation, suggesting that both the onset and progression of reproductive development were influenced by prevailing climatic conditions. The climatic variables during the 30-day

period preceding flowering initiation also exhibited considerable variation among years (Table 1). Mean growing degree days (GDD30D) ranged from 5.11 to 13.16°C day<sup>-1</sup>, with an average of 8.37°C day<sup>-1</sup>. Cumulative rainfall (RF30D) varied widely between 4.37 and 285.48 mm, averaging 125.92 mm, indicating large differences in moisture availability among flowering seasons. Relative humidity (RH30D) ranged from 50.30 to 88.73%, with a mean of 76.53%, while mean solar radiation (SRAD30D) varied from 13.46 to 23.42 MJ m<sup>-2</sup> day<sup>-1</sup>, averaging 18.18 MJ m<sup>-2</sup> day<sup>-1</sup>.

Table 1

Descriptive statistics of climatic variables during the 30-day period preceding flowering initiation.

| Variable                                                                                                                                                                                                                                                                       | Minimum | Mean   | Median | Maximum |
|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------|--------|--------|---------|
| GDD30D (°C day <sup>-1</sup> )                                                                                                                                                                                                                                                 | 5.11    | 8.37   | 8.26   | 13.16   |
| RF30D (mm)                                                                                                                                                                                                                                                                     | 4.37    | 125.92 | 118.90 | 285.48  |
| RH30D (%)                                                                                                                                                                                                                                                                      | 50.30   | 76.53  | 79.72  | 88.73   |
| SRAD30D (MJ m <sup>-2</sup> day <sup>-1</sup> )                                                                                                                                                                                                                                | 13.46   | 18.18  | 17.76  | 23.42   |
| GDD30D = growing degree days accumulated during the 30 days preceding flowering initiation; RF30D = cumulative rainfall during the preceding 30 days; RH30D = mean relative humidity during the preceding 30 days; SRAD30D = mean solar radiation during the preceding 30 days |         |        |        |         |

The distributions of climatic variables differed markedly among years (Fig. 2). Annual boxplots showed substantial variation in thermal accumulation, rainfall, atmospheric humidity, and incoming solar radiation during the pre-flowering period. Rainfall exhibited the greatest interannual variability, with several years characterized by high precipitation accompanied by elevated relative humidity and reduced solar radiation. In contrast, years with lower rainfall generally experienced higher solar radiation and lower atmospheric humidity. Growing degree days also varied among years but showed comparatively narrower dispersion than rainfall.

## Climatic determinants of flowering initiation

The effects of climatic conditions during the 30-day period preceding flowering initiation were evaluated using a linear mixed-effects model incorporating genotype and year as random effects (Table 2). The model explained a substantial proportion of the observed variation in flowering initiation, with a marginal R<sup>2</sup> of 0.682 and a conditional R<sup>2</sup> of 0.886, indicating that both climatic predictors and hierarchical random effects contributed to model performance. The fitted model showed good overall fit (AIC = 12,181.6; BIC = 12,223.1; log-likelihood = - 6,082.8). Among the fixed climatic predictors, relative humidity (RH30D) was the strongest determinant of flowering initiation (Estimate = 5.058 ± 0.226, t = 22.409, P < 0.001). Cumulative rainfall (RF30D) also showed a significant positive association with flowering initiation (Estimate = 0.061 ± 0.019, t = 3.155, P = 0.0016), while solar radiation (SRAD30D) had a smaller but significant positive effect (Estimate = 1.629 ± 0.692, t = 2.354, P = 0.0187). In contrast, growing degree days (GDD30D) were not significantly associated with flowering initiation (Estimate = 1.314 ±

1.106,  $t = 1.188$ ,  $P = 0.235$ ). Variance partitioning demonstrated marked differences in the contributions of random effects. The variance attributable to year (907.59) greatly exceeded that associated with genotype (14.13), whereas the residual variance was 515.18 (Table 2). Year-to-year variation was approximately 64-fold greater than genotype-level variation, indicating substantial interannual heterogeneity in flowering phenology after accounting for measured climatic variables.

Table 2  
Linear mixed-effects model of pre-flowering climatic effects on flowering initiation and variance components

| Predictor / model component                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                | Estimate | SE     | Test statistic | P       |
|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------|--------|----------------|---------|
| <b>Fixed effects</b>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       |          |        |                |         |
| Intercept                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  | -260.379 | 28.847 | $t = -9.026$   | < 0.001 |
| GDD30D                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     | 1.314    | 1.106  | $t = 1.188$    | 0.235   |
| RF30D                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      | 0.061    | 0.019  | $t = 3.155$    | 0.0016  |
| RH30D                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      | 5.058    | 0.226  | $t = 22.409$   | < 0.001 |
| SRAD30D                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    | 1.629    | 0.692  | $t = 2.354$    | 0.0187  |
| <b>Random-effects variance</b>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             |          |        |                |         |
| Genotype                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   | 14.13    | 3.76†  | —              | —       |
| Year                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       | 907.59   | 30.13† | —              | —       |
| Residual                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   | 515.18   | 22.70† | —              | —       |
| <b>Model fit</b>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           |          |        |                |         |
| AIC                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        | 12,181.6 | —      | —              | —       |
| BIC                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        | 12,223.1 | —      | —              | —       |
| Log-likelihood                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             | -6,082.8 | —      | —              | —       |
| Marginal $R^2$                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             | 0.682    | —      | —              | —       |
| Conditional $R^2$                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          | 0.886    | —      | —              | —       |
| <p>GDD30D = growing degree days accumulated during the 30 days preceding flowering initiation; RF30D = cumulative rainfall during the preceding 30 days; RH30D = mean relative humidity during the preceding 30 days; SRAD30D = mean solar radiation during the preceding 30 days; SE = standard error; AIC = Akaike information criterion; BIC = Bayesian information criterion. †Values represent standard deviations (SD) of the corresponding random-effect variance components. Marginal <math>R^2</math> represents variance explained by fixed effects, whereas conditional <math>R^2</math> represents variance explained by the full model including fixed and random effects. Significance is based on the reported <math>t</math> tests; <math>P &lt; 0.05</math> was considered statistically significant.</p> |          |        |                |         |

# Genotype-specific variation in flowering tendency

Genotype-specific flowering tendencies were estimated using Best Linear Unbiased Predictions (BLUPs) derived from the linear mixed-effects model after accounting for annual environmental variation. The BLUP estimates revealed substantial differences in flowering behaviour among the 251 sugarcane wild and allied genotypes, indicating considerable genetic variation in reproductive phenology despite the strong influence of annual climatic variability (Fig. 3). BLUP values exhibited a continuous distribution ranging from strongly negative to positive estimates, reflecting a gradient from consistently early- to consistently late-flowering genotypes. Genotypes with negative BLUP values flowered earlier than the population average after adjustment for environmental effects, whereas positive BLUP values indicated relatively delayed flowering. The distribution of BLUP estimates showed that most genotypes clustered around the population mean, while relatively few genotypes consistently exhibited extremely early or extremely late flowering behaviour. This pattern suggests that flowering phenology among sugarcane wild relatives is characterized by continuous quantitative variation rather than discrete flowering classes. Although genotype-specific variation was evident, the magnitude of genetic variation remained substantially smaller than the year-to-year variation estimated by the mixed-effects model, indicating that environmental variability contributed more strongly to flowering initiation than intrinsic genetic differences. The earliest predicted flowering genotypes included IND 00-999, SES 257, IND 85-527, SES 185, and IND 89-639, whereas IND 11-1617, IND 90-828, IND 04-1350, IND 85-607, and SES 361 consistently exhibited the latest predicted flowering (Table 3).

Table 3  
Genotypes exhibiting the earliest and latest predicted flowering initiation  
based on genotype-specific BLUPs

| Rank               | Genotype     | BLUP    | Flowering tendency |
|--------------------|--------------|---------|--------------------|
| Earliest flowering |              |         |                    |
| 1                  | IND 00-999   | -8.0672 | Earliest           |
| 2                  | SES 257      | -7.1937 | Very early         |
| 3                  | IND 85-527   | -7.1134 | Very early         |
| 4                  | SES 185      | -6.5916 | Very early         |
| 5                  | IND 89-639   | -6.1437 | Very early         |
| 6                  | SES 228      | -5.9255 | Very early         |
| 7                  | SES 223      | -5.8228 | Very early         |
| 8                  | IND 82-281   | -5.7653 | Very early         |
| 9                  | IND 84-353   | -5.7609 | Very early         |
| 10                 | Eri 3198     | -5.6948 | Very early         |
| Latest flowering   |              |         |                    |
| 233                | IND 05-1398  | 6.2411  | Very late          |
| 234                | Neyraudia cl | 6.2491  | Very late          |
| 235                | IND 05-1401  | 6.2497  | Very late          |
| 236                | IND 05-1394  | 6.5069  | Very late          |
| 237                | IND 90-771   | 6.9206  | Very late          |
| 238                | SES 361      | 7.0751  | Very late          |
| 239                | IND 85-607   | 7.2238  | Very late          |
| 240                | IND 04- 1350 | 7.2879  | Very late          |
| 241                | IND 90-828   | 8.3499  | Very late          |
| 242                | IND 11- 1617 | 10.4075 | Latest             |

## Nonlinear climatic responses associated with flowering initiation

Generalized additive models (GAMs) revealed significant nonlinear relationships between flowering initiation and all four climatic variables evaluated during the 30-day pre-flowering period (Table 4, Fig. 4). Effective degrees of freedom (EDF) ranged from 8.59 to 8.74, indicating highly nonlinear responses

rather than simple linear associations. Growing degree days (GDD30D) exhibited the strongest nonlinear effect on flowering initiation ( $F = 101.41$ ,  $P < 0.001$ ), followed by relative humidity (RH30D) ( $F = 65.80$ ,  $P < 0.001$ ), solar radiation (SRAD30D) ( $F = 42.79$ ,  $P < 0.001$ ), and cumulative rainfall (RF30D) ( $F = 19.35$ ,  $P < 0.001$ ). The GAM provided excellent predictive performance, explaining 90.5% of the total deviance with an adjusted  $R^2$  of 0.902. The fitted model also showed a relatively low REML score (5919.8) and Akaike Information Criterion (AIC = 11,721.3), indicating good model fit for describing nonlinear climatic influences on flowering phenology. The smooth response functions identified distinct climatic optima associated with the earliest predicted flowering initiation. Flowering was predicted to occur earliest under approximately 10.1 GDD day<sup>-1</sup>, 132.8 mm cumulative rainfall, 80% relative humidity, and 18 MJ m<sup>-2</sup> day<sup>-1</sup> solar radiation (Fig. 4). Outside these climatic ranges, flowering initiation became progressively delayed, reflecting nonlinear responses across environmental gradients.

Table 4

Nonlinear effects of 30-day pre-flowering climatic conditions on flowering initiation estimated using generalized additive models.

| Component                                                                                                                                                                                                                                                                                                                               | Smooth term / Statistic | EDF / Value | Ref. df | F-value | P-value |
|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------|-------------|---------|---------|---------|
| Nonlinear effect                                                                                                                                                                                                                                                                                                                        | s(GDD30D)               | 8.64        | 8.92    | 101.41  | < 0.001 |
| Nonlinear effect                                                                                                                                                                                                                                                                                                                        | s(RF30D)                | 8.60        | 8.92    | 19.35   | < 0.001 |
| Nonlinear effect                                                                                                                                                                                                                                                                                                                        | s(RH30D)                | 8.74        | 8.95    | 65.80   | < 0.001 |
| Nonlinear effect                                                                                                                                                                                                                                                                                                                        | s(SRAD30D)              | 8.59        | 8.93    | 42.79   | < 0.001 |
| Model performance                                                                                                                                                                                                                                                                                                                       | Adjusted R <sup>2</sup> | 0.902       | —       | —       | —       |
| Model performance                                                                                                                                                                                                                                                                                                                       | Deviance explained (%)  | 90.5        | —       | —       | —       |
| Model performance                                                                                                                                                                                                                                                                                                                       | REML score              | 5,919.8     | —       | —       | —       |
| Model performance                                                                                                                                                                                                                                                                                                                       | AIC                     | 11,721.3    | —       | —       | —       |
| Model performance                                                                                                                                                                                                                                                                                                                       | Sample size (n)         | 1,330       | —       | —       | —       |
| GDD30D = growing degree days accumulated during the 30 days preceding flowering initiation; RF30D = cumulative rainfall during the preceding 30 days; RH30D = mean relative humidity during the preceding 30 days; SRAD30D = mean solar radiation during the preceding 30 days; SE = standard error; AIC = Akaike information criterion |                         |             |         |         |         |

## Climatic conditions associated with early flowering

To characterize the environmental conditions under which flowering occurred most rapidly, climatic variables corresponding to the earliest 10% of flowering events were compared with the overall climatic means (Table 5). Early flowering events were associated with moderately greater thermal accumulation ( $9.1 \pm 0.8$  GDD day<sup>-1</sup>) than the overall mean (8.37 GDD day<sup>-1</sup>). Cumulative rainfall preceding early

flowering averaged  $128 \pm 24$  mm, which was similar to the overall mean (125.9 mm). Relative humidity during the earliest flowering events averaged  $70.5 \pm 4.2\%$ , approximately 6 percentage points lower than the overall average (76.5%). Likewise, solar radiation averaged  $16.8 \pm 1.4$  MJ m<sup>-2</sup> day<sup>-1</sup>, which was slightly lower than the overall mean (18.2 MJ m<sup>-2</sup> day<sup>-1</sup>). These climatic conditions define the environmental window under which flowering initiation occurred earliest within the study population.

Table 5  
Climatic conditions associated with the earliest 10% of flowering events

| Variable                                                                                                                                                                                                                                                                       | Earliest 10% flowering                              | Overall mean |
|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------------------------------------|--------------|
| GDD30D                                                                                                                                                                                                                                                                         | $9.1 \pm 0.8$                                       | 8.37         |
| RF30D                                                                                                                                                                                                                                                                          | $128 \pm 24$ mm                                     | 125.9        |
| RH30D                                                                                                                                                                                                                                                                          | $70.5 \pm 4.2\%$                                    | 76.5         |
| SRAD30D                                                                                                                                                                                                                                                                        | $16.8 \pm 1.4$ MJ m <sup>-2</sup> day <sup>-1</sup> | 18.2         |
| GDD30D = growing degree days accumulated during the 30 days preceding flowering initiation; RF30D = cumulative rainfall during the preceding 30 days; RH30D = mean relative humidity during the preceding 30 days; SRAD30D = mean solar radiation during the preceding 30 days |                                                     |              |

## Effects of pre-flowering climatic conditions on flowering probability

The mixed-effects Cox proportional hazards model identified significant effects of pre-flowering climatic conditions on flowering probability (Table 6). All climatic variables except GDD30D significantly influenced the timing of flowering initiation. Growing degree days showed a significant positive association with flowering probability (HR = 1.217, 95% CI = 1.075–1.379, P = 0.00197), indicating that increasing thermal accumulation increased the instantaneous probability of flowering initiation. Likewise, cumulative rainfall significantly increased flowering probability (HR = 1.005, 95% CI = 1.004–1.007, P < 0.001). In contrast, relative humidity significantly reduced flowering probability (HR = 0.821, 95% CI = 0.802–0.840, P < 0.001), while solar radiation also exhibited a significant negative association with flowering probability (HR = 0.856, 95% CI = 0.797–0.920, P < 0.001).

Table 6

Mixed-effects Cox proportional hazards model evaluating the effects of pre-flowering climatic conditions on flowering initiation

| Predictor                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 | $\beta$          | SE      | Hazard ratio (HR) | 95% CI      | z       | P       |
|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------|---------|-------------------|-------------|---------|---------|
| GDD30D                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    | 0.197            | 0.0637  | 1.217             | 1.075–1.379 | 3.094   | 0.00197 |
| RF30D                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     | 0.005            | 0.00076 | 1.005             | 1.004–1.007 | 6.606   | < 0.001 |
| RH30D                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     | –0.197           | 0.0117  | 0.821             | 0.802–0.840 | –16.793 | < 0.001 |
| SRAD30D                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   | –0.156           | 0.0367  | 0.856             | 0.797–0.920 | –4.246  | < 0.001 |
| <b>Model performance</b>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  |                  |         |                   |             |         |         |
| Number of observations                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    | 1,330            | —       | —                 | —           | —       | —       |
| Number of flowering events                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                | 1,330            | —       | —                 | —           | —       | —       |
| Concordance index (C-index)                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               | 0.787            | —       | —                 | —           | —       | —       |
| Likelihood-ratio test                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     | $\chi^2 = 1,352$ | —       | —                 | —           | —       | < 0.001 |
| Wald test                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 | $\chi^2 = 942$   | —       | —                 | —           | —       | < 0.001 |
| Score test                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                | $\chi^2 = 1,854$ | —       | —                 | —           | —       | < 0.001 |
| <p><math>\beta</math> = Cox regression coefficient; SE = standard error; HR = hazard ratio; CI = confidence interval; GDD30D = growing degree days accumulated during the 30 days preceding flowering initiation; RF30D = cumulative rainfall during the preceding 30 days; RH30D = mean relative humidity during the preceding 30 days; SRAD30D = mean solar radiation during the preceding 30 days. HR &gt; 1 indicates an increased instantaneous probability of flowering initiation, corresponding to earlier flowering, whereas HR &lt; 1 indicates a reduced flowering hazard and delayed flowering. The C-index indicates the model's discriminatory ability to rank the relative timing of flowering events.</p> |                  |         |                   |             |         |         |

## Delayed climatic influences on flowering initiation

Distributed lag nonlinear models (DLNMs) demonstrated that climatic conditions experienced during the 30-day period preceding flowering initiation exerted delayed effects on flowering phenology. Response surfaces indicated that both the magnitude and timing of climatic exposure influenced flowering initiation, with lag effects differing among climatic variables. Thermal accumulation exerted the strongest influence approximately 10–20 days before flowering initiation, whereas rainfall exhibited the greatest influence during the 15–25 day pre-flowering period. Relative humidity showed persistent lag

effects across much of the 30-day window, while solar radiation primarily influenced flowering during the final two weeks before flowering initiation. These lag-response surfaces demonstrate that flowering phenology was regulated not only by climatic magnitude but also by the timing of environmental exposure before panicle emergence. Distributed lag nonlinear model response surfaces illustrating delayed effects of GDD30D, RF30D, RH30D, and SRAD30D on flowering initiation (Fig. 5a) and contour plots identify regions of strongest lag-response, highlighting the combinations of GDD values and lag periods that most strongly influence flowering initiation (Fig. 5b). These results indicate that flowering initiation depends not only on climatic magnitude but also on the timing of environmental exposure preceding flowering.

## Discussion

Flowering phenology is among the most climate-sensitive plant traits and is widely recognized as an important indicator of ecological responses to environmental variability and climate change (Parmesan and Yohe 2003; Cleland et al. 2007; Forrest and Miller-Rushing 2010). By integrating linear mixed-effects models, genotype-specific BLUPs, generalized additive models (GAMs), mixed-effects Cox proportional hazards models, and distributed lag nonlinear models (DLNMs), this study demonstrates that flowering initiation in sugarcane wild relatives is regulated by multiple interacting climatic drivers acting through nonlinear and time-dependent mechanisms. Unlike conventional analyses that assume linear responses, our results show that flowering timing depends on the combined effects of atmospheric humidity, rainfall, thermal accumulation, and solar radiation, while interannual climatic variability contributes substantially more to flowering behaviour than genetic differences among genotypes.

Among the climatic variables examined, relative humidity consistently emerged as the strongest determinant of flowering initiation. The mixed-effects model identified RH30D as the most influential fixed effect, and the Cox model showed that increasing humidity significantly reduced flowering probability (HR = 0.821,  $P < 0.001$ ). GAM analysis further revealed pronounced nonlinear responses, indicating that flowering responds to humidity through threshold-dependent rather than linear mechanisms. Atmospheric humidity influences transpiration, stomatal conductance, carbohydrate transport, and hormonal regulation, all of which affect floral induction (Chuine 2010; Richardson et al. 2013). High humidity is generally associated with persistent cloud cover and reduced evaporative demand, conditions that favour prolonged vegetative growth and delay reproductive transition. Similar humidity-mediated effects have been reported in tropical perennial species and grassland ecosystems (Polgar and Primack 2011), suggesting that atmospheric moisture is a major regulator of flowering phenology in sugarcane wild relatives.

Rainfall during the 30-day pre-flowering period also significantly increased flowering probability, indicating that adequate moisture promotes reproductive development by supporting nutrient uptake, assimilate transport, and cell expansion (Richardson et al. 2013). However, the GAM demonstrated a nonlinear response, with flowering being favoured under moderate rather than excessive rainfall. The predicted optimum (approximately 133 mm) closely matched the rainfall observed during the earliest

flowering events ( $128 \pm 24$  mm), suggesting that moderate moisture provides favourable conditions for floral induction. Excessive rainfall likely delays flowering indirectly by increasing atmospheric humidity and reducing radiation, reinforcing the importance of considering climatic variables collectively rather than independently.

Thermal accumulation is traditionally regarded as the principal driver of flowering through the accumulation of growing degree days (Chuine 2010). Although GDD30D was not significant in the linear mixed-effects model after accounting for genotype and year effects, both the GAM and Cox model identified it as an important predictor of flowering. The GAM indicated a strong nonlinear response ( $F = 101.41$ ,  $P < 0.001$ ), while the Cox model showed that increasing GDD significantly enhanced flowering probability ( $HR = 1.217$ ,  $P = 0.002$ ). The optimum predicted thermal accumulation (approximately  $10.1$  GDD  $\text{day}^{-1}$ ) closely corresponded with the mean GDD observed during the earliest flowering events. These findings indicate that flowering occurs only after sufficient thermal accumulation has been achieved and highlight the importance of nonlinear modelling for describing temperature responses. Similar threshold behaviour has been reported across temperate forests, alpine vegetation, and perennial grasslands (Basler 2016; Chuine and Régnière 2017).

Solar radiation also significantly influenced flowering phenology, although its effect was weaker than that of humidity. Higher radiation reduced flowering probability in the Cox model, while the GAM indicated that flowering was optimized at intermediate radiation levels (approximately  $18 \text{ MJ m}^{-2} \text{ day}^{-1}$ ). Moderate radiation likely provides sufficient photosynthetic carbon assimilation to support reproductive development, whereas excessive radiation may increase canopy temperature and evaporative demand, delaying flowering. Similar interactions between radiation, atmospheric moisture, and phenology have been observed in forest ecosystems, where flowering reflects integrated climatic conditions rather than irradiance alone (Richardson et al. 2013; Liu et al. 2015).

A major outcome of this study is the demonstration that interannual environmental variation exerted a much greater influence on flowering phenology than genotypic differences. Variance attributable to year was approximately 64 times greater than genotype variance, and inclusion of random effects increased model explanatory power from 68.2% to 88.6%. These findings indicate that flowering phenology in sugarcane wild relatives is highly environmentally plastic, consistent with reports from forest trees, alpine vegetation, and perennial grasses where annual climatic variability explains most phenological variation (Parmesan 2006; Piao et al. 2019).

Despite the strong environmental influence, BLUP analysis identified genotypes that consistently flowered earlier or later after accounting for annual climatic variation. Early-flowering accessions such as IND 00-999 and SES 257 and late-flowering genotypes such as IND 11-1617 and IND 90-828 demonstrated stable flowering tendencies across years, indicating the presence of heritable variation for flowering time. Because BLUPs separate genetic effects from environmental noise (Henderson, 1975; Piepho et al., 2008), these estimates provide valuable information for selecting parental lines with reliable flowering behaviour in breeding programmes. The integrated analytical framework used in this

study provides important advantages over conventional correlation or regression analyses. Mixed-effects models quantified the relative contributions of climatic and random environmental effects, BLUPs estimated genotype-specific flowering behaviour, GAMs captured nonlinear climatic responses, Cox models quantified flowering probability, and DLNMs identified delayed climatic influences during the 30-day pre-flowering period. Together, these complementary approaches provide a more realistic description of flowering phenology and improve prediction of reproductive responses under variable environmental conditions.

From a practical perspective, the climatic optima identified in this study provide quantitative guidance for flowering management. Flowering initiation was favoured under approximately 10.1 GDD day<sup>-1</sup>, 132.8 mm cumulative rainfall, around 80% relative humidity, and 18 MJ m<sup>-2</sup> day<sup>-1</sup> solar radiation during the pre-flowering period. Combined with genotype-specific BLUP estimates, these results can support flowering synchronization, parental selection, and conservation of wild germplasm. However, these climatic thresholds should be regarded as predictive estimates derived from the observed environmental range and require validation across additional environments.

## **Declarations**

### **Conflict of Interest**

The authors declare that they have no conflicts of interest

## **Author Contribution**

CJ conceived and designed the study. VA, KD and MP collected and curated the flowering and climatic datasets and performed the statistical analyses. CJ and VA developed the analytical framework, interpreted the results, and wrote the first draft of the manuscript. CP contributed to data interpretation and statistical validation. SA contributed to the interpretation of results and critically revised the manuscript. All authors reviewed, edited, approved the final version of the manuscript, and agreed to be accountable for all aspects of the work.

## **Acknowledgement**

The authors express their gratitude to the Director, ICAR SBI, Coimbatore, for the constant support to conduct research on germplasm conservation. Research was supported by the Indian Council of Agricultural Research, Department of Agricultural Research and Education, Government of India.

## **Data Availability**

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## Figures

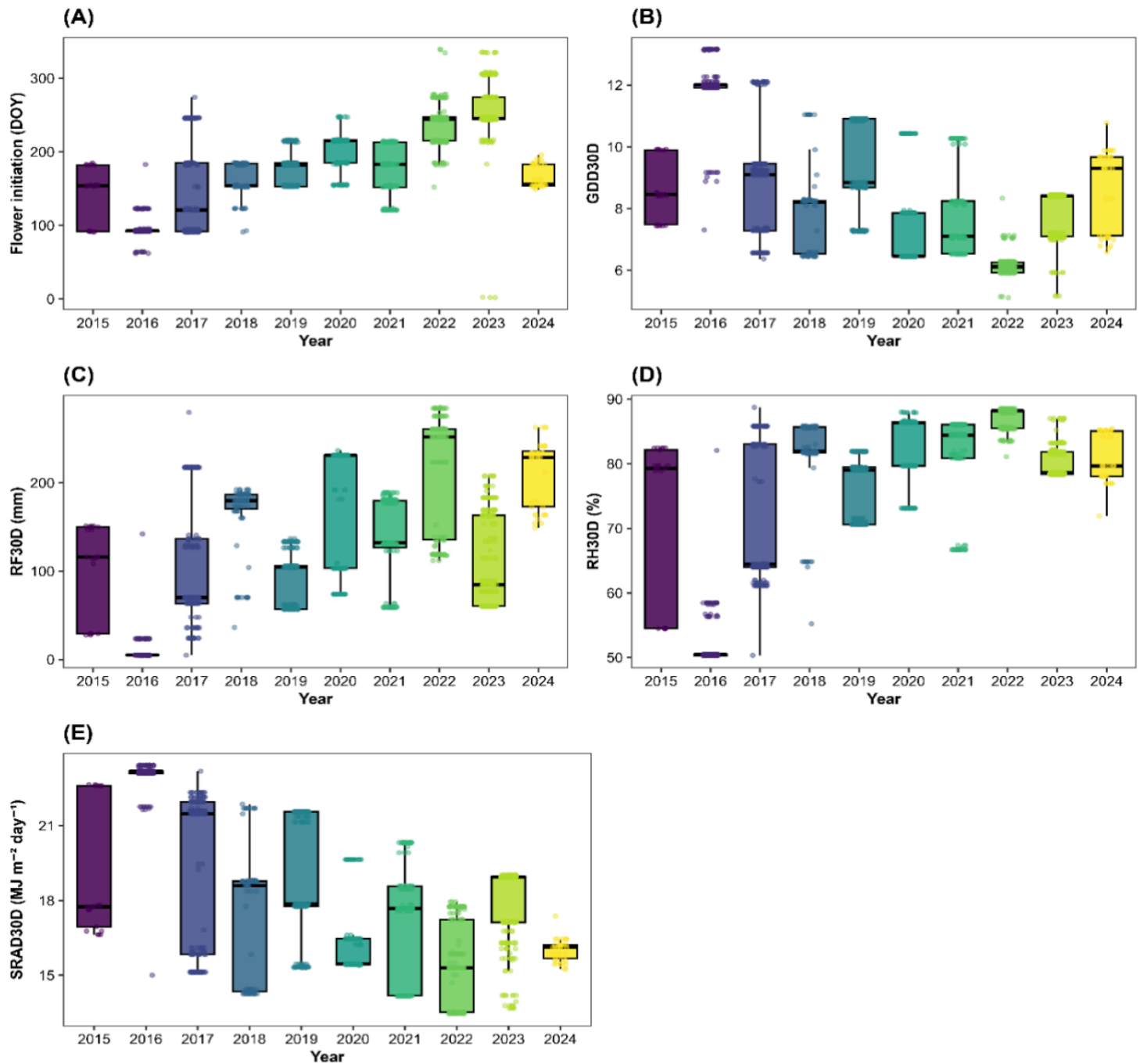


**Figure 1**

**Interannual variation in flowering initiation and climatic variables during the 30-day period preceding flowering initiation.**

Boxplots illustrate annual variation in (A) flowering initiation (DOY), (B) growing degree days (GDD30D), (C) cumulative rainfall (RF30D), (D) relative humidity (RH30D), and (E) solar radiation (SRAD30D).

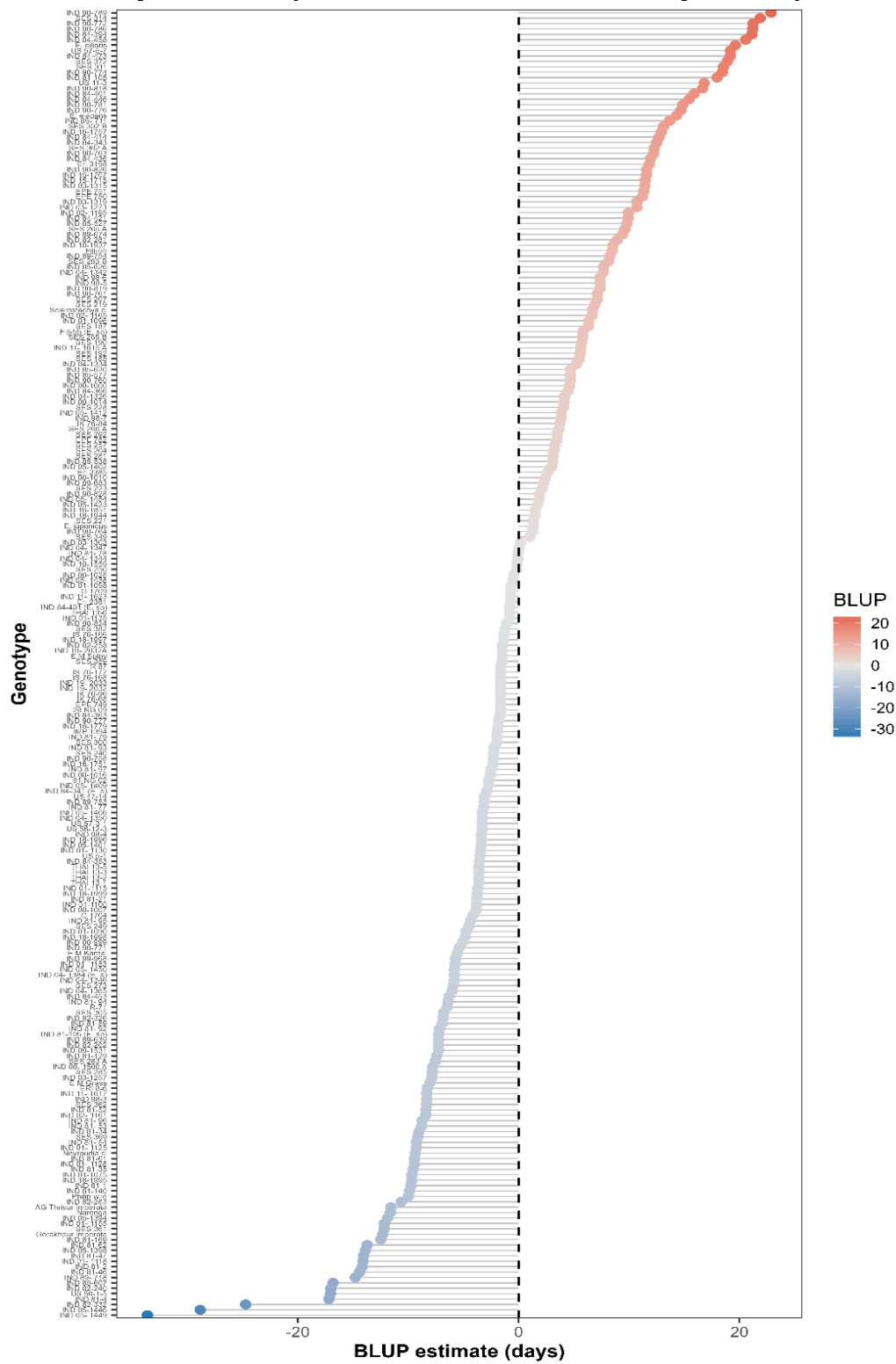


**Figure 2**

**Best Linear Unbiased Predictions (BLUPs) showing genotype-specific flowering tendencies among 251 sugarcane wild relatives**

Negative BLUP values indicate relatively earlier flowering, whereas positive values indicate delayed flowering after accounting for year-to-year environmental variation.

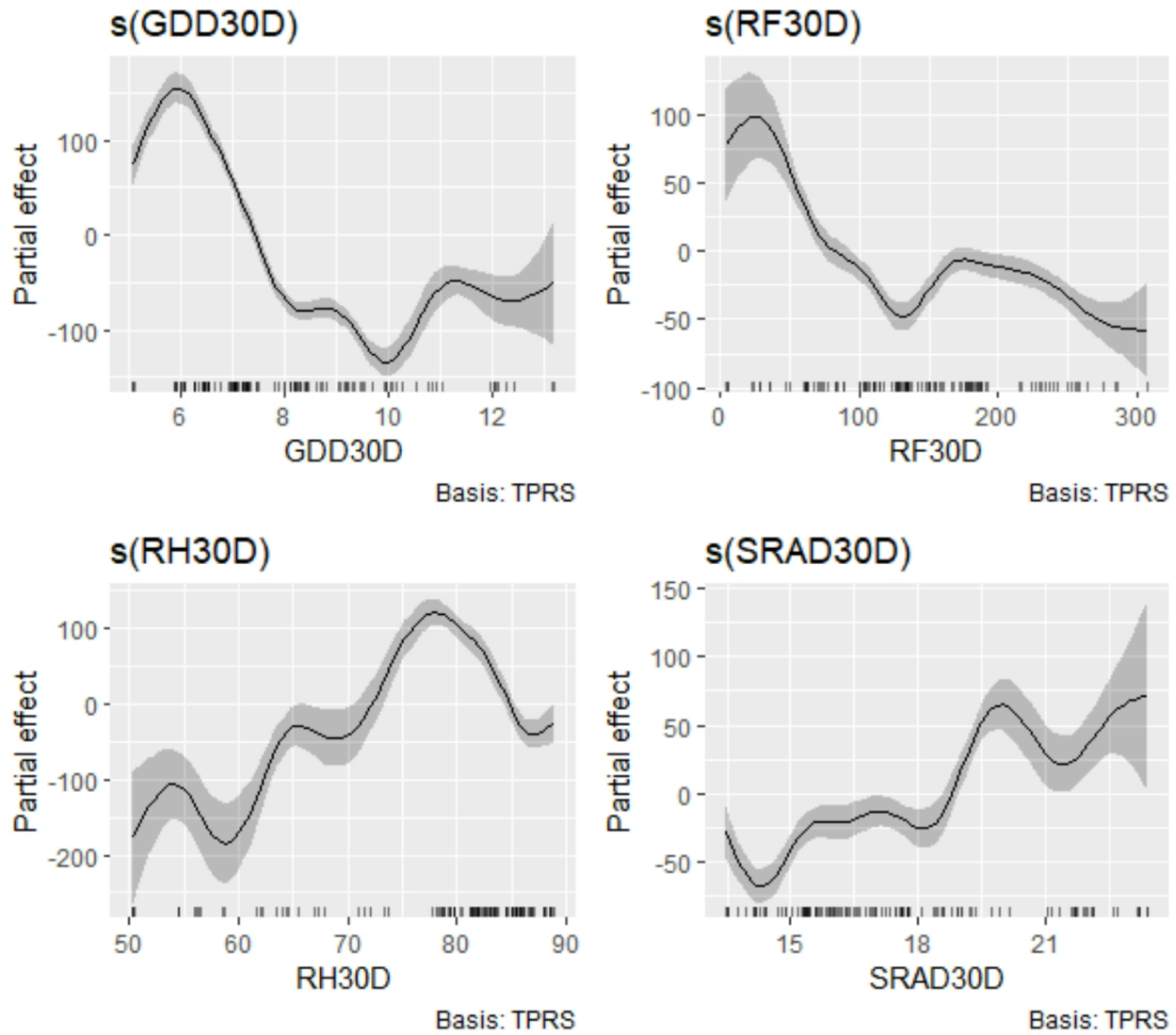


**Figure 3**

Generalized additive model smooth functions describing nonlinear relationships between flowering initiation and (A) GDD30D, (B) RF30D, (C) RH30D, and (D) SRAD30D.

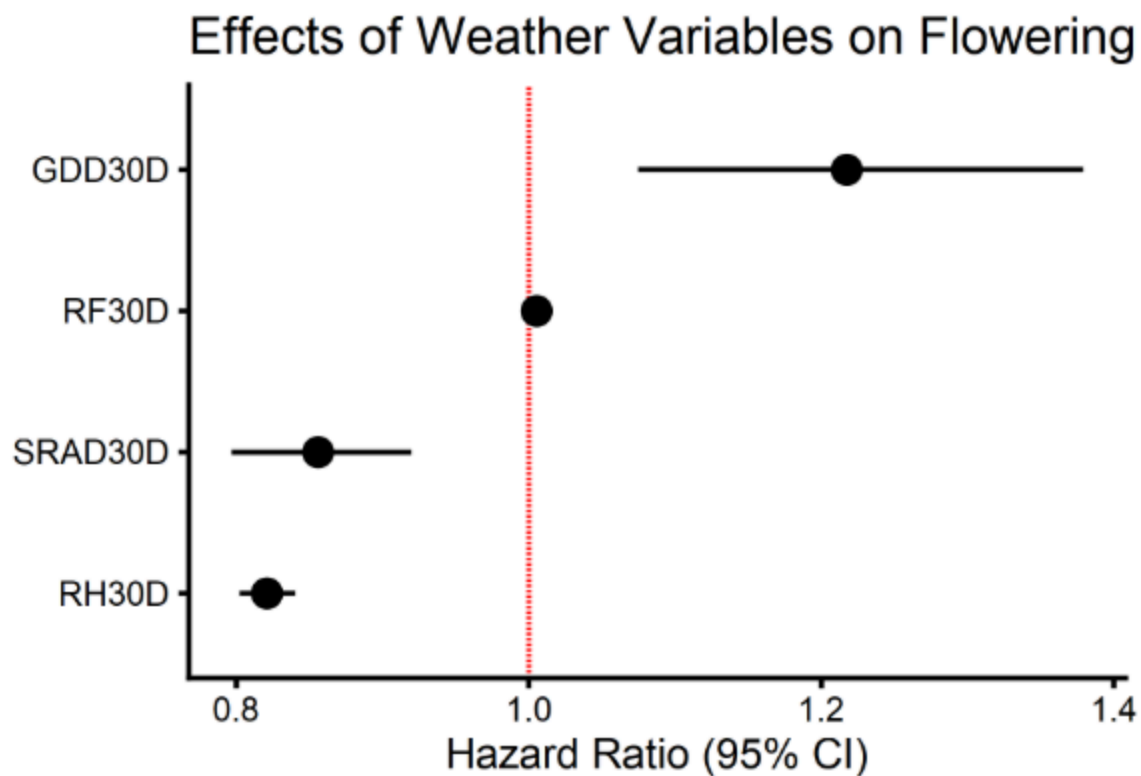


Figure 4

**Forest plot showing hazard ratios (HR) and 95% confidence intervals for climatic variables affecting flowering initiation.**

Hazard ratios greater than one indicate an increased probability of flowering initiation (earlier flowering), whereas hazard ratios less than one indicate a reduced flowering probability (delayed flowering). Error bars represent 95% confidence intervals.

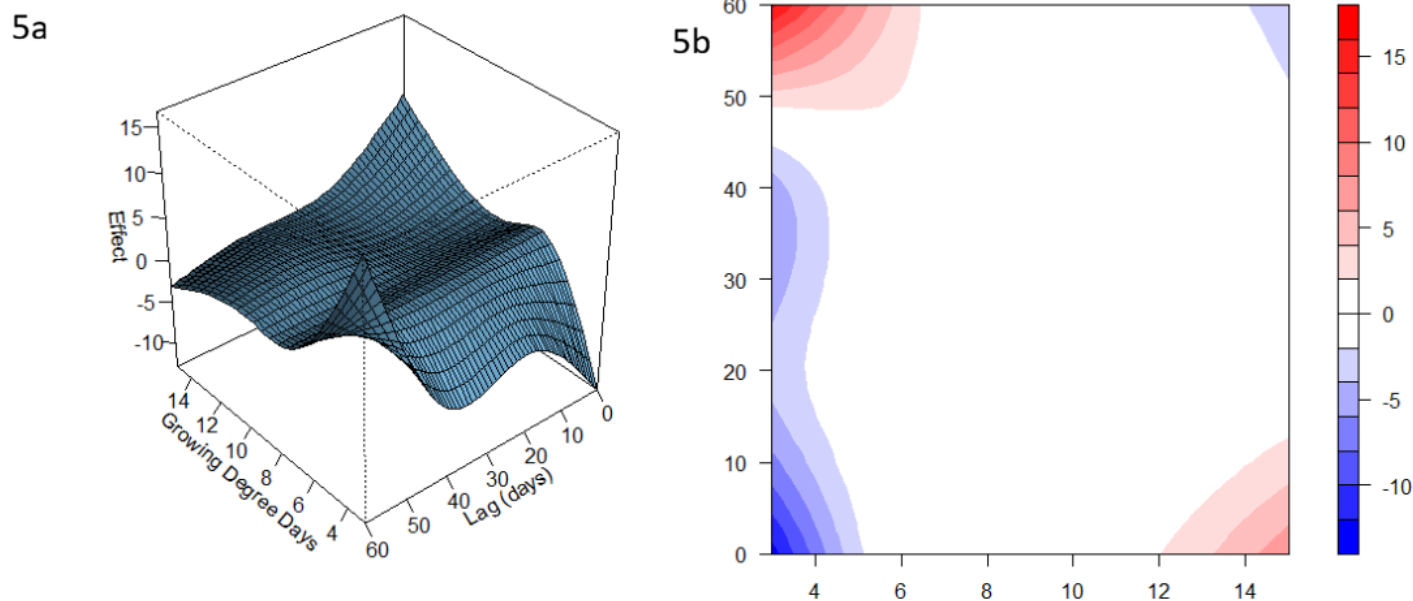


**Figure 5**

**5a. The three-dimensional surface illustrates how the effect of GDD changes simultaneously with both exposure magnitude and lag time.**

Peaks identify lag periods where accumulated heat has the greatest influence on flowering initiation. Distributed lag nonlinear model response surfaces illustrating delayed effects of GDD30D, RF30D, RH30D, and SRAD30D on flowering initiation

**5 b. Contour plots identify regions of strongest lag-response, highlighting the combinations of GDD values and lag periods that most strongly influence flowering initiation.**

## Supplementary Files

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