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Field evaluation of a model of photothermal flowering responses in a world lentil collection

Abstract A model to predict flowering time in diverse lentil genotypes grown under widely different photothermal conditions was developed in controlled environments. The present study evaluated that model with a world germ plasm collection of 369 accessions using two field environments in Syria and two in Pakistan. Photoperiod alone accounted for 69% of the variance in $1/f$, the reciprocal of time (d) from sowing to flower. In contrast, temperature alone did not account for a significant proportion of variation in flowering time due to the exposure of plants to supra-optimal temperatures in the late-sown Syrian trial. With the model mean pre-flowering values of photoperiod and temperature combined additively to account for 90.3% of the variance of $1/f$ over accessions. The correlation of field-derived estimates of temperature sensitivity of accessions to glasshouse-derived estimates was significant at $P = 0.05$, but the equivalent correlation for estimates of photoperiodic sensitivity was higher at $P < 0.01$. Flowering in the field was better measured as time from sowing to 50% plants in flower rather than time to first bloom or its node number. Dissemination of the lentil crop following domestication in West Asia to the lower latitudes such as Ethiopia and India has depended on selection for intrinsic earliness and reduced sensitivity to photoperiod. Movement from West Asia to the higher latitudes accompanied by spring sowing has resulted in a modest reduction in photoperiod sensitivity and an increase in temperature sensitivity.

Introduction

Considerable effort in current breeding programs is being expended to match crop phenology to the target environment. The most common approach is pragmatic and involves growing many genotypes in several different environments in order to test phenological adaptation. Our approach with lentil (*Lens culinaris* Medikus) has been to develop a simple descriptive model from a few controlled environments and to use this model for prediction of response in other natural environments. We initially investigated the response of flowering in six genotypes to a wide range of photothermal conditions in controlled environement cabinets (Summerfield et al. 1985). The flowering response was found to be governed by both temperature and photoperiod and to be well described by the relation

$$1/f = a + b\overline{T} + cP, \tag{1}$$

where f is the time (days) from sowing to first flowering, $\overline{T}$ and P are the respective values of mean temperature and photoperiod during that period, and a, b and c are genotypic constants. The model was subsequently validated on a wide range of germ plasm (231 accessions with an average R^2 of 0.852) at a factorial combination of two photoperiods (13 and 16 h d^{-1}) and two temperature regimes (24 °C/13 °C and 18 °C/9 °C) provided by controlled-environment glasshouses (Erskine et al. 1990). This most recent study aimed to test the model in the field.

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Materials and methods

A total of 369 germ plasm accessions [inclusive of those used by Erskine et al. (1990)] were evaluated for time to flowering at ICARDA, Tel Hadya, Syria (36° 01′ N and 37° 20′ E) and at the National Agriculture Research Centre, Islamabad, Pakistan (33° 40′ N and 33° 00′ E). The accession comprised 25 randomly selected germ plasm accessions from the following major lentil-producing countries: Afghanistan, Chile, Egypt, Ethiopia, Greece, India, Iran, Jordan, Lebanon, Pakistan, Syria, Turkey and the former Soviet Union together with elite accessions from the ICARDA breeding program and registered varieties.

In Syria, the experiment was sown in a split-plot design with sowing dates of 30 December 1986 and 2 February 1987 as main plots and accessions as sub-plots in two replications. In Pakistan, a randomized block design was used for the sowing on 11 November 1986 and on 30 November 1991 in two replications. Plots were single, 1-m-long rows with inter-row distances of 30 and 37.5 cm in Pakistan and Syria, respectively. On the basis of past experience *Rhizobium* inoculation was neither necessary nor undertaken; plants were well nodulated in both locations.

In all cases the time to flower (days) was recorded from sowing to when 50% of the plants in a plot had come into bloom. Additionally, at Tel Hadya, the time from sowing (days) to the first open flower in a plot and its node number were recorded, as was the time (days) from sowing to 90% pod maturity.

Analyses of variance for each location were undertaken on flowering data of those accessions that flowered in all four environments, and a combined analysis was done. The broadsense heritabilities of the three characters measuring flowering at Tel Hadya were derived as described in Allard (1960).

Using the mean over replicates, we transformed accession means for times from sowing to flower (f) to rates of progress toward flowering with the reciprocal ($1/f$). Daily weather data were used from each environement to calculate the mean temperature ($\bar{T}$) between sowing and time to flowering for each line by summation over the n days to flower: $\bar{T} = \Sigma T/n$. The mean photoperiod (P) was calculated on the basis of daily values (p) (civil sunrise to sunset; Goodspeed 1975) as: $p = \Sigma p/n$. Linear regressions of mean values of temperature and photoperiod on to $1/f$ were calculated independently. The multiple linear regression in Eq. 1 was applied to each accession as in Erskine et al. (1990). One-way analyses of variance on the basis of country of origin were undertaken for the estimates of a, b, and c, and the coefficient of multiple determination (R^2) was calculated using accessions from those countries with more than 5 representative accessions (a total of 298 accessions).

The estimates of a, b, and c from these field trials were compared to those reported by Erskine et al. (1990) by correlation over common accessions.

Results

Islamabad provided the two warmest environments for the vegetative plants with mean temperatures prior to

flowering of $\bar{T} = 13.2$ °C in 1986/1987 and 12.6 °C in 1991/1992, to which the greatest contrast was an early sowing at Tel Hadya with a mean temperature of $\bar{T} = 9.8$ °C (Table 1). There was, however, a striking increase in temperature in Tel Hadya from mid-March (when the mean 7-day temperature was 5.7 °C) to mid-May when the corresponding value reached 24.1 °C (Fig. 1). For mean photoperiod prior to flowering, the shortest daylength regime was in Islamabad in 1986/1987 with $P = 10.5$ h d^{-1}, and the longest days ($P = 12.1$ h d^{-1}) were experienced following late sowing in Tel Hadya.

A total of 63 accessions did not flower in Pakistan within 145 days in 1987 and 150 days in 1992 when the investigations were terminated. These accessions were therefore excluded from the analysis; they comprised germ plasm accessions from Afghanistan (18), Turkey (8), the former Soviet Union (8), Ethiopia (7), Greece (7), Lebanon (6), Iran (4), Pakistan (3), and Chile (2).

Mean time to 50% flowering was longest (125 days in Islamabad in the 1986/1987 season. In Syria, sowing date had a major effect on flowering, being 121 days for early sowing but 91 days for late sowing (Table 1). The interaction between accessions and environments for time to flowering was highly significant ($P = 0.001$) in the combined analysis of variance. However, the accessions mean square value was 9 times greater than the mean square value of the interaction.

In Syria, times to first flower and to 50% flowering and also the node number of the first flower were greater in early-sown crop than in the late-sown crop. In contrast, the time from 50% flowering to maturity was unaffected by sowing date (Table 1). There were strongly significant differences between accessions in all of the characters measured. The broadsense heritabilities in Syria of times to first flower, to 50% flowering, and to maturity were strong at $h^2_{bs} = 0.91$, 0.90 and 0.80, respectively; whereas the broadsense heritability for node number of the first flower was weak at $h^2_{bs} = 0.18$. Phenotypic correlations among these characters showed strong, probably pleiotropic, associations between the trio of characters – times to first flower , to 50% flowering, and to maturity – and a poor association of node number of the first flower with the other phenological characters (Table 2).

Table 1 Means and standard errors over accessions of different phenological characters at two sowing dates in Tel Hadya, Syria in the 1986/1987 and at Islamabad, Pakistan in the 1986/1987 and 1991/1992 seasons, together with mean temperature ($\bar{T}$) and photoperiod (P) from sowing until the onset of flowering

Character	Tel Hadya		Islamabad		SE
	Date 1	Date 2	1986/1987	1991/1992	
Time to 50% flower (days)	121	91	126	120	0.6
Time to first flowering (days)	117	88			1.0
Node no. first flower	9.7	8.6			0.3
Time to maturity (days)	161	131			0.9
Mean temperature ($\bar{T}$) (°C)	9.8	11.0	13.2	12.6	
Mean photoperiod (P) (h d^{-1})	11.4	12.1	10.5	10.6	

Table 2 Phenotypic correlations among phenological measurements over 369 accessions and two sowing dates at Tel Hadya

	Time to 50% flowering (days)	Time to first flower (days)	Node no. first flower
Time to first flowering (days)	0.988**		
Node no. of first flower	0.351**	0.360**	
Time to maturity (days)	0.935**	0.936**	0.328**

** $P < 0.01$

Regressions of mean temperature prior to flowering on rate of progress to flowering $(1/f)$ were non-significant $(P > 0.05)$ for a random sample f 50 accessions. In contrast, regressions of mean photoperiod prior to flowering on $1/f$ gave a mean $R^2 = 0.69$ over the same 50 randomly selected accessions. The multiple linear, additive regression model of mean values of photoperiod and temperature prior to flowering improved the fit greatly, accounting on average for a remarkable 90.3% of the variance of $1/f$ over 306 accessions. Nevertheless, the fit of the multiple regression model varied over accessions with a standard deviation of 13.6%.

The mean value for the constant a $(\times 10^4)$ was -280.5 with a range over accessions of 9.5 to -605.8. Sensitivity to temperature $(b \times 10^4)$ ranged from 1.0–17.9 over accessions with a mean of 6.99. For photoperiod, the mean sensitivity $(c \times 10^4)$ was 25.7 with a range of 2.8–43.8 over accessions. The R^2 values for individual accessions from the multiple regression were positively correlated with the estimates of c $(r = 0.469, P < 0.01)$ and b $(r = 0.303, P < 0.01)$; i.e., the accessions with the best fit to the model were those in which the onset of flowering was most sensitive to photoperiod and temperature.

A one-way analysis of variance for time to flowering on the basis of country of origin showed significant $(P < 0.001)$ differences amongst country means (Table 3). Early flowering was a characteristic of germ plasm from Egypt, Ethiopia, and India; accessions from Afghanistan, Iran and Turkey were typically the latest to flower. There were strongly significant differences among countries in the fit of the multiple regression model. Accessions from the former Soviet Union and Turkey showed the poorest fit to the model, whereas accessions from Chile, Ethiopia, and Syria showed the best fit (Table 3). Differences between countries in the mean response to temperature (b), photoperiod (c) and the constant a were substantial $(P < 0.001;$ Fig. 2, Table 3).

When the estimates of a, b and c from this field study were compared with a previous glasshouse trial (Erskine et al. 1990) a good association between estimates for both the response to photoperiod (c) and the constant (a) became apparent (Table 4). The correlation between field and glasshouse estimates of the response to temperature (b) was significant at $P = 0.05$ and so weaker than the equivalent ones for the estimates of a and c.

Discussion

The current step in the sequence of model validation, following testing on a wide range of germ plasm under glasshouse conditions, involved a similarly wide range of germ plasm grown in the field in four contrasting field environments. The overall fit of the model was remark-

Table 3 Number of accessions (No. acc.), mean time (days) from sowing to flowering, means of the fit (mean R^2) of the multiple repression, responses to temperature (b) $(\times 10^4)$ and photoperiod (c) $(\times 10^4)$, and constant (a) $(\times 10^4)$ of germ plasm from different countries with their standard deviations, together with the F value from one-way analysis of variance by origin
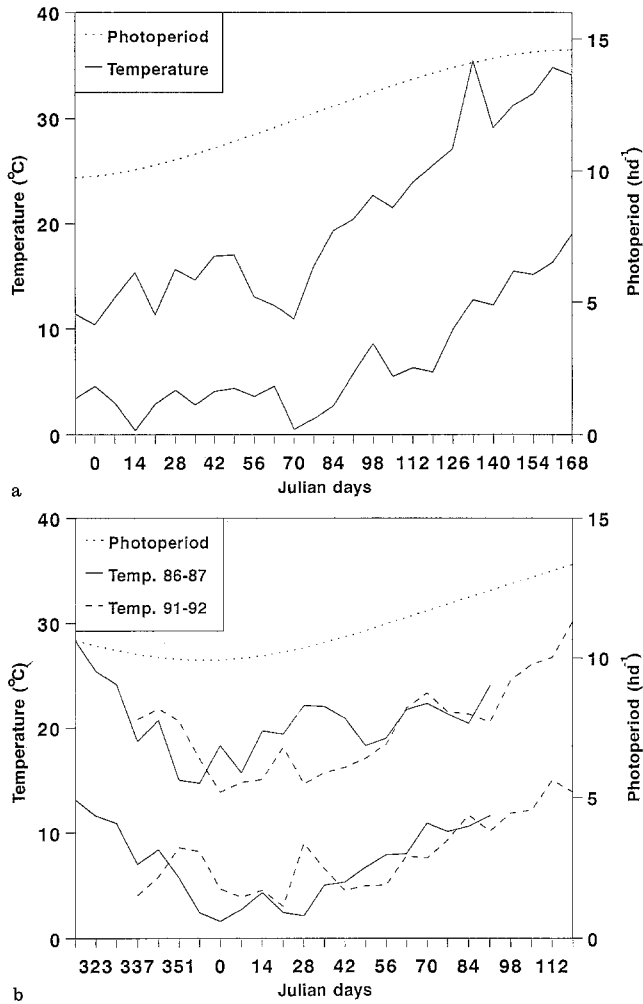
Country	No. acc.	Time to flowering (days)	SD	R^2	a	SD	b	SD	c	SD
Afghanistan	7	127	2.3	0.87	-295	135	6.6	3.5	26.1	9.1
Chile	17	124	2.3	0.98	-336	78	7.9	2.4	28.7	4.5
Egypt	26	105	2.5	0.91	-183	44	7.2	1.6	17.9	3.5
Ethiopia	19	104	1.3	0.96	-241	49	7.9	1.0	22.6	3.8
Greece	16	122	5.2	0.90	-322	120	7.2	3.7	28.5	6.6
India	27	105	1.1	0.90	-223	71	7.2	2.6	21.5	4.0
Iran	21	125	4.2	0.89	-326	108	7.6	2.5	28.0	7.2
Jordan	27	108	2.5	0.88	-286	56	7.0	2.0	27.2	3.9
Lebanon	19	117	7.6	0.91	-290	86	6.3	1.8	27.1	6.4
Pakistan	22	117	7.3	0.92	-290	78	5.7	1.9	27.8	6.4
Syria	27	115	2.2	0.96	-331	55	6.5	1.6	29.8	6.5
Turkey	16	124	3.1	0.85	-323	125	7.5	3.7	27.8	8.1
Soviet Union	17	122	6.8	0.83	-295	133	8.5	3.4	25.1	8.4
ICARDA	37	112	4.8	0.87	-270	65	6.0	2.1	25.5	5.9
F value[a]		70.8***		2.06*	6.69***		2.31**		7.28***	

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$
[a] Degrees of freedom of F:13, 284

a

b

Fig. 1 A,B Weekly (7-day) means of maximum and minimum temperature (°C) and of photoperiod (inclusive of civil twilight) (h d^{-1}) from sowing to flowering based on Julian calender days for Tel Hadya (spanning both sowing dates) (**A**) and Islamabad (**B**) in the 1986–1987 and 1991–1992 seasons

a

b

Fig. 2A,B Mean values for accessions of different countries of origin for time from sowing to flowering (d) and the constant (a) ($\times$ 10^4) (**A**) and responses ($\times$ 10^4) to temperature (b) and photoperiod (c) (**B**). The respective means for the countries of West Asia are joined in order to illustrate the variation inherent in the region where the crop was domesticated. *Afg* Afghanistan, *Chl* Chile, *Egy* Egypt, *Eth* Ethiopia, *Grc* Greece, *Ind* Indian, *Irn* Iran, *Jor* Jordan, *Leb* Lebanon, *Pak* Pakistan, *Syr* Syria, *Tur* Turkey, *Rus* former Soviet Union. ICA ICARDA selections

ably good in the field with mean photoperiod and temperature prior to flowering collectively accounting for 90.3% of the variance of $1/f$. Photoperiod alone accounted for 69% of the variance of $1/f$, whereas temperature alone did not account for a significant portion of the variation in flowering. Clearly, in these circumstances photoperiod was the predominant influence on flowering, prompting the question of why was the fit of temperature data poorer than that for photoperiod? One possible reason for the poorer fit regarding temperature is that the maximum difference in mean temperature between environments was only 3.4 °C, which was encompassed by wide fluctuations of daily minima and maxima. In contrast, photoperiod varied by 95 min over environments. Then again, in May 1987 at Tel Hadya, temperatures were high prior to flowering in some late accessions in the late-sown trial, reaching a maximum daily value of 38.8 °C and a mean daily value of 27.7 °C (Fig. 1). Such extreme temperature values are certainly supra-optimal for lentil (i.e., the genotypic coefficients in Eq. 1 no longer apply). These conditions
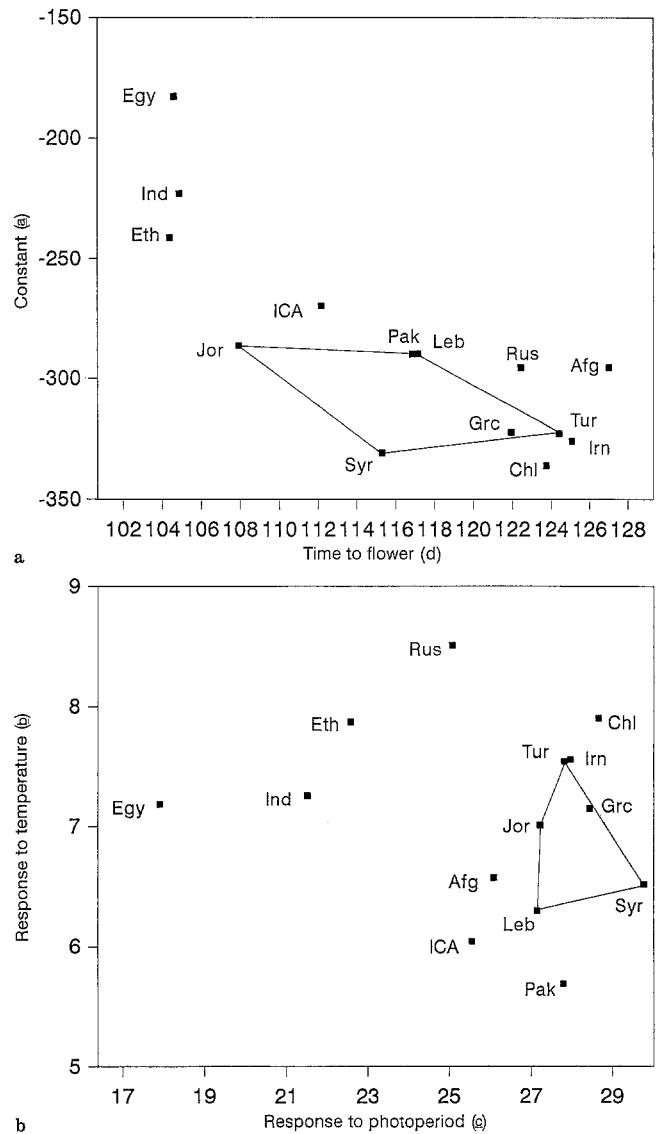
Table 4 Correlations between field and glasshouse estimates of flowering responses (sensitivity) to temperature (b) and photoperiod (c) and the constant (a) in Eq. 1 over 199 accessions common to both trials

Environment/parameter	Field		
	a	b	c
Glasshouse a	0.350**	−0.044	−0.332**
Glasshouse b	0.071	0.157*	−0.123
Glasshouse c	−0.358**	0.001	0.365**
Field c	−0.917**	0.306**	
Field b	−0.561**		

* $P < 0.05$, ** $P < 0.01$

are not experienced in lentil-producing regions in Syria where local cultivars are sown at the agronomically optimum date (December–January).

The lentil was domesticated in West Asia (Cubero 1981; Ladizinsky 1979), from whence it spread. The variation within West Asia for time to flowering was relatively high (diamond in Fig. 2A). The distributions of country means for time to flowering and the genotypic constants *a*, *b*, and *c* illustrate the response to selection for adaptation to new ecological environments following the spread of the crop from its center of origin. Dissemination to lower latitudes such as those in Egypt, India, and Ethiopia has been accompanied by an increase in the constant (*a*) (Fig. 2A) and a reduction in the photoperiodic response (Fig. 2B); sensitivity to photoperiod must have been actively selected against during that spread. Obligate photoperiodic control of the onset of flowering ensures that flowering starts annually in the same calendar period, irrespective of fluctuations in temperature. Consequently, selection against photoperiodic control in a long-day plant such as lentil implies an adaptation to relatively short days, which occur at low latitudes and which would otherwise delay flowering to an unacceptable extent. Under such conditions the crop relies rather more on temperature to ensure that flowering occurs at an ecologically and agronomically appropriate time.

In an earlier study on lentil germ plasm from fewer countries, the sensitivity of the crop to photoperiod was found to be related to latitude of origin, i.e., materials from the extreme latitudes were more sensitive to *P* (Erskine et al. 1990). However, it was unclear whether there was a continuous association of photoperiod sensitivity with latitude of provenance or two separate cohorts; namely, Group 1, represented by Mediterranean/temperate germ plasm with a strong response to photoperiod, and Group 2, Sub-tropical germ plasm with a reduced response. Our study gives evidence of two groups contrasting in intrinsic earlines (of which the value *a* is an approximate guide) and photoperiodic response (*c*), i.e., Mediterranean/temperature germ plasm with large values of both the constant *a* and sensitivity to photoperiod (*c*) and a second group of sub-tropical germ plams with a much smaller response to photoperiod and a reduced value of *a*. There is also evidence that flowering of the sub-tropical group is more temperature sensitive than much of the Mediterranean/temperate group. Germ plasm from Pakistan showed a more Mediterranean/temperature response than a characteristic sub-tropical response, possibly because the germ plasm sampled was largely of highland, rather than lowland, sub-tropical origin. Unfortunately, passport data are insufficient to test this possibility.

The spread of the crop into higher latitudes, for example into the former Soviet Union, has resulted in a small reduction in the photoperiodic response, probably reflecting the change in sowing date from winter to spring. The temperature response of the germ plasm

from the former Soviet Union ranked the highest among country means in this field study but ranked the lowest among country means in previous glasshouse research (Erskine et al. 1990). Indeed, in the latter study 5 accessions from the former Soviet Union had negative responses to temperature (i.e., their rates of progress to flowering at 19°–20°C was less rapid than at 14°–15°C in the glasshouse), suggesting that the optimum temperature for rate of development toward flowering had been transgressed and that it was substantially cooler for this material than for other germ plasm. In the cooler field trials reported here, supraoptimal temperatures were probably only encountered for brief periods in contrast to the glasshouse trial where such temperatures were a daily feature. In short, the greater temperature sensitivity of germ plasm from the former Soviet Union in the present study is a more realistic evaluation than the lower values from the glasshouse trial where the particularly cool optimal temperatures of this specific germ plasm were broached each day. This difference between field and glasshouse temperature response contributed substantially, as expected, to the atypically low correlation between the *b* values calculated from the two trials.

In the food legumes times to flowering are described in several different ways. The most common field descriptor is the average time from sowing or emergence for 50% of the plants in a plot to produce their first open flower (e.g., IBPGR 1985). In our work in growth cabinets the mean time from sowing for the first open flower to appear has been consistently used (Summerfield et al. 1985). In the field pea, (*Pisum sativum* L.) and because early cultivars characteristically produce their first flower at nodes between 5–11 and late cultivars generally flower at node 14 or above (Gritton 1980), the node number of the first flower is sometimes used. Our study with lentil has shown that the vegetative node of the first flower has a low heritability, making it unsuitable as a scale of the measurement of flowering. As time to first flowering may be biased by the presence of an individual atypically early plant, we now consider that 50% flowering is a preferable characteristic for field use since it is a central measure of population distribution that discounts possible extreme genetic variants within a plot.

The major evolutionary force in the spread of the domesticated lentil is known to have been selection pressure for an appropriate phenology (Erskine et al. 1989). The present study has not only validated a photothermal model (Eq. 1) over a wide range of germ plasm in diverse field environments, but it has also shown that dissemination of lentil to new environments has caused selection for different regionally-specific balances between photoperiod and temperature for the control of flowering.

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