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# Araştırma Makalesi/Research Article (Original Paper) Genetic Analysis of Flowering in Maize based on Calendar and Thermal Time

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Abstract: The objectives of this study were to investigate the genetic effects on flowering traits in maize and to determine the suitable families for potential use in the future breeding studies. Six generations (P1, P2, F1, F2, BcP1F1, BcP2F1) of five different maize families were used as plant material in this study. These materials were generated in the years of 2011 and 2012, than evaluated in 2013. Four flowering traits (days to tasseling, days to pollen shading, days to silking and anthesis silking interval) were investigated. Type of gene action, narrow heritability values, effective gene number and genetic gain from selection for investigated traits were determined by the Generation Mean Analysis method. A considerable variation for the evaluated traits was found among the families and their generations. The results of the study showed that few genes had effect on the evaluated flowering traits. Additive gene action was preponderance for most of the flowering traits, while dominance and epistatic interactions were significant just in two families. In terms of the significance of gene effects, the results were similar from both calendar and thermal time calculations, but not for the heritability estimations. Some of the families (IHOxMo17, IHPxHya, Mo17xIHO) showed potential for effective selection for flowering traits.

Key words: Genetic effects, Growing degree days, Zea mays

## Mısırda Çiçeklenmenin Gün ve Termal Süreye Göre Genetik Analizi

Özet: Bu çalışmanın amaçları, mısırda çiçeklenme özelliklerine etki eden gen tipinin incelenmesi ve ilerde yapılacak ıslah çalışmalarında kullanıma uygun olan aileler tespit edilmesidir. Çalışmada materyal olarak beş farklı aileye ait altı farklı nesil (P1, P2, F1, F2, BcP1F1, BcP2F1) kullanılmıştır. Bu materyal 2011 ve 2012 yılında oluşturulmuş ve 2013 yılında değerlendirme denemesine alınmıştır. Dört çiçeklenme özelliği (tepe püskülü çıkarma, polen dökme, koçan püskülü çıkarma ve koçan püskülü çıkarma polen dökme aralığı) gün ve termal süre bazlı olarak ele alınmıştır. İncelenen özelliklerde gene etki tipi, dar anlamda kalıtım derecesi, etkili gen sayısı ve seleksiyona karşı ilerleme değeri Nesil Ortalama Analizi (NOA) metodu ile belirlenmiştir. İncelenen özellikleri e ait nesiller arasında dikkate değer bir değişim saptanmıştır. Çalışma sonuçları incelenen çiçeklenme özellikleri üzerine az sayıda genin etkili olduğunu göstermiştir. Çiçeklenme özelliklerinin çoğunda eklemeli gen etkilerinin rol oynadığı bulunurken yalnızca iki ailede dominans ve epistatik interaksiyonlar önemli bulunmuştur. Gen etkilerinin önemliliği bakımından gün sayısı ve termal sure hesaplamaları arasında benzer sonuçlar elde edilmiş, fakat kalıtım derecesi tahminleri farklılık göstermiştir. Bazı ailelerin (IHOxMo17, IHPxHya, Mo17xIHO) çiçeklenme özellikleri için seleksiyon

Anahtar kelimeler: Gen etkileri, Günlük gelişim derecesi, Zea mays

## Introduction

Flowering is an important feature for maize in terms of cultural practices. Early or extra early maize genotypes have advantages in stressful conditions, such as drought (Troyer 1983; Frei 2000), although late flowering genotypes tend to be higher yielding (Giesbrecht 1960). Early flowering genotypes also fit better in

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intercropping systems (Lopes et al. 1995). Therefore, shifting flowering time is one of the traits maize breeders focus on.

Several standard observations are commonly used to determine the flowering timing of breeding materials in maize, such as; days to tasselling, days to pollen shading, days to silking, and anthesis-silking interval (Xie et al. 2010). These observations are generally based on calendar time. Alternatively, thermal units or growing degree days (GDD) can be used to evaluate such traits (Stewart et al. 1998). Amount of total heat required (GDD) for plants to progress through phenological stages is calculated with different methods (Baker and Reddy 2001). GDD method is considered to give more reliable results in estimating the transition time between generative stages, as compared with calendar time (Miller et al. 2001). Thus, it is hypothesized that choice of method may produce different results in maize genetics research.

Understanding the genetic effects on flowering traits is important to effectively shift the flowering time in the desired direction, and to choose the best selection strategy to achieve this target. Different statistical methods have been used in genetic effect calculations for this purpose. Generation Mean Analysis (GMA) is one of the methods to analyze genetic effects on a given trait (Mather and Jinks 1977). This method is able to detect the genetic effects that are undetectable in diallel analysis. In the scientific literature, GMA has been used in numerous studies targeting to determine the genetic effects on flowering traits in maize. These studies used data based on calendar time (Hema et al. 2001; Hefiny et al. 2010; Iqbal et al. 2011; Sher et al. 2012), while we could not find any study using thermal time data. Diallel analysis is another method that could be used in genetic analysis of flowering traits. There are examples of studies utilized diallel analysis based on thermal time data (Ahmad and Saleem 2003; Rood and Major 1980), as well as calendar time (Lopes et al. 1995). However, these studies used either thermal or calendar time data. To the best of our knowledge, there is no study that compares genetic estimations that use calendar time and thermal time together.

The objectives of this study were i) to determine the genetic effects on flowering traits in maize, ii) to compare the use of calendar and thermal time data in genetic analysis, iii) to determine the families with suitable flowering habits for potential use in the future breeding studies.

#### Material and Methods

In this study, five parental lines were used to obtain 6 generations of 5 families. F1 generations were developed by crossing parental lines in 2011. F2s and backcrosses were made in 2012. The information about the families and their generations are summarized in Table 1.

Familiy #	P1	P2	F1	F2	BcP1F1	BcP2F1
1	IHO	Mo17	IHOxMo17	IHOxMo17	(IHOxMo17)xIHO	(IHOxMo17)xMo17
2	IHP	B73	IHPxB73	IHPxB73	(IHPxB73)xIHP	(IHPxB73)xB73
3	IHP	Mo17	IHPxMo17	IHPxMo17	(IHPxMo17)xIHP	(IHPxMo17)xMo17
4	IHP	Нуа	IHPxHya	IHPxHya	(IHPxHya)xIHP	(IHPxHya)xHya
5	Mo17	IHO	Mo17xIHO	Mo17xIHO	(Mo17xIHO)xMo17	(Mo17xIHO)xIHO

Table 1	. Plant materi	al used i	n this study.
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Notes: P1 is female parent, P2 is male parent, F1 is cross of P1 and P2, F2 is selfing generation of F1, BcP1F1 is backross of F1 with P1 parent and BcP2F1 is backross of F1 with P2 parent.

The evaluation trial was carried out in Çanakkale. Each generation of families was planted with a 70x20 cm plant density in 2-row plots, on May  $17^{\text{th}}$  2013. Row length was 2 meters. The plots were fertilized with a total of 180 kg ha<sup>-1</sup> nitrogen and 80 kg ha<sup>-1</sup> phosphorus. Nitrogen application (ammonia form) was made in 2 occasions (i.e., before planting and before flowering) while phosphorus was given before planting. The soil of experimental area was loamy with organic matter content of 1.27%, with lime 12.4% and pH of 7.88. Corresponding available P<sub>2</sub>O<sub>5</sub> and K<sub>2</sub>O contents were 37.8 kg ha<sup>-1</sup> and 541.0 kg ha<sup>-1</sup>, respectively. Drip irrigation was used on a weekly basis.

Days to tasseling, to pollen shading, and to silking were recorded when at least 50% of the plants in a row reached that stage. Anthesis-silking interval (ASI) values were found by subtracting the anthesis date from silking date.

For the thermal time calculations, daily values of growing degree days (GDD) were computed according to Stewart et al. (1998). Total GDD values for each flowering event were determined by summing the daily GDD values for a given genotype. GDD for ASI was found with the subtraction of GDD for anthesis from GDD for silking. The daily minimum and maximum temperatures (Figure 1) were collected from HOBO microclimate station (Onset Computer Corporation, USA).



Figure 1. Changes of daily temperature in the experimental area.

General variance analysis was done using proc GLM procedure of SAS V8 software (SAS Institute, 1999). To compare the generations of each family, Tukey's multiple comparison test was used. The quantitative genetic analysis of flowering traits for five families consisting of six generations (P1, P2, F1, F2-derived, BcP1F1 and BcP2F1) was performed using SASQuant macro in SAS (Gusmini et al. 2007). Generation means and variances, computed on 60 individual plants for each generation, were combined to estimate the gene effects (Foolad and Lin 2001, Mather and Jinks 1977). Additive, dominance, and epistatic effects were partitioned according to Hayman's GMA procedure (Gamble 1962, Hayman 1958). Main genetic components consisted of additive variance [a] and dominance variance [d]. Interaction components (epistasis) were described as additive+additive [aa], additive+dominance [ad], and dominance+dominance [dd]. Number of effective factors was calculated with Lande's Method III (Gusmini et al. 2007). Narrow sense heritability estimates were classified into three classes (low =0-30%, moderate =30.1-60%, and high >60%), as suggested by Robinson et al. (1949). The expected gain from selection (at 5% intensity) was calculated by Gusmini et al. (2007), using the selection differential (k) equal 2.06 for 5% selection intensity and narrow sense heritability.

#### **Results and Discussion**

Results of variance analysis showed that generation means for all traits were significantly different (p<0.05) for all families, except ASI (Table 2).

		2	0		5 5				
FN	DF	DA-CT	DA-TT	DA-CT	DA-TT	DS-CT	DS-TT	ASI-CT	ASI-TT
1	5	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0251	0.0099
2	5	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0339	0.0267
3	5	0.0036	0.0078	0.0016	0.0015	0.0100	0.0108	0.4125	0.4172
4	5	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.2012	0.1387
5	5	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0007	0.0004

Table 2. Probability values of general variance analysis by the families.

Notes: FN:Family number, DF: Degrees of freedom, DT:Days to tasselling, DA:Days to anthesis, DS:Days to silking, ASI:Anthesis silking interval, CT: Calendar time, TT: Thermal time.

Family3 and Family4 had no significant variation between their generations for this trait. The parental lines had a considerable variation for flowering traits (Table 3). Regarding bi-parental combinations, parental differences were found between 3.4-9.7 for days to tasseling, 3.2-8.5 for days to anthesis, 1.2-6.3 for days to silking, 0-2.2 for ASI. The correspondent thermal time calculations for these values were 46-135 °Cd, 43-119 °Cd, 16-84 °Cd, and 2.8-34.8 °Cd for days to tasseling, days to anthesis, days to silking and ASI, respectively. Mather and Jinks (1977) suggested the use of contrasting parents in GMA. The broad ranges we observed indicate that the choice of parents were appropriate for this analysis. Most F1s showed lower values for

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flowering events than F2s, except for ASI. This is because F1s are more homogenous genotypes compared to F2s, where genetic segregating occurs. Backcrosses with female parent (MBCa) had higher values than those with male parent for days to tasseling and days to anthesis in some families. In those crosses, female parents had higher values than males (Table 3). This result was expected because backcrossing increases the frequency of the alleles of the recurrent parent (Gonzalez et al. 2014). In some families, however, backcrosses did not resemble to the recurrent parent for days to silking and ASI (Table 3). This suggests environmental effects may have a significant role on the phenotype of these traits.

Table 3. The mean values of families and their six generations as computed with calendar and thermal time data.

Calendar Time (Day)							Thermal Time (°Cd)						
	FN	MPa	MPb	MF1	MF2	MBCa	MBCb	MPa	MPb	MF1	MF2	MBCa	MBCb
	<u>1</u>	58.0c	67.7a	59.3c	60.8c	58.2bc	63.7b	729cd	864a	748cd	769bc	726d	806b
2 2	2	63.8ab	67.2a	61.3b	67.2a	61.3b	65.3a	812ac	858a	777bc	858a	774c	828ab
l Stiller	3	63.8a	59.2c	62.2ac	59.8bc	62.8ab	61.3ac	812a	745b	789ab	755b	793ab	772ab
D <sup>°</sup>	84	63.8a	58.0b	57.5b	59.7b	62.8a	56.7b	812a	729bc	722bc	752b	798a	705c
L	5	67.7a	58.0c	59.7bc	60.0bc	62.5b	58.7c	864a	729c	752bc	752bc	788b	733c
	1	59.7cd	68.2a	59.8cd	61.8c	59.0d	64.7b	752cd	871a	755cd	783c	743d	824b
to	<u>2</u>	64.8ab	68.0a	61.8ab	67.5a	62.0b	65.3ab	826ab	869a	784b	862a	786b	833ab
the	3	64.8a	59.8b	62.0ac	61.5bc	63.2ab	61.8ac	826a	755bc	786ac	779bc	802ab	784ac
Dĩ V	<b>4</b>	64.8a	59.7b	58.5b	60.0b	63.3a	57.5b	826a	752b	736b	757b	805a	722b
	5	68.2a	59.7c	59.8c	60.8bc	63.3b	59.3c	871a	752c	754bc	769b	805c	748c
	1	64.7bc	71.0a	62.0c	65.3bc	62.5c	67.3ab	824bc	908a	786c	832bc	793c	860ab
to to	a 2	67.8ab	69.0a	64.7b	70.3a	64.5b	67.8ab	866ab	882a	824b	899a	822b	866ab
tys 11-i:	3	67.8a	62.8b	63.7b	64.0ab	65.7ab	64.5ab	866a	798b	810b	815ab	837ab	822ab
ũ S	54	67.8a	64.7ab	62.7b	64.2b	67.8a	61.8b	866a	824ab	795b	817b	867a	784b
	5	71.0a	64.7b	62.3b	63.8b	65.3b	64.0b	908a	824b	791b	812b	833b	815b
	1	5.0a	2.8ab	2.2b	3.5ab	3.5ab	2.7ab	72.2a	37.3b	31.4b	49.0ab	50.2ab	35.8b
	2	3.0a	1.0b	2.8ab	2.8ab	2.5ab	2.5ab	40.6a	12.8b	40.2a	37.3ab	35.6ab	33.6ab
IS 3	3.0a	3.0a	1.7a	2.5a	2.5a	2.7a	40.6a	43.4a	23.8a	35.9a	34.5a	37.8a	
4	4	3.0a	5.0a	4.2a	4.2a	4.5a	4.3a	40.6a	72.2a	59.4a	60.1a	61.2a	61.6a
	5	2.8bc	5.0a	2.5c	3.0ac	2.0c	4.7ab	37.3bc	72.2a	36.7bc	42.6ac	27.6c	67.0ab

Notes: For each traits, statistically significant differences were showed with different letters in each familiy. First and last letter showed in group letters (i.e. abc=ac). FN: Family number, MPa:Mean of female parent, MPb:Mean of male parent, MF1:Mean of F1,MF2:Mean of F2, MBCa:Mean of backcross with female parent, MBCb:Mean of backcross with male parent.

Genetic effect estimations about flowering traits are summarized in Table 4. The mean effect showed significant differences from zero for all traits in all families (Table 4). Most of dominance effects and interaction components for families were not significant. However, dominance and epistatic effects were significant in Families 2 and 4. A careful inspection of Table 4 reveals that additive type gene action plays a more effective role on flowering traits than other effects (Table 4). This finding was in accordance with the results of other researchers (Atanaw et al. 2006; Hefiny 2010; Hema et al. 2001). On the other hand, dominance and non-additive effects were found to be significant in some other studies (Sher et al. 2012; Irshad ul Hag 2014). These contrasting findings suggest that different material may have different genetic effects on flowering traits. The sign of genetic effect estimations offer more detailed information on the type of epistatic effect (Kearsey and Pooni 1996), as well as allelic dispersion in parents (Mather and Jinks 1977). Negative sign of the dominance effects in the families (Families 4 and 5), where additive gene action was significant suggests that favorable and unfavorable alleles controlling the flowering traits come from different parents (Table 4). In Family2, dominance gene effects were significant, and it had opposite sign of dominance+dominance effect; which implies a duplicate epistasis for days to tasseling and days to silking (Table 4). If this family was to use in selection, it should be started in advanced generations or a few generations of selfing should precede the selection process for allele fixation. Although significance of gene effects that we calculated from calendar and thermal time were similar, in some families we obtained values with opposite signs (Table 4). This point should be taken into consideration when selecting for flowering traits.

		<u>.</u>		-		*				
	FN	m	а	d	aa	ad	dd	$h^2$	EF	GS05
to ing	1	60.8**	-5.5**	-3.17	0.33	-0.67	0.30	0.64	-4.2	2.3
	2	67.2**	-4.0	-19.5*	-15.3*	-2.33	15.7	-0.3	-0.7	-1.1
iys sel CT	3	59.8**	1.50	9.67	9.00	-0.83	-10.0	-0.18	0.5	-0.8
Da Da	4	59.7**	6.17**	-3.08	0.33	3.25	-2.50	0.61	-0.6	1.5
	5	60.0**	3.83*	-0.83	2.33	-1.00	0.33	-2.1	8.5	-5.5
<b>b</b> 0	1	769.1**	-79.1**	-61.0	-12.2	-11.3	37.0	0.64	-5.8	33.1
) to	2	857.8**	-54.0	-285.6*	-227.5*	-31.1	246.7	-1.45	2.4	-71.8
uys sel	3	755.2**	20.9	118.4	108.1	-12.4	-102.1	-0.84	0.3	-55.2
Da Cas	4	752.5**	93.0**	-51.1	-2.50	51.5	-20.6	0.65	-0.6	23.6
	5	752.2**	55.0	-10.0	34.4	-12.9	20.2	-13.24	2.1	-316
	1	61.83**	-5.67**	-4.08	0.00	-1.42	0.17	1.65	-8.1	8.7
to ssis	2	67.5**	-3.33	-19.92	-15.33*	-1.75	17.17	-1.36	-0.6	-4.6
the	3	61.5**	1.33	3.67	4.0	-1.17	-5.33	-2.81	0.7	-8.0
Ý Dĩ	4	60.0**	5.83**	-2.08	1.67	3.25	-1.83	-0.14	-0.8	-0.3
	5	60.83**	4.0*	-2.08	2.0	-0.25	0.17	-0.07	2.5	-0.2
	1	783.4**	-81.4**	-56.2	0.60	-21.85	-2.11	1.68	-9.6	127.6
to ssis	2	862.2**	-46.7	-274.6	-210.8*	-25.01	235.13	-1.76	-0.5	-78.4
ys the	3	778.9**	18.2	53.1	56.97	-17.3	-76.03	-2.32	0.7	-97.8
An Dã	4	757.0**	83.2**	-25.7	27.24	46.22	-32.7	-0.06	-0.9	-2
,	5	769.1**	57.5*	-27.4	29.72	-2.09	-3.59	0.09	2.4	4.8
	1	65.3**	-4.83*	-7.5	-1.67	-1.67	1.67	0.93	1.0	6.2
o to	2	70.3**	-3.33	-20.42*	-16.67*	-2.75	18.17	-41.75	0.1	-44.4
uys CT	3	64.0**	1.17	2.67	4.33	-1.33	-6.67	-3.68	0.3	-13.6
Si De	4	64.2**	6.0**	-0.92	2.67	4.42*	-4.17	1.07	-0.2	4.0
	5	63.8**	1.33	-2.17	3.33	-1.83	-1.67	1.08	1.4	6.7
	1	832.4**	-67.03*	-103.5	-23.35	-24.9	22.08	0.91	0.9	81.2
o ng	2	899.4**	-44.65	-271.9*	-221.5*	-36.9	241.5	-37.46	0.1	-560
uys Ikij	3	814.7**	14.91	35.92	58.03	-19.21	-89.88	-3.5	0.3	-179
Si De	4	817.1**	82.74**	-17.25	32.74	61.62*	-52.38	1.12	-0.2	58.6
	5	811.6**	18.17	-26.47	48.63	-23.95	-28.7	1.12	1.3	97.9
	1	3.50**	0.83	-3.42	-1.67	-0.25	1.50	-4.33	0.1	-9.4
_ ~	2	2.83**	0.00	-0.50	-1.33	-1.00	1.00	-0.15	0.2	-0.4
ASI	3	2.50**	-0.17	-1.00	0.33	-0.17	-1.33	-2.81	0.0	-4.8
~ 🗉	4	4.17**	0.17	1.17	1.00	1.17	-2.33	1.67	-0.1	5.9
	5	3.00**	-2.67*	-0.08	1.33	-1.58	-1.83	1.05	2.1	3.6
	1	49.0**	14.41	-47.25	-23.95	-3.05	24.19	-5.13	0.2	-141
	2	37.3**	2.02	2.74	-10.76	-11.88	6.37	-0.41	0.2	-14.7
ASI	3	35.9**	-3.33	-17.1	1.06	-1.91	-13.86	-2.84	0.0	-65.7
,	4	60.1**	-0.40	8.44	5.50	15.39	-19.68	1.64	-0.1	81.7
	5	42.6**	-39.32*	0.90	18.91	-21.86	-25.11	1.02	2.1	49.6

Table 4. Genetic parameter estimates for flowering traits as computed with calendar and thermal time data.

Notes: TT: termal time, CT: calendar time. \*,\*\* statistically significant at 0.05 and 0.01 respectively. FN is family number, m is mean effect, a is additive, d is dominance, aa is additive+additive, ad is additive+dominance, dd is dominance+dominance effects. h<sup>2</sup> is narrow sense heritability. EF indicates the number of effective factors and GS05 indicates the genetic gain from selection at 5% intensity.

If heritability is high for a trait, individual plant selection could be practiced in early generations to achieve genetic gain. Otherwise, replicated trials and multiple locations in more advanced generations are needed in selection programs (Kumar and Wehner 2013). High values of narrow sense heritability were observed in Families 1 and 4 for days to tasseling, in Familyl for days to silking, in Families 1, 4 and 5 for days to anthesis, and in Families 4 and 5 for ASI. Our results showed that individual plant selection may be practiced in early generations of abovementioned families (Table 4). Narrow sense heritability estimations in our study are in consistence with the results of other studies (Noor et al. 2013). Nevertheless, our heritability values for flowering traits are higher than those in some other studies (Dawod et al. 2012; Lopes et al. 1995). These differences may be due to the fact that those studies used diallel analysis for the heritability calculations. Heritability estimates based on thermal time calculations were found to be higher than those of calendar day estimations. This is probably due to differences in odd lots of thermal time and calendar date calculations.

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Because this study was carried out at only one location, this effect may be negligible; however, it may be important for studies that are carried out in multi-location trials. Furthermore, negative heritability estimates could be a result of the negative estimations of genetic and additive variances in GMA. Negative estimates of heritability should be omitted (Gusmini et al. 2007).

Estimation for the number of effective factors (EF) showed that all of the investigated flowering traits were controlled by few genes (Table 3). This finding is similar to the results of previous studies (Lima et al. 2008). Lopes et al. (1995) reported that gene number (effective factor) for flowering traits varied from one to nineteen in the different studies. For most families, our numbers were consistent with that reported range. In some families, small number of effective factor for observed traits also estimated in our study (Table 3). Gallais and Rives (1993) indicated that the number of effective factors could be underestimated with comparison to the actual number. They argue that the number of effective factors can only be truly estimated when one of the parents has favorable, while the other has unfavorable alleles.

Table 4 shows the theoretical genetic enhancement at 5% selection intensity for each trait. It was found that, for days to tasseling, genetic enhancement may be obtained by selection only in Family1 and Family4. Family1 (8.7 days) may also have a higher theoretical enhancement potential for days to anthesis by selection practices. Based on our estimations, it is possible to obtain genetic gain from Families 1, 4 and 5 for days to silking, and from Families 4 and 5 for ASI (Table 4). These estimations are consistent with the results of Noor et al. (2013), who determined the realized genetic gain in different flowering traits. However, our estimations are theoretical values and the previously mentioned families should be tested for more accurate results prior to selection experiments.

In conclusion, this research showed that additive type gene action is more important in the genetic control of flowering traits in the genotypes used here. From this material, selection may be effective in Family1 for both days to tasselling and days to anthesis. Family 4 and 5 show potential for days to silking and ASI. Calendar and thermal time based calculations gave similar results in terms of the significance of genetic estimations. However, it was found that the sign of genetic effects, heritability estimates and effective factor number estimations could vary based on the calculation method. This point should be taken into consideration in maize breeding studies for flowering traits. The estimated values of genetic effects are valid for the populations used in this study. Longer term trials in multiple locations may provide more comprehensive results.

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