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Research Article

Reliability of Nonlinear least square broken stick model in quantifying the effects of temperature and photoperiod on flowering of pigeonpea genotypes (*Cajanus cajan* (L.) Millsp.)

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Abstract

Temperature and photoperiod are two major environmental determinants that affect the flowering time. The information on the effect of temperature and photoperiod on flowering response in pigeonpea is limited and needs updating for new genotypes. The present study aimed to assess the reliability of the Nonlinear least square broken stick model to quantify photothermal effects in pigeonpea (*Cajanus cajan* (L.) Millsp.) genotypes. Data at 50 % flowering (FL) from pot, field, and temperature-controlled glasshouse experiments under eight sowing dates were analysed using regression models to describe the individual effect of temperature and photoperiod and photothermal models to quantify the combined effect. The critical photoperiod (P_{ce}) and optimum temperature (T_o)predicted by the Nonlinear broken stick model for 50 % FL ranged from 12.4 - 13.4 h and 21.0 - 23.5 °C, respectively. The higher P_{ce} reported for extra-early flowering genotype (QPL 1001) indicates that their insensitiveness to a range of photoperiod regimes compared to QPL 941 and ICP 14425 (medium duration). Further, the results also revealed that the time to 50 % FL of genotype QPL 1001 was strongly sensitive to the temperature at sub-optimal range ($T < T_o$), with warmer temperatures accelerating reproductive development. In contrast, QPL941 and ICP 14425 were sensitive to supra-optimal temperature ($T > T_o$), with flowering being delayed in warmer temperatures. The parameters (T_o and P_{ce}) derived from Nonlinear least square broken stick model can be used as a proxy to identify photoperiod insensitivity in pigeopea genotypes.

Keywords: Critical photoperiod, Nonlinear least square broken stick model, Optimum temperature, Photothermal model

INTRODUCTION

Pigeonpea [*Cajanus cajan* (L.) Millsp.] is a drought-tolerant crop widely grown in semi-arid and tropical regions of East and Southeast Asia and East Africa (Sharma *et al.,* 2023 and Li *et al.,* 2023) and extensive adaptability to a range of soil types (Akshaya *et al.,* 2023). Deep rooting characteristics help to tolerate drought and water stress (De *et al*., 2023). However, over the years, there has been a significant fluctuation in pigeonpea yield and productivity due to environmental factors, including temperature, photoperiod and humidity (Sridhara *et al.,* 2023). Yield loss due to drought in pigeonpea ranged from 20 - 50 % (Hemavathy *et al.,* 2023). Pigeonpea has gained increased interest among Australian farmers as an alternative summer legume crop for the Northern grain growing regions due to its ability to perform under harsh environments (Rachaputi *et al*., 2018). With unpredictable rainfall, intermitted drought conditions and temperature variability, the early maturing genotypes are promising options for avoiding climatic extremes. Pigeonpea comprises a wide range of maturity classes closely related to flowering time. Quantifying the effect of temperature and photoperiod during flowering is essential for genotypic selection in a specific environment (Chauhan *et al.,* 1998).

Phenology or crop development is mainly driven by temperature and photoperiod. The differences in the thermal time across sowing dates, in turn, mean that the sowing date affects the time of flowering (Tiwari *et al.,* 2017. Accumulated photothermal unit was found be positively correlated with floral initiation and 50 % flowering, pod initiation and physiological maturity (Sindhu *et al*., 2017).Silim *et al*. (2006) reported that the T_o to induce flowering as 24.7 ^oC, 23.0 ^oC, 22.2 ^oC and 18.3 ^oC for extra-early, early, medium and long duration pigeonpea genotypes, respectively. Omanga *et al.* (2008) emphasized that the effect of temperature on flowering of pigeonpea can be associated with that of photoperiod. Turnbull (1987) showed that warm (> 28 ^oC) and cooler temperatures (< 20 ^oC) delayed flowering and the optimum temperature for floral initiation in pigeonpea was approximately 24 ^oC. In another pigeonpea experiment, temperature and photoperiod had substantial effects on flowering over the range of 16 - 32 ^oC and 10 - 14 h, respectively. The responses of floral bud initiation in pigeonpea genotypes to temperature and photoperiod were strongly nonlinear with the optimum temperature between 20 - 24 ^oC and optimum photoperiod 12 - 14 h (McPherson, 1985). Execssive heat and drought conditions advanced flowering in early and medium duration pigeonpea genotypes (Vanaja *et al*., 2015).

Pigeonpea is generally considered a quantitative short-day plant (Saxena *et al.,* 2021; Hussain *et al*., 2022), which means that floral initiation only occurs when the photoperiod falls below the critical photoperiod (Summerfield, 1985., Major, 1990 and Roberts, 1996). Carberry (2001) reported that pigeonpea genotype exhibits quantitative short-day response in which flowering does not occur when photoperiod exceeds a critical photoperiod. McPherson (1985) found that the longest day length treatment of 14 h decreased the rate of development towards 50 % FL in pigeonpea. Reported mean values of minimum (critical), optimum and maximum (ceiling) photoperiods for flowering in pigeonpea genotypes were 11.11, 13.00 and 12.28 h (Akinola & Whiteman, 1974). Maturity class in pigeonpea genotypes was linked with the time of flowering which was highly influenced by photoperiod (Carberry *et al.,* 2001; Omanga, 1995). Flowering time was determined mainly by the timing of transition from juvenile to reproductive phase determined by photoperiod (Hussain *et al*., (2022). Further, Chauhan *et al*. (2009) showed that photoperiod influenced synchronisation of flowering and dry matter partitioning led to differences in grain yield across seasons.

The limitations in previous studies, such as lack of photoperiod range at the upper threshold level of > 13 h, the sensitivity of genotypes to photoperiod and analytical framework in relation to flowering in pigeonpea genotypes led to discrepancies in genotypic classification and estimation of threshold levels of photoperiod (Carbery, 2001; Omanga, 1995; Silim, 2006; Summerfield, 1985).

The rate of development towards flowering has been described as a function of daily mean temperature and photoperiod using an additive linear model (Carbery, 2001; Omanga et al., 2008; Rao, 2002, Summerfield *et al.,* 2008a). However, the Nonlinear model captures the nonlinearity of the temperature and photoperiod on crop development (Soltani *et al.,* 2006). The objectives of this study were :(1) To quantify the effect of temperature and photoperiod on 50 % flowering, (2) To assess the reliability of non linear least square broken- stick model to identify photoperiod insensitive genotypes and (3) To characterize the genotypes based on their photothermal response to the flowering of pigeonpea genotypes.

MATERIALS AND METHODS

Pot, field, and glasshouse experiments were conducted at the Gatton campus of The University of Queensland, Australia, between November 2017 and September 2019, using six pigeonpea genotypes listed in Table 1.

Table 1. Experimental details of the present study

Experiment Type	Experiment No.	Sowing date	Genotypes
Fields	S1	3 Nov.2017	Quest, QPL 1001, QPL 941, ICPL 86022, ICPL 88039 and ICP 14425
	S2	6 Dec.2017	
	S3	9 Jan.2018	
	S4	16 Feb.2018	
	S5	13 Mar.2018	
Pot	S1	3 Nov.2017	Quest, QPL 1001, QPL 941, ICPL 86022, ICPL 88039 and ICP 14425
	S2	6 Dec.2017	
	S3	9 Jan.2018	
	S4	16 Feb.2018	
	S5	13 Mar.2018	
Pot – TGH	1	11 Jul.2019	Quest, QPL 1001and ICP 14425
	2	2 Aug.2019	
	3	9 Sep.2019	

‘UQ’ University of Queensland; ‘TGH’, temperature-controlled glass house; ‘QPL’, Queensland pigeonpea Line; ‘ICPL’, ICRISAT Pigeonpea line, ‘ICP’, ICRISAT Pigeonpea germplasm.

All genotypes were obtained from the Australian Grains Gene Bank (AGGB), Horsham, Victoria, Australia.

Field experiment

The following in-field experiment was conducted on-site at the Crop Research Unit, Gatton, Queensland (Latitude 27.6° S, 152.3° E). The research site was rotary-hoed twice at weekly intervals to the tilling depth of 45 cm. Basal fertiliser Incitec Pivot Fertilisers®, CK-88 (N: K:S = 15.1:4:11.5:13.6) was applied 30 days before planting. Each plot size was 4.0 m in length × 1.2 m in width, a spacing of 0.5 m between rows and × 0.15 m within rows. A drip irrigation system was set up using 'T' tapes (Rivulis®, 340 LPH/100 m at 0.55 BAR) and irrigated weekly in summer (November to March) and reduced to fortnightly starting from April to June. In each subplot, 1 m^2 area was assigned for phenology measurement data collection.

Pot experiment

Under natural temperature and photoperiod conditions

The following pigeonpea pot experiment was conducted at the University of Queensland, Gatton campus plant nursery. Five seeds were planted in 4.8 L, 27 cm diameter plastic pots filled with UQ-23 potting media. The UQ-23 potting media is a commercial mix that consists of well-decomposed tree bark combined with slow-release fertilisers. The pots were irrigated using an automated overhead misting system that operated twice daily for 10 minutes. Experimental pots were rotated every 15 days to avoid the positional effect. The experiments were inspected every second day for plant growth.

Temperature-controlled glasshouse experiment

The pigeonpea field and pot experiments, as well as another pot experiment, were conducted in a temperature-controlled glasshouse at The University of Queensland, Gatton campus. The three sowing dates 11 July 2019, 2 Aug. 2019, and 9 Sep. 2019 were replicated thrice. Seeds of selected three Quest, QPL 1001 and ICP 14425 genotypes were used and sown in 4.8 L plastic pots arranged in split-plot design. Pigeonpea plants were grown at a constant mean air temperature of 26.1 °C, ranging from 25.2 - 28.6 °C under natural photoperiods of 11.2 h - 14.7 h.

The details of location, sowing dates and genotypes corresponding to each sowing date for field, pot and temperature-controlled glasshouse experiments conducted at the Gatton campus of The University of Queensland, Australia (27.6° S, 152.3° E, 94 MSL) using six pigeonpea genotypes in seasons 2017/2018 and 2019/2020 are mentioned in Table 1.

The time to 50 % flowering was recorded in both field and pot experiments. Hourly data on the minimum and maximum air temperatures were obtained from an automatic weather station installed within 0.5 km distance from the experimental sites. The daily photoperiod included civil twilight computed by APSIM day-length calculator (APSIM-16941, Latitude -27.56 S and solar angle -6°).

Statistical analysis

Analysis of variance

Both pot and field experiments used a split-plot design. Five sowing dates of 3 Nov.2017, 6 Dec.2017, 9 Jan.2018, 16 Feb.2018 and 13 Mar.2018 were treated as main plots. The genotypes of Quest, QPL 941, QPL 1001, ICPL 86022, ICPL 88039 and ICP 14425 were treated as sub-plots. Data were analysed with 'R' Studio (Version 4.0.3) for general analysis of variance (ANOVA) for thermal time to 50 % FL (50 % of plants with at least one open flower). The mean sums of squares accounted by the dates of sowing (E), genotypes (G) and their interactions (G × E) were estimated and tested for statistical significance at the '***' P <0.001, '**' P < 0.01 and '*' P < 0.05.

Application of models

In this study, all four models were fitted adequately to describe the effects of temperature and photoperiod. The root-means-square deviations (RMSD) and regression of predicted versus observed days from sowing to flowering were compared. Parameters were estimated for each genotype through an iterative optimisation process that minimises residual sums of squares (Faraway, 2009).

A. |Linear thermal response model (Model 1)

Responsiveness to mean Temperature (T) alone was described as,

$$\frac{1}{f} = a + bT$$

when $P < P_c$……………. (1)

In which, f is the time to 50 % FL ($^{o}Cd^{-1}$), 'T' is the mean pre-flowering temperature, and b are specific to genotypes and have negative values at the supra-optimal range.

For this model to be effective the genotypes had to either be insensitive to photoperiod or all the photoperiods tested were shorter than the critical photoperiod (P_c) for that genotype (Imrie and Lawn, 2008; Silim, 2006; Summerfield *et al.,* 2008b).

B.| Linear photoperiod model (Model 2)

This model is based on photoperiod alone, in which the time of flowering is predicted, assuming the absence of effects of temperature.

$$\frac{1}{f} = a' + b'P$$

when $T_b < T < T_o$……… (2)

Where,

f is the time from sowing to 50 % FL, and *P* is the mean pre-flowering photoperiod. The values of *a'* and *b'* are specific to genotypes and take negative values at the supra-optimal range of the mean temperature.

C. | Linear photothermal model (Model 3)

It has been proven for many crop species that the rate of progress towards flowering can be described as a simple linear function of photoperiod and temperature (Summerfield *et al.,* 2008a).

Responsiveness to Temperature (T) and photoperiod (P) is given by:

$$\frac{1}{f} = a'' + b''T + cP \qquad \text{............ (3)}$$

Where *f* is the number of days to the first flower, *P* is the mean pre-flowering photoperiod (h d^{-1}) and a" , *b"*, *c and d* are genotype-specific constants.

D. | Nonlinear least-square broken-stick model (Model 4)

A Nonlinear least-square broken-stick model was fitted to the responses defined by Model 1 and 2 to evaluate the effect of photoperiod and temperature. This model allows for the estimation of the apparent optimal temperature and critical photoperiod for individual genotypes (Faraway, 2009).

$$\frac{1}{f} = a_1 + b_1(T_o - T) + b_2(T - T_o) + c_1(P - P_c) \qquad \text{...... (4)}$$

Where a_1, b_1, b_2, and *c* are genotypic constants. The positive and negative regression lines for the respective data sets were finally compared to reveal differences and similarities between genotypes and to estimate the optimum temperature and ceiling photoperiod for flowering.

The critical photoperiod was calculated as the photoperiod at which the thermal and photoperiod planes intersect and the maximum delay in flowering occurs (Carbery, 2001; Summerfield *et al.,* 2008b). The mode 4 was validated by fitting regression parameters (a_1, b_1, b_2 and c_1) derived from the pot experiment were used to predict the observed days to 50 % FL in the field experiment.

RESULTS AND DISCUSSION

Growing environment

The five sowing dates of both field and pot experiments provided a wide range of environmental conditions to examine the flowering response of pigeonpea genotypes. The mean photoperiod decreased from 14.4 - 12.1 h during flowering in all sowings. In sowing date 3 Nov.2017 (S.1) and sowing date 6 Dec.2017 (S.2), the crop grew under increasing photoperiods and flowering occurred under decreasing photoperiod conditions. However, in sowing dates 9 Jan.2018, 16 Feb.2018 and 13 Mar.18 (S.3, S.4 and S.5, respectively), plants were exposed to decreasing photoperiod throughout the crop cycle (Fig. 1).

Quantifying the effect of temperature and photoperiod at the time to 50% flowering

The sowing dates (3 Nov.2017 and 6 Dec.2018) with higher mean temperatures of 23.8 - 25.3 °C and longer

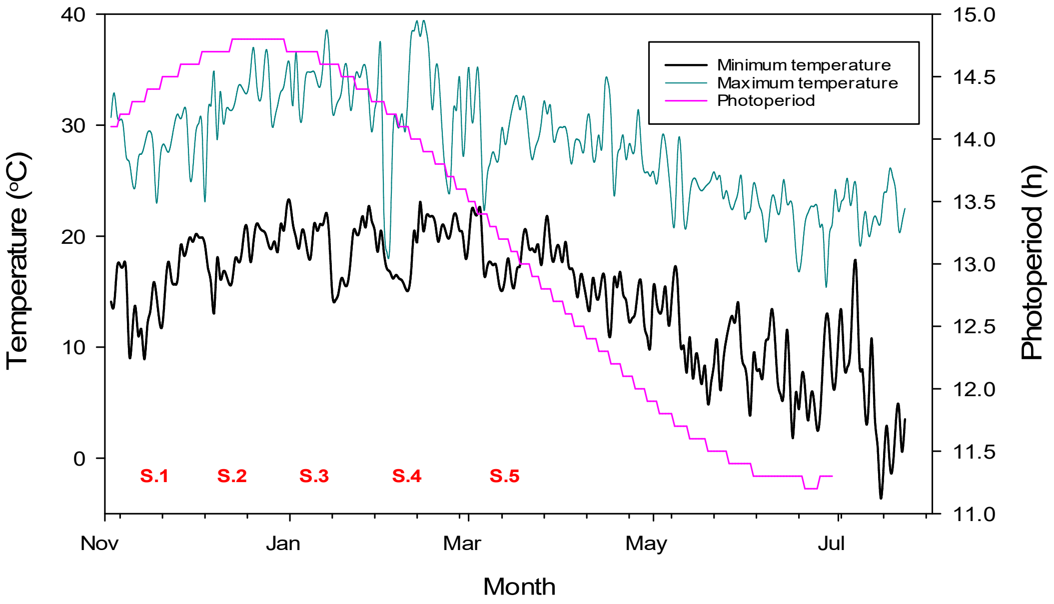

Fig. 1. *Showing daily maximum air temperature (green solid line), minimum air temperature (black solid line) and mean photoperiod (purple line) across the pigeonpea sowing season of November 2017 - July 2018. Where S.1 to S.5 are the 5 sowing dates (3 Nov.2017, 6 Dec.2017, 9 Jan.2018, 16 Feb.2018 and 13 Mar.2018, respectively) of the pigeonpea pot and field experiments conducted at the Gatton campus, University of Queensland*

photoperiods of 14.2 - 14.6 h were characterised by delayed flowering in all six genotypes. There was a steady decrease in thermal time to 50 % FL with a decrease in photoperiod. The earliest flowering occurred when the mean temperature and photoperiod were 20.0 °C and 12.3 h, respectively. Most synchronous flowering among genotypes was observed at S.5 (13 March.2018) (Fig. 2).

Thermal response model (Model 1)

The early flowering genotypes of QPL 1001 ($b = 2.98 \times 10^{-4}$) and ICPL 86022 ($b = 3.38 \times 10^{-4}$) showed a strong response to temperature. In contrast, the long-duration genotype QPL 941 was relatively less sensitive to temperature ($b = 0.39 \times 10^{-4}$) variation. Conversely, the genotype ICP 14425 ($b = -8.53 \times 10^{-4}$) showed a strong negative response to mean temperature (Table 2). However, temperature alone could not adequately explain the environmental control of flowering. The coefficient of determination of the model was only 58 % (Table 2).

Photoperiod response model (Model 2)

Results showed that the genotypes except Quest, QPL 941 and ICP 14425 indicated a positive relationship between the rate of development and the mean photoperiod. Based on photoperiod response model, genotype QPL 1001 and ICPL 86022 were relatively insensitive to photoperiod (b' is 0.136 and 0.146 × $10^{-3,}$ respectively).

The differences in time of flowering for QPL 941and ICPL 88039 were mainly associated with differences in

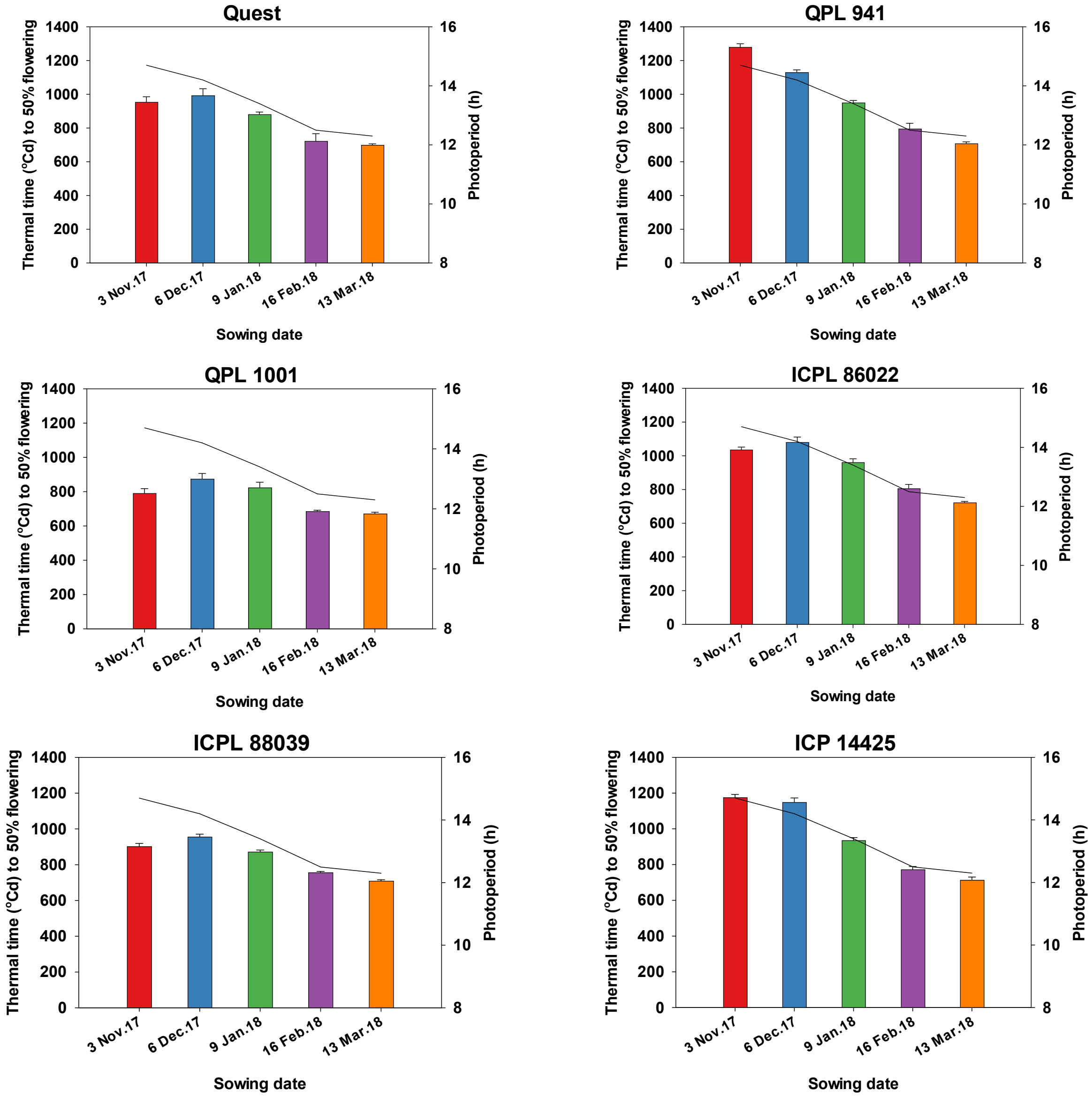

Fig. 2. *Showing thermal time (°C d) to 50 % flowering and photoperiod (h) of pigeonpea genotypes of Quest, QPL 941, QPL 1001, ICPL 86022, ICPL 88039 and ICP 14425 at five sowing dates at the University of Queensland, Gatton Campus during 2017 and 2018. Vertical bar represents standard error of mean*

photoperiod. In contrary, genotype ICP 14425 appears to be responsive to both temperature and photoperiod (Table 2). However, neither temperature nor photoperiod alone was able to explain the environmental control of flowering adequately. The coefficient of determination of the models (1 and 2) were 45 % and 52 % for the tested genotypes (Table 2).

Linear additive model (Model 3)

The linear additive model showed a significant improvement in the goodness of fit of 94 % for temperature and photoperiod with the rate of development towards flowering (Table 2). This model shows that the temperature (*b"*) coefficient explained the observed variation in the rate of progress towards 50 % FL, indicating that it was a very important factor controlling the rate of phenological development for the genotypes Quest, QPL 1001, ICPL 86022, ICPL 88039 and ICP 14425 ($b'' > c$) except for QPL 941 ($b'' < c$). All the genotypes responded negatively and with more homogeneity in the sub-optimal range. However, there was a large variation between genotypes in the direction and magnitudes of sensitivity to photoperiod. All genotypes responded identically to temperature except for ICP 14425 ($P < 0.05$) (Table 2).

Though the linear additive model quantified the relative sensitivity to temperature and photoperiod, it failed to estimate the temperature and photoperiod thresholds for 50 % FL. Thus, nonlinear model (Model 4) was fitted to the data of 50 % FL with Temperature and photoperiod.

Nonlinear least square broken stick model (Model 4)

The estimated optimum temperatures for 50 % FL ranged from 21.0 to 23.5 ^{0}C (Table 3). Mean temperatures from sowing to 50 % FL experienced in the first four sowing dates (November to February) were supra-optimal for all the genotypes ($T > T_o$). Supra-optimal temperatures hastened the rate of development towards 50% flowering (1/*f*) for QPL 941 and ICPL14425 with a value of $b_1 > b_2$. However, for the remaining genotypes of Quest, QPL 1001, ICPL 86022 and ICPL 88039 1/*f* was delayed in the supra-optimal temperature range of > 21.5 ^{0}C with the value of $b_1 < b_2$. There was a distinct pattern in the photoperiod response (c_1) slopes among genotypes. The estimated c_1 values for extra-short duration genotypes QPL 1001 (-1.32) and ICPL 86022 (-1.98) were lower than short-duration genotypes except ICPL 88039 (-1.40). The higher c_1 values for Quest (-2.35), QPL 941 (-3.37) and ICP 14425 (-3.37) were observed. The critical photoperiod varied from 12.4 to 13.4 h. The highest critical photoperiod was observed for ICPL 86022; no critical photoperiod was detected for QPL 1001.

Validating Nonlinear least square broken stick model

Table 2. Regression parameters (10^{-3}) and coefficient of estimates (r^2) for model 1, model 2 and model 3 of six pigeonpea genotypes of Quest, QPL 941, QPL 1001, ICPL 86022, ICPL 88039 and ICP 14425. Parameters *a, a'* and *a"* are intercepts; *b, b'* and *b"* are slopes for the temperature plane; *c* is the slope of the photoperiod plane of Model 3.

Genotype	Parameter Estimate			R^2
Linear thermal (Model 1)				
	A	B		
Quest	-7.14	0.356	-	
QPL 941	2.39	-0.111	-	
QPL 1001	-9.37	0.524	-	0.45
ICPL 86022	-13.16 *	0.619 *	-	
ICPL 88039	-4.67	0.202	-	
ICP14425	15.70 ***	-5.83	-	
Linear photoperiod (Model 2)				
	a'	b'		
Quest	-10.8	0.88	-	
QPL 941	2.2	-0.178	-	
QPL 1001	-15.6	0.136 **	-	
ICPL 86022	-18.5	0.146 **	-	0.52
ICPL 88039	-9.9	0.73	-	
ICP 14425	-24.2 ***	-0.62 ***	-	
Linear photothermal (Model 3)				
	a"	b"	C	
Quest	60.1	-4.4	-3.01	
QPL 941	209.0 ***	-7.8	-17.3**	
QPL 1001	1.1	-2.7	1.66	
ICPL 86022	1.3	-2.2	2.35	0.93
ICPL 88039	36.4	-3.5	-0.67	
ICP 14425	79.3 ***	-6.50***	10.4***	

† Significance level used: '***' 0.001 '**' 0.01 '*' 0.05.'ND' = Not Detected

Table 3. Regression parameters (10^{-3}), coefficient of estimates (r^2) and residual sums of square error (RMSE) for nonlinear broken stick model for the estimation of optimum temperature (T_o) and ceiling photoperiod (P_{ce}) from nonlinear least square model to explain temperature and photoperiod response of six different pigeonpea genotypes Quest, QPL 941, QPL 1001, ICPL 86022, ICPL 88039 and ICP 14425 (Parameter a_1 is an intercept; b_1 and b_2 are slope for the temperature plane at suboptimal and supra optimal range respectively and c_1 is the slope of the photoperiod plane)

Genotype	**Parameter**							
	a_1	b_1 ($T < T_o$)	b_2 ($T > T_o$)	c_1	P_{ce} (h)	T_o (^{0}C)	R^2	RMSE
Quest	19.8	1.65	-0.11	-2.35	12.4	23.5	0.89	7.7
QPL941	15.7	2.50	-0.47	-3.37	12.8	21.0	0.98	6.5
QPL1001	37.1	3.75	-0.01	-1.32	n.d	22.1	0.82	7.0
ICPL86022	17.6	2.34	-0.01	-1.98	13.4	21.9	0.96	3.9
ICPL86039	16.6	2.13	-0.07	-1.40	12.6	21.5	0.90	7.0
ICP14425	17.9	2.20	-0.49	-3.37	13.1	22.0	0.97	6.5

† Significance level used: '***' 0.001 '**' 0.01 '*' 0.05. ND' = not detected.

The coefficients for the temperature and photoperiod response to 50 % FL defined through Nonlinear broken stick model using the data from the pot experiment were used to predict time to 50 % FL under field conditions using pre-flowering temperature and photoperiod data. These observed days were predicted well by Model 4 with no significant differences between fitted and observed values (Fig. 3).

Comparison of the pigeonpea field experiment and the temperature-controlled glasshouse experiment for 50 % FL

Time to 50 % FL data from the pigeonpea temperature-controlled glasshouse experiment were fitted separately to thermal and photoperiod models (Model 1 & Model 2) for selected three Quest, QPL 1001 and ICP 14425 genotypes.

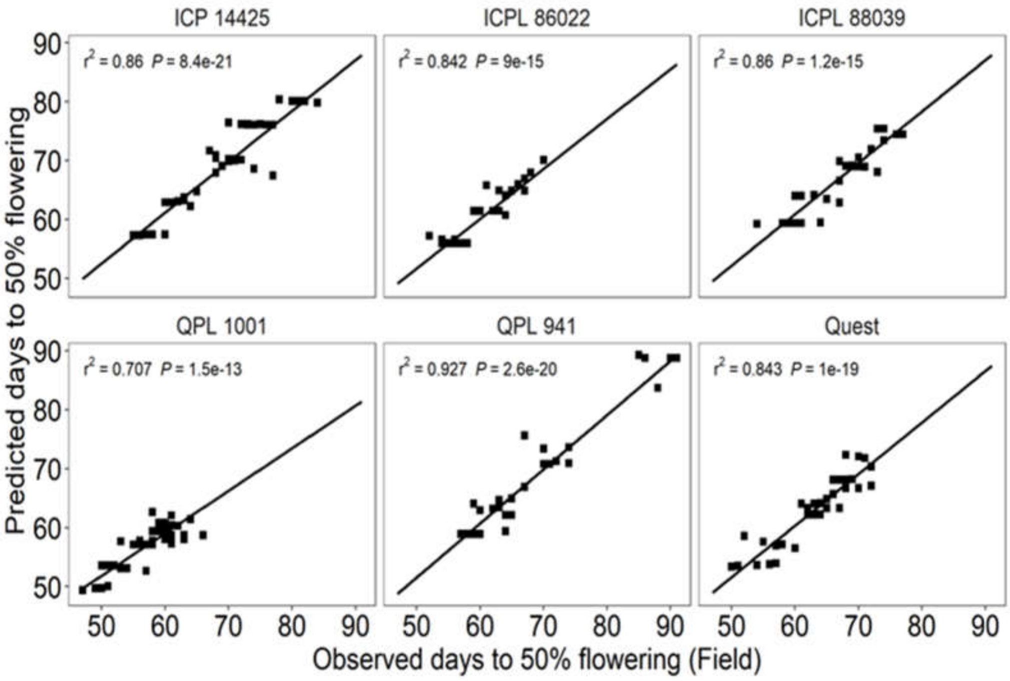

Fig. 3. *Observed time (days) in the field experiment and predicted time (days) to 50 % FL for pigeonpea genotypes tested using nonlinear least square broken stick model (Model 4) with coefficient of determination (R^2) at $P < 0.001$.*

Table 4. Regression parameters for fitting thermal response and photoperiod response models for pigeonpea temperature-controlled growth chamber experiment at Gatton campus, University of Queensland of three diverse pigeonpea genotypes Quest, QPL 1001 and ICP 14425.

Genotype	**a (10^{-2})**	**b**	**R^2**	**a' (10^{-2})**	**b' (10^{-3})**	**R^2**
Quest	0.32 (0.038)	ND	0.78	-0.92 (0.035) *	0.88 (0.026) **	0.98
QPL 1001	0.45 (0.053)	ND		0.76 (0.033) *	-0.25 (0.024)	
ICP 14425	1.20 (0.032)	ND		3.03 (0.027) ***	-1.36 (0.020) ***	

† Significance level used: '***' 0.001 '**' 0.01 '*' 0.05., 'ND' - Not detected, *a and a'* = intercept, *b* and b' = slope, standard error of estimate given in parenthesis.

The parameter estimates of temperature (a and a') and photoperiod (b and b') for each model are given in Table 4. No temperature effect was detected for any of these genotypes. However, the genotypic responses to the photoperiod were significant at $P < 0.05$. Accordingly, genotypes showed differential sensitivity to photoperiod from marginal (b' = -0.25, QPL 1001) to relatively high (b' = 0.88, ICP 14425).

Genotypic classification

The number of days and thermal time required for 50 % FL varied among genotypes across the sowing dates in response to photoperiod. Among the genotypes tested QPL 1001 was the earliest in flowering with 771 ^{0}C d, followed by ICPL 86022 and Quest at 849 ^{0}Cd and 840 ^{0}Cd, respectively (Table 5).

Pigeonpea is inherently a short-day plant with a range of maturity classes which are associated with photoperiod sensitivity. Identifying and developing photoperiod-insensitive genotypes are crucial in expanding its cultivation to new production systems and geographical regions. No specific and standardised method exists to evaluate photoperiod sensitivity in pigenpea (Hussain *et al*., 2022). The present study assesses the reliability of Nonlinear least square broken stick model to predict photoperiod sensitivity in pigeonpea genotypes.

Table 5. Photoperiod sensitivity based on days and thermal time to 50 % FL

Genotype	50 % flowering		Class	Photoperiod sensitivity
	Days	Thermal time (°C d)		
QPL 941	73^{a}	1004^{a}	Late flowering (71 - 80 d)	Acutely
ICP 14425	71^{b}	978^{b}	Late flowering (71 - 80 d)	Acutely
ICPL 88039	68^{c}	921^{c}	Early flowering (61 - 70 d)	Fairly
ICPL 86022	62^{d}	849^{d}	Early flowering (61 - 70 d)	Fairly
Quest	62^{d}	840^{d}	Early flowering (61 - 70 d)	Fairly
QPL 1001	56^{e}	771^{e}	Extra-early (< 60 d)	Slightly
l.s.d	1.5	16.8		

† Treatments with the same letter are not significantly different at $P < 0.05$.

The genotypes exhibited differences in their responses to the photoperiod as indicated by significant genotypes × treatment interactions. Based on the coefficient of determination (r^2), photothermal models (Model 3 and Model 4) were suitable for predicting the interactive effect of temperature and photoperiod on flowering in pigeonpea genotypes. However, using a nonlinear least square broken stick model (Model 4) to quantify photothermal parameters such as critical photoperiod and optimum temperature resulted in satisfactory robustness (Faraway, 2009). The present study confirmed that time to 50 % FL in pigeonpea was sensitive to photoperiod at varying degree. The photoperiod effects on the rate of development to 50 % FL ($1/f$) were significant amongst the genotypes ($P < 0.05$). The major finding of this study was that the extra-early flowering pigeonpea genotype (QPL 1001) was less sensitive to photoperiod within the range tested (12.3 - 14.7 h). In contrast, late flowering genotypes (QPL 941 and ICP 14425) were highly sensitive to photoperiod. They showed a quantitative response in which flowering delayed beyond the ceiling photoperiod (P_{ce}). The present study's findings agree with the findings of the studies conducted on pigeonpea using linear broken stick model (Summerfield *et al.,* 2008b) and contrast to the findings of Carbery *et al*. (2001). The Model 4 predicted ceiling photoperiod (P_{ce}) for these genotypes ranged from 12.4 to 13.4 h. However, it (Model 4) failed to predict P_{ce} for the extra-early flowering genotype (QPL 1001), indicating it's insensitivity to photoperiod. Earlier studies reported that P_{ce} for most pigeonpea genotypes was 13.1 h (Sharma,1980).

Summerfield *et al.*(2008) also reported a mean value of P_{ce} for four selected genotypes as ≤ 13 h at a mean temperature of 20 °C. The genotypes are classified into different maturity groups and respond differently in different regions because they vary in the degree of sensitivity to photoperiod, temperature, and interactions (Hussain *et al*., 2022). In the present study, the differences in ceiling photoperiod ($P < 0.001$) for 50 % FL between genotypes indicated their adaptation to the different photoperiod × temperature regimes. In fact, the higher P_{ce} reported for extra-early and early genotypes indicates their insensitiveness to a range of photoperiod regimes. The predicted optimum temperature for the rate of development to 50 % FL ($1/f$) estimated in this present study using a nonlinear broken-stick model (Model 4) ranged from 21 to 23.5 °C. This value was consistent with the values reported earlier on pigeonpea using a multiple linear regression model (McPherson, 1985, Omanga *et al.,* 2008). A study conducted in a controlled environment showed that warm (> 28 ^{0}C) and cold (< 20 ^{0}C) delayed floral initiation and found that the optimum temperature for flowering in early maturing genotype was around 24 ^{0}C (Turnbull and Ellis, 1987). These temperature optima are much lower than those recorded for other tropical legumes such as cowpea, mung bean and soybean (Summerfield *et al.,* 2008a). This study also showed that temperature and photoperiod were additives for the photoperiod sensitive genotypes (ICP 14425 and QPL 941, Table 3).

The results of the present confirmed that the rate of development to 50 % FL ($1/f$) of late flowering genotypes was highly sensitive to temperature at the supra-optimal range ($T > T_o$), while extra-early flowering genotypes were less sensitive (Table 3). This finding implies that the development of extra-early flowering genotypes will tolerate warmer conditions than late flowering genotypes. Conversely, extra-early flowering genotypes (QPL 1001 and ICPL 86022) were highly sensitive to the variation in temperature at the sub-optimal range. A study on the effects of temperature and photoperiod on the flowering of pigeonpea genotypes showed that the modern early-maturing genotypes were relatively more tolerant of supra-optimal temperatures on the rate of phenological progression to flowering (Omanga *et al.,* 2008). Yadav *et al.* (2021) reported that the extra-short duration pigeonpea genotypes have the highest optimum temperature and warm temperature, shortening the time of flowering.

The temperature-controlled experiment data helped define the understanding of observed pot and field responses. The fact that the controlled temperature treatment under natural day-length conditions showed that the extra-early flowering genotype QPL 1001 was less sensitive to photoperiod than the late flowering genotype ICP 14425 implied that photoperiod plays an important role in determining the time of flowering of late flowering genotypes. Further, the coefficient of determination for the regression between time to 50 % FL and photoperiod was much stronger (r^2 = 0.92) than for temperature (r^2 = 0.74). However, adding the photothermal model results confirmed that temperature and photoperiod were important environmental variables to drive flowering in pigeonpeas. Results showed that due to the lack of significant genotypic variability in optimum temperature and ceiling photoperiod, pigeonpea had a specific narrow range of adaptation to temperature and photoperiod.

The prediction of flowering in the field using the data from the pot experiment was proved to be realistic, with the analysis of variance showing only 1 % of the variation in the predicted flowering date explained by the experimental type. It shows that pot and field results were highly correlated. Results suggest that pot experiments can be used to assess the phenology of new genotypes and can be extrapolated to the field in a location.

The phenological classification depends on the locations and seasons (Omanga *et al*., 2008). Extra-early flowering pigeonpea genotypes were more sensitive to temperature than a late flowering types (Saxena *et al.,* 2021). In contrast, the rate of development of late flowering genotypes was faster in cooler environments. Therefore, the rate of progress towards flowering is not only a genotypic character but also influenced by the growing environment (Carbery *et al.,* 2001). The six genotypes investigated in the present study were classified as extra-early, early, and late flowering genotypes. Accordingly, the genotype QPL 1001 was classified as extra-early to flower in the most inductive environment ('a_1'= 3.71 in Model 4) and best suited for warmer environments (heat tolerant). Compared with medium and long-duration genotypes, extra-early and early-duration types take less time to flower and mature with a higher harvest index (Vales *et al*., 2012). Knowledge of flowering response to temperature and photoperiod and its origin would be useful in planning pigeonpea breeding programs and selecting genotypes for specific environments (Saxena *et al.,* 2021).

The Nonlinear least square broken stick model used in this study was more appropriate to explore the response to the photo-thermal effect than the methods (Linear broken stick model) reported in previous studies on pigeonpea genotypes (Carbery *et al.,* 2001; Summerfield *et al*., 1985) as it captured the nonlinearity of the temperature and photoperiod response. Further, this model was successfully applied in determining cardinal temperatures for germination and seedling emergence on pigeonpea genotypes (Mahendraraj *et al*., 2021). However, the nonlinear least square broken stick model estimated the optimum temperature and ceiling photoperiod based on parameter values. There were insufficient photoperiod data at the lower end (< 11 hrs) to predict critical photoperiod. The present study recommends that growing under a lower photoperiod range (8 - 10 h) would complement existing data. Hence, the prediction of flowering to other sites and seasons outside the study region needs environmental parameterisation.

Conclusion

The results of the present study confirmed the genotypic variability in response to temperature and photoperiod for 50 % FL in pigeonpea (*Cajanus cajan* (L.) Millsp.). Flowering occurred only when genotypic-specific mean temperature and mean photoperiod conditions were met. This study showed that the parameters (optimum temperature and ceiling photoperiod) derived from Nonlinear least square broken stick model (Model 4) can be used as a proxy to identify photoperiod-insensitive pigeonpea genotypes. The study also showed that the extra-early flowering genotype (QPL 1001) was insensitive and early flowering was least sensitive (Quest, ICPL 86022 and ICPL 88030) to photoperiod. These results suggest that the genotypes exhibited responses to temperature and photoperiod that were linked to their maturity group. The present findings are of considerable importance in quantifying photoperiod sensitivity for genotypic adaptation to subtropical environments since photoperiod exceeds 13 h between latitude 20° S - 30° S.

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Data availability

The data supporting this study's findings are available from the corresponding author, [Mahendraraj. S], upon reasonable request.

Conflict of Interest

The authors declare that they have no conflict of interest.

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