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Flowering in Sulla (*Hedysarum coronarium* L. cv. Carmen) and Persian clover (*Trifolium resupinatum* L. cv. Laser) as affected by sowing date in a mediterranean environment

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Abstract

A reliable prediction of flowering time of forage legumes is an important factor which determines management decisions in order to optimize production and utilization. The experiments, were conducted during two successive growing seasons, following a split plot randomized complete block design with three replications, seven main plots (sowing dates) and two sub-plots (sulla and Persian clover). Linear models were used to relate the rate of progress towards flowering (*1/f*) to mean air temperature and mean photoperiod from sowing to early and full flowering. Evaluation of flowering time was also based on days after sowing (DAS), day of year (DOY-age in days from 1 January), growing degree days (GDD-amount of heat units above a species-specific base temperature), photothermal index (PTI) and photothermal time (PTT). Temperature and photoperiod strongly affect time to flowering, but not in the same way for the two species. The thermal and photothermal models accounted for most (80-85% and 82-87% respectively) of the variation observed in time to flowering for the two flowering stages. The responses of *1/f* to temperature were significant for the two species, whereas the sensitivity to photoperiod was significant only for sulla. Time to reach early and full flowering was best correlated with DAS and DOY for sulla, while DAS and GDD predicted more accurate flowering time for Persian clover. GDD or PTT for sulla and DOY or PTI for Persian clover were not as highly correlated with dates to reach both flowering stages.

Keywords: Base temperature; day length; flowering time; photothermal index; photothermal time.
Abbreviations: BF-beginning of flowering; DAS-days after sowing; DOY-day of year; EF-end of flowering; GDD-growing degree days; PTI-photothermal index; PTT-photothermal time.

Introduction

Forage legumes are an important component of Mediterranean grasslands with a significant ecological and economic role. The benefits of legumes in livestock production systems are well documented (Frame and Laidlaw, 2005). These include nitrogen (N) fixation, high nutritive value and high voluntary feed intake, improvement of soil structure and reduction of soil erosion (Bilalis et al., 2009). As a result of the above advantages, the wide use of forage legumes may lead to reduced energy consumption and environmental pollution, increased biodiversity and improved agricultural sustainability (Rochon et al., 2004). Legume breeding and agronomic research has focused on relatively few species, mainly lucerne (*Medicago sativa*), white clover (*Trifolium repens*) and red clover (*Trifolium pretense*), while information on the performance of alternative legume species well adapted to low input sustainable systems and biological livestock production is limited (Sulas, 2005; Sölter et al., 2007; Basu et al., 2009). Furthermore the ontogeny of each species needs to be well matched to their target environments, with rapid growth habit in the late winter season, in order to ensure good adaptation, longer supply and maximized yield. Of particular interest are adaptive traits, such as seed dormancy, seed size, hardiness of seeds and flowering time (Martiniello and Ciola, 1993; Rochon et al., 2004; Ali et al., 2009). Flowering is a particularly important event in crop development and flowering time plasticity is a common adaptive feature of annual crops to various climatic conditions, making the ability to predict time of flowering of particular cultivars when grown in different locations, seasons and weather conditions, a potentially powerful tool in order to optimize the choice of cultivar, sowing time and agronomic management (Summerfield et al., 1991; Lawn et al., 1995). Phenology in a wide range of annual legumes is principally controlled by responsiveness to photoperiod and air temperature, although other factors such as water and light availability are of secondary importance (Bernier and Périlleux, 2005). A family of simple linear models has been proposed to quantify the effects of temperature and photoperiod on flowering in various long- and short-day plants. In these models, when time to flowering (*f*) is transformed into developmental rate of progress towards flowering (*1/f*), the responses to temperature and/or photoperiod are found to be linear and can be expressed simultaneously as response surfaces. Furthermore the effects of temperature and photoperiod have proved to be additive, with little evidence of interactions between the environmental variables (Roberts and Summerfield, 1987). Despite the large number of experiments concerning various field crops, there is little information regarding forage legume development (Evans et al., 1992; Del Pozo et al., 2000; Butler et al., 2002; Iannucci et al., 2008). In Greece, forage production is mainly based on lucerne (*Medicago sativa*) and secondarily on common vetch (*Vicia sativa*) and berseem clover (*Trifolium alexandrinum*), while other species such as subterranean and Persian clovers (*Trifolium subterraneum* and *Trifolium resupinatum*), birdsfoot trefoil (*Lotus corniculatus*) or sainfoin (*Onobrychis viciifolia*) play a minor role in pastoral livestock systems (Papastylianou et al., 2010). In the present study sulla, an adventitious herbaceous legume in our country and Persian clover, a minor crop, were chosen for evaluation because of their high productivity in low input sustainable production systems. Sulla, or French honeysuckle (*Hedysarum coronarium* L.), is well adapted to semi-arid environments in clay and calcareous soils, produces forage mainly during early spring and autumn and is useful for grazing, haying and ensiling (Sulas, 2005). It is a good source of protein for livestock and has moderate levels of condensed tannins which are thought to prevent bloat and posses anti-helminthic properties (Borreani et al., 2003; Ramirez-Restrepo and Barry, 2005). Persian clover (*Trifolium resupinatum* L.) is a vigorous winter annual legume, cultivated in pure stands or in mixtures with cereals for hay production or grazing. It is adapted to a wide range of soil conditions and tolerates poor drainage and mildly saline soils (Knight, 1985). The objectives of this study were to a) use the linear models in order to quantify the effects of temperature and photoperiod on flowering and b) evaluate different parameters (DAS, DOY, GDD, PTI, PTT) as predictors of flowering time of sulla and Persian clover grown over a range of sowing dates across 2 years.

Results

The climatic conditions during the growing season in the years 2007, 2008 and 2009 were quite different and this had a significant impact on the results (Fig 1). In 2008-2009 the autumn and late winter were warmer than 2007-2008 and the winter and early spring were wetter. The seven sowing dates provided a wide range of environmental conditions to examine the performance of the two species. As the sowing time was delayed from mid autumn (October) to late winter (January), the crops were exposed to higher values of temperature and longer photoperiod, resulting in a shortening of the number of days to the beginning of flowering (Fig 2A,B). The number of days to full flowering followed the same pattern between the two species. Plants in the first growing season were exposed to cooler temperatures, resulting in a delay for flowering which was expressed more in the early sowing dates. For all species, the interval from sowing to early and full flowering (expressed as the inverse of duration) was strongly ($R^2 \geq 0.80$, $P<0.001$ Table 1) and linearly related to mean air temperature (Fig 3). Extrapolation of the regression lines gave a base temperature T_b of 4.1 and 4.4 ^oC for sulla and 6.6 and 6.8 for Persian clover for each phase (BF and EF). The rate of progress from sowing to BF and EF flowering stages showed a linear relationship with photoperiod which was more significant for sulla than for Persian clover ($R^2 \geq 0.86$ and $R^2 \geq 0.53$ respectively). Values of P_b were lower for sulla than for Persian clover, while thermal units T_t, which have to be accumulated above the base temperature for flowering to occur, showed lower values for Persian clover. In all cases higher values for P_b and T_t were recorded for the EF flowering stage. Temperature alone explained much of the observed variation in rate of progress to flowering (*1/f*) ($R^2 \geq 0.80$, $P<0.001$ Table 1). When temperature and photoperiod were both included in the analysis, significant responses of *1/f* to mean air temperature and photoperiod were detected, especially for sulla ($R^2 \geq 0.82$, $P<0.001$ Table 1). The parameters b' and c' were positive and significant for sulla, indicating that rate of progress to flowering was accelerated by warmer temperature and longer photoperiod. In contrast, in Persian clover the responses of *1/f* to temperature (expressed as b') were highly significant, whereas the sensitivity to photoperiod (expressed as c') was lower. Sowing date influenced DAS, DOY, GDD, PTI and PTT to reach BF and EF flowering stage (Table 2). Sulla reached both flowering stages earlier than Persian clover and the differences between BF and EF flowering stages were 7 and 6 days for sulla and Persian clover respectively. As sowing date was delayed, the time from sowing to each flowering stages decreased, which resulted in progressively shorter growing seasons (Fig 4A). Days after sowing were highly and negatively correlated to DGS at which the two flowering stages occurred (r=-0.97 and r=-0.98, $P<0.001$ for sulla and Persian clover respectively). Similarly as sowing date was delayed, the DOY to reach both the developmental stages was later for Persian clover than for sulla. Days of the year were highly correlated to DGS (r=0.93 and r=0.91, $P<0.001$ for sulla BF and EF and r=0.80 and r=0.78, $P<0.001$ for Persian clover BF and EF) and increased linearly more rapidly for sulla than Persian clover with time (Fig 4B). The use of GDD reveals the influence of temperature on flowering. Persian clover required about 322 and 379 fewer GDDs to reach the BF and EF flowering stages than sulla (Table 2). There was a tendency for the GDD values to decrease as sowing dates were postponed, showing a significant quadratic response between DGS and GDD for both species (Fig 4C). However the relationship was not as accurate as DAS and DOY, especially for sulla. Growing degree days only accounted for 78 and 57% of the variation in sulla BF and EF stages, while the explained variation was higher for Persian clover (85 and 77% for BF and EF stages respectively). PTI and PTT are climatic parameters that combine the influence of heat units and day length. Sulla required 144 and 173 more PTI units (or 121 and 169 more PTT units) to reach the early and full flowering stages than Persian clover (Table 2). Although the regression equations for PTI and PTT followed the same trend as GDD, they were not significant in all cases (Fig 4 D,E). The coefficients of determination for PTI were higher for Persian clover (R^2=0.56 and R^2=0.40 for BF and EF stages), in contrast to PTT for sulla (R^2=0.65 and R^2=0.56).

Discussion

Forage legumes showed great variability in flowering time, which is a variable of critical importance to pasture management in seasonal highly-unpredictable environments such as most Mediterranean-type ecosystems (Del Pozo and Aronson, 2000; Monks et al. 2010). The present study revealed that temperature and photoperiod affected flowering response in sulla and Persian clover, consistent with previous results with other forage legumes (Evans et al., 1992; Butler et al., 2002; Iannucci et al., 2008). The linear models described by Roberts and Summerfield (1987) accounted for most (80-87%) of the variation observed in time to flowering in the field. In agreement with experiments on forage legumes carried out for several years by Iannucci et al. (2008), the photothermal model provided a better description of the data for sulla than for Persian clover, as illustrated by R^2 (Table 1). In all cases, sensitivity to temperature (coefficients b and b') was positive indicating that rate of development toward flowering was hastened by warmer temperatures. Additionally, the variation in the coefficients b and b' between the two species may reflect differences in vernalization response, which was capable of delaying flowering in Persian clover (de Ruiter and Taylor, 1979). The parameter estimates for sensitivity to photoperiod (coefficients c and c') were positive, consistent with the two species being quantitative long-day plants. There was, however, large variation between species in the magnitude of c', whereas the variation of the coefficients between flowering stages was small. Although the estimates of the parameters b' and c' for Persian clover in this study are in agreement with the results of other field trials (Iannucci et al., 2008) estimates from experiments in controlled environments with a Persian clover cultivar originating in the Lebanon agreement with the results of other field trials (Iannucci et al., 2008) estimates from experiments in controlled environments with a Persian clover cultivar originating in the Lebanon show greater sensitivity to photoperiod than to temperature,

Table 1. Values of constants (x10^{-4}) and coefficients of determination (R^2) of models derived from regression of the rate of progress to flowering (*1/f*) against mean air temperature (model 1), photoperiod (model 2) and both mean air temperature and photoperiod (model 3), base temperature (T_b), base photoperiod (P_b) and thermal time (T_t) for sulla and Persian clover.

Species	FS	Model 1				
		α[a]	b	R^2	T_b (°C)	T_t (°Cday)
Sulla	BF[b]	-25.9*	6.4***	0.85***	4.1	1570.6
	EF	-26.7*	6.0***	0.80***	4.4	1659.4
Persian clover	BF	-51.1**	8.2***	0.83***	6.2	1215.2
	EF	-54.2**	8.0***	0.83***	6.8	1248.3
		Model 2				
		a	c	R^2	P_b (h)	
Sulla	BF	-87.8***	12.7***	0.86***	6.91	
	EF	-91.4***	12.6***	0.87***	7.21	
Persian clover	BF	-101.0	13.3	0.56	7.61	
	EF	-149.3	17.7	0.53	8.45	
		Model 3				
		α'	b'	c'	R^2	
Sulla	BF	-64.6*	2.7*	7.5*	0.87***	
	EF	-76.8**	1.9*	9.2*	0.87***	
Persian clover	BF	-94.5	8.0***	3.9	0.83***	
	EF	-76.2	7.5***	2.4	0.82***	

Significant at *P<0.05; **P<0.01; ***P<0.001; [a]*a* and α': day^{-1}; *b* and b': $°C^{-1}$; *c* and c':h^{-1}; [b]BF: beginning of flowering; EF: end of flowering.

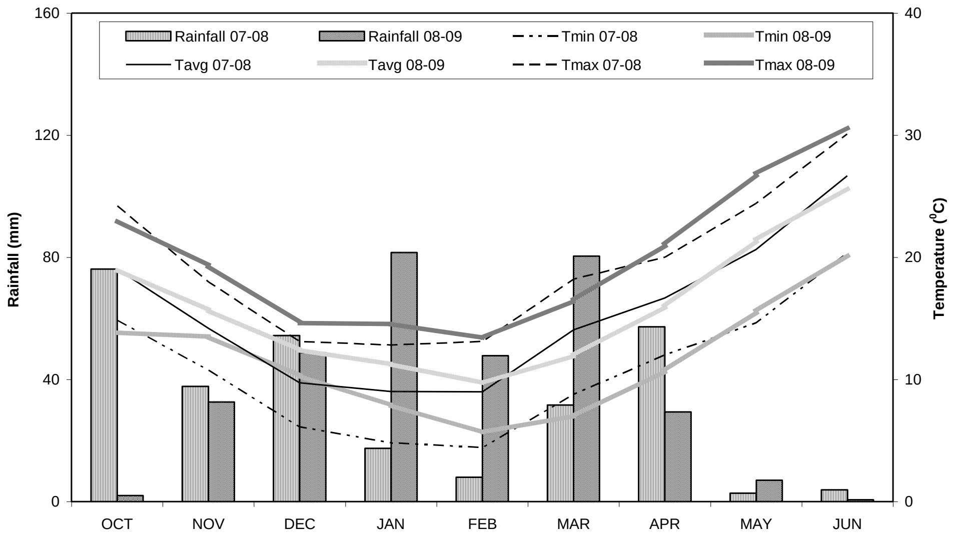


Fig 1. Monthly means of maximum, minimum and average temperature (°C) and total rainfall (mm) for the experimental site during 2007-2008 and 2008-2009 growing seasons.

suggesting large variation among cultivars (Keatinge et al., 1998). Base temperature, base photoperiod and thermal time were specific to each species and developmental stage. According to Iannucci et al. (2008) the estimates of the base temperature (T_b) for sulla were 3.9 and 4.8 °C and for Persian clover 5.7 and 5.2 °C for early and full flowering stages respectively. For the computation of GDD, Borreani et al. (2003) also used a base temperature of 5 °C for sulla. In addition, de Ruiter and Taylor (1979), examining the photothermal characteristics for different legumes species in controlled environment experiments, reported that *Trifolium resupinatum* plants did not flower in short photoperiods (8h day^{-1}). The values mentioned above were within the range of the estimates in the current study. DAS and DOY were closely correlated to sowing dates for sulla, while DAS and GDD showed more accuracy for Persian clover as predictors of time to reach BF and EF flowering stages. Furthermore, the number of PTT required to reach flowering was linked to sowing dates only for sulla, while the flowering stages were less accurately predicted by PTI. Similar results have been reported for other plant species (Butler et al., 2002)

Materials and methods

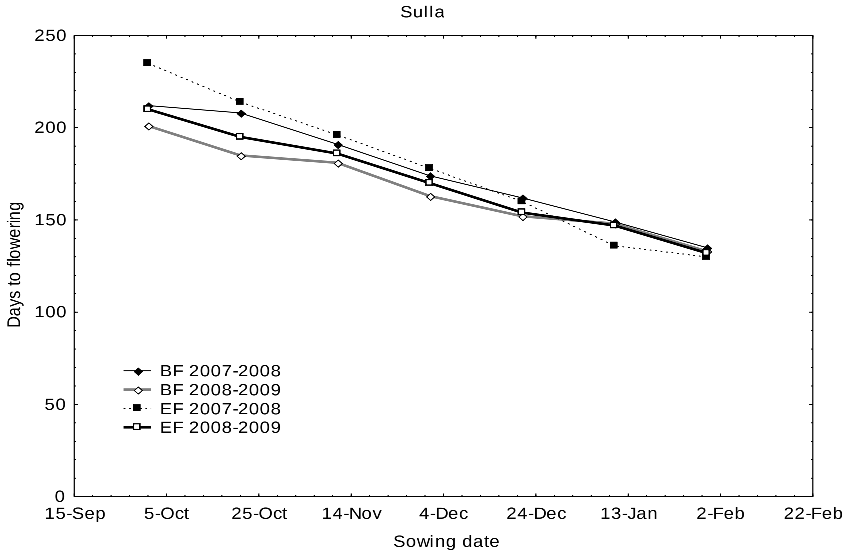
Experimental site

A field experiment was carried out at the Agricultural University farm located in Athens (southern Greece: latitude 37°58′N, longitude 23°32′E, altitude 30 m above sea level) during the 2007-2008 and 2008-2009 growing seasons.

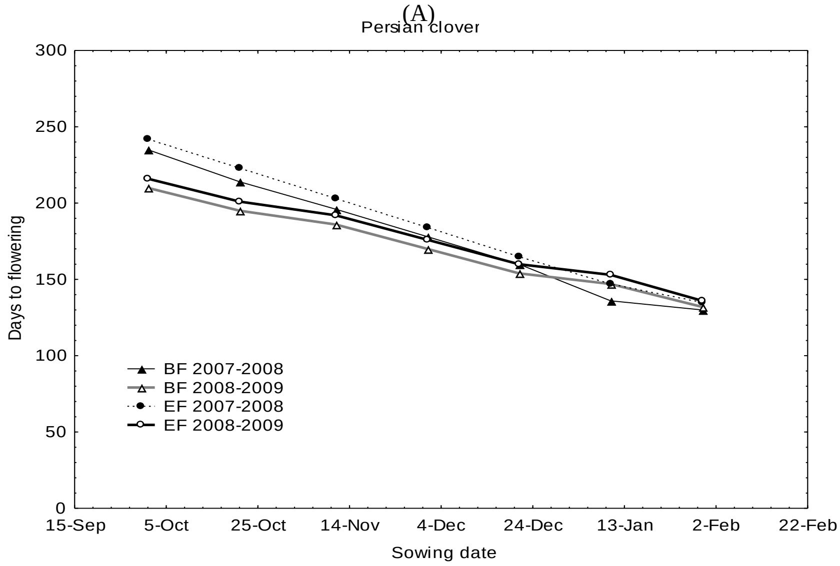
Table 2. Days after sowing (DAS), day of year (DOY), growing degree days (GDD), photothermal index (PTI) and photothermal time (PTT) to reach the early (BF) and full (EF) flowering stages for sulla and Persian clover.

Species	DAS(day)	DOY(day)	GDD(^{o}Cday)	PTI(h^{o}Cday)	PTT(h^{o}Cday)
BF					
Sulla					
Mean	171.0	140.7	1629.3	783.7	490.1
SDV	26.2	17.7	163.0	70.0	71.3
Persian clover					
Mean	174.5	144.2	1307.4	640.1	369.5
SDV	33.0	12.1	192.3	77.8	53.5
EF					
Sulla					
Mean	178.5	147.1	1702.9	833.4	511.6
SDV	27.1	17.5	146.7	68.1	76.3
Persian clover					
Mean	180.9	150.6	1323.8	660.7	342.8
SDV	33.4	11.9	176.1	75.3	56.7

SDV: standard deviation.



(A)



(B)

Fig 2. Variation in number of days to early (BF) and full (EF) flowering of sulla (A) and Persian clover (B) sown on seven sequential dates during 2007-2008 and 2008-2009 growing seasons.

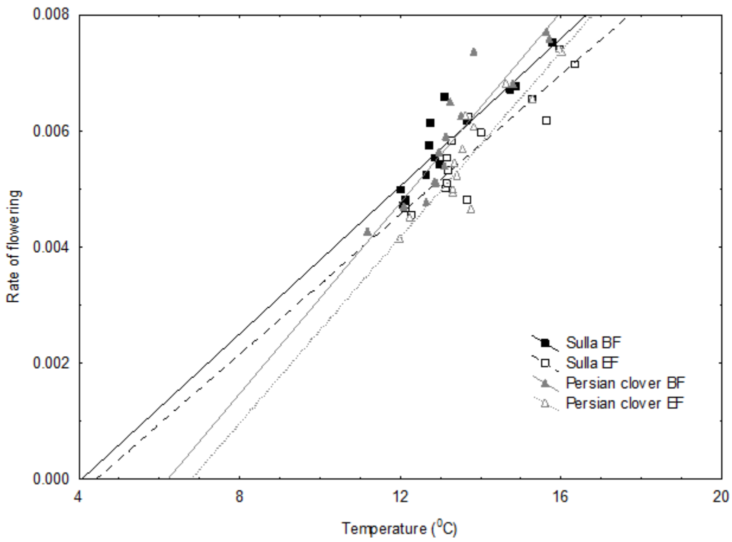


Fig 3. The fitted linear regressions between mean air temperature and rate of progress from sowing to BF and EF flowering stages for sulla and Persian clover.

The soil was clay loam (29.8 % clay, 34.3 % silt and 35.9 % sand) with pH 7.17, NO_3-N 12.4 mg kg^{-1} soil, available P 13.2 mg kg^{-1} soil, available K 201 mg kg^{-1} soil and 1.17 % organic matter.

Plant material and experimental treatments

Two forage legumes, sulla (*Hedysarum coronarium* L. cv. Carmen) and Persian clover (*Trifolium resupinatum* L. cv. Laser) were evaluated to determine the time from sowing to flowering. Treatments consisted of seven sowing dates at about 20-day intervals from October through January.

Experimental design

The experiments were arranged as split-plot design with sowing dates in the main plot and forage species in the subplot in a randomized complete block design replicated three times. Each plot consisted of 5 equally spaced rows, 5 m in length and 0.3 m apart. Seeds were planted by hand in a mixture with sand at a rate of 25 kg ha^{-1} and 12 kg ha^{-1} for sulla and Persian clover respectively. Chemical fertilizers, (30 kg ha^{-1} of nitrogen as ammonium sulfate 21-0-0 and 100 kg ha^{-1} of P_2O_5 as biphosphate 0-20-0) had been applied two weeks before seeding. Soil-water availability was maintained close to field capacity for all treatments using sprinkler irrigation when required. Weeds were removed by hand, when necessary. No *Rhizobium* inoculation was applied and the plants were well nodulated in all treatments.

Measurements and calculations

Plots were monitored at 2-3 day intervals to estimate the beginning and the end of flowering (days on which 10% and 100% of the plants from the middle rows of the plots had at least one open flower, BF and EF respectively). Mean daily temperature was obtained by averaging maximum and minimum daily temperatures which were recorded by an automatic weather station located close to the experimental field. The photoperiod was defined as the time in hours from sunrise to sunset and the day lengths were collected from the Institute for Environmental Research of the National Observatory of Athens. For the Athens experimental site day length varies from 9 h 32 min on 21 December to 14 h 49 min on 21 June. The mean daily temperature (T) and photoperiod (P) were calculated for each of the flower stages (BF and EF) in each year. The rate of plant development ($1/f$), defined as the inverse of duration from sowing to beginning (BF) or end flowering stages (EF), was related to mean diurnal temperature (T, °C), to mean photoperiod (P, hd^{-1}), or to both, using three linear models, the thermal time model $1/f=\alpha+bT$ (1), the photoperiodic model $1/f=a+cP$ (2) and the photothermal model $1/f=\alpha'+b'T+c'P$ (3) where α, b, a, c, α', b' and c' are constants specific for each species (Roberts and Summerfield, 1987). The regression coefficients from the Eqn 1 and Eqn 2 can be related to base temperature T_b, base photoperiod P_b and thermal time T_t as $T_b=-\alpha/b$, $P_b=-a/c$ and $T_t=1/b$. The constants of both models were estimated through linear regression, using Statsoft software (2007), for each species. Data from each growing season were analyzed together, after checking for homogeneity (Bartlett's test). The accumulated growing degree days (GDD) between sowing dates (stage A) and the flowering stages BF and EF (stage B) were calculated as the sum of the difference between the mean daily T and a base temperature (T_b estimated from Eqn 1, different for each flowering stage), using Eqn 4.

$$GDD = \sum_{\text{Stage A}}^{\text{Stage B}} (T-T_b) \quad (4)$$

Negative values were not included in the summation. A photothermal index (PTI) over the same periods was calculated by summing the proportion of day length per 24-h period multiplied by the total heat units (Masle et al., 1989) as in Eqn 5,

$$PTI= \sum_{\text{Stage A}}^{\text{Stage B}} [DL_i /24(T- T_b)] \quad (5)$$

where DL_i is the number of daylight hours in each day, T is the mean daily temperature and T_b is the base temperature estimated from Eqn 1, different for each flowering stage. Temperature corrected for photoperiod (T_{pp}) was calculated from the method of Gallagher et al. (1983) as in Eqn 6,

$$T_{pp} = [(T-T_b)(P-P_b)]/(24-P_b) \quad (6)$$

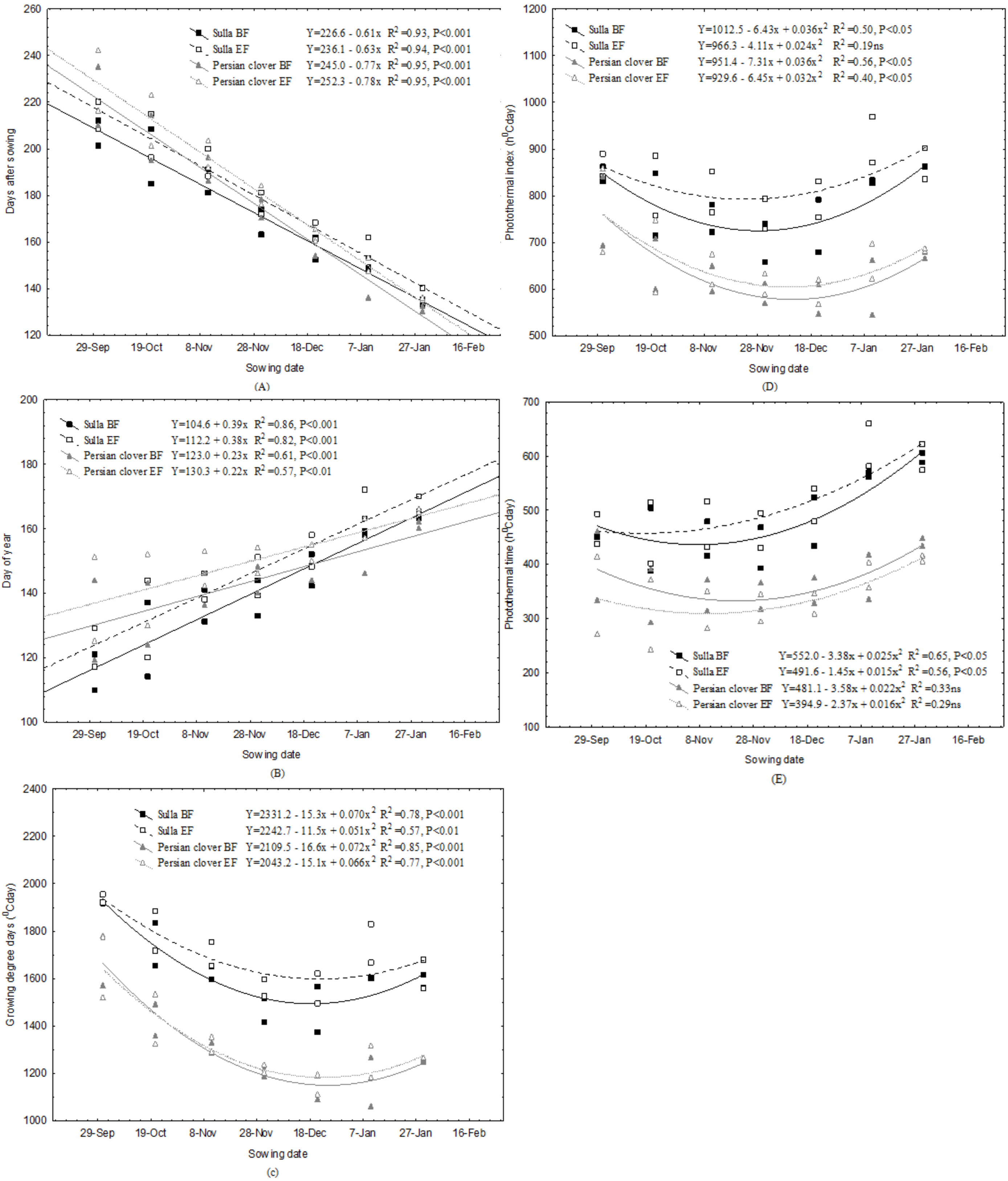


Fig 4. Relationship between day of the growing season (DGS) starting 1 September and A) days after sowing (DAS), B) day of year (DOY), C) growing degree days (GDD), D) photothermal index (PTI), and E) photothermal time for sulla and Persian clover to reach BF and EF flowering stages.

where T and P are the mean temperature and mean photoperiod over the stage being considered, while T_b and P_b are the base temperature and the base photoperiod estimated from Eqn 1 and Eqn 2 respectively. The Eqn 6 is used to estimate the accumulated photothermal time (PTT) by summing each increment of daily T_{pp} between sowing dates and the flowering stages BF and EF.

Statistical analysis

The relationship between sowing date based upon day of the growing season (DGS) starting from 1 September, day of year (DOY) starting from 1 January, days after sowing (DAS), growing degree days (GDD), photothermal index (PTI) and photothermal time (PTT) to reach 10% and 100% flower stages were determined by multiple regression and analysis of variance procedures (Steel and Torrie, 1980). The determination coefficients (R^2) reported in this paper were adjusted for degrees of freedom.

Conclusion

The results of the current study confirm that differential genotypic sensitivity to temperature and photoperiod can explain most of the variation in flowering behaviour of the species in consideration in the field. Further, knowledge of the parameter estimates using linear thermal and photothermal models in different environmental circumstances (e.g. in different locations and for different sowing dates) will provide an accurate prediction of flowering process in order to optimize management decisions, production and utilization.

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