

Article

Effective Seed Yield and Flowering Synchrony of Parents of CIMMYT Three-Way-Cross Tropical Maize Hybrids

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Abstract: Genotype, environmental temperature, and agronomic management of parents influence seed yield in three-way cross hybrid maize seed production. The objective of this research was to generate information on the seed production of six three-way cross hybrids and their progenitors, adapted to tropical lowlands. Data on days to—and duration of—flowering, distance to spike and stigmas, and seed yield of five female single crosses and five male inbred lines were recorded for different combinations of four planting densities and four sowing dates in Mexico. The effect of planting density was not significant. The male inbred line T10 was the earliest and highest seed yield and T31 the latest, occupying second place in yield. The single crosses T32/T10 and T13/T14 were the earliest and had the highest effective seed yield. At the earliest sowing date, the females were later in their flowering, accumulated fewer growing degree days (GDD), and obtained higher yields since the grain-filling period coincided with hot days and cool nights. To achieve greater floral synchronization and therefore greater production of hybrid seed, differential planting dates for parents are recommended based on information from the accumulated GDD of each parent. The three-way cross hybrids were classified according to the expected seed yield of the females and the complexity in the synchronization of flowering of their parents.

Keywords: *Zea mays* L.; single-cross female; growing degree days; hybridization; seed production



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1. Introduction

In 2011, the International Maize Improvement Consortium for Latin America (IMIC-LatAm) was created in Mexico within the Sustainable Modernization of Traditional Agriculture Project (MasAgro) agreement between the International Maize and Wheat Improvement Center (CIMMYT) and the Ministry of Agriculture, Livestock, Rural Development, Fisheries and Food (SAGARPA). National research centers and Mexican seed companies participate in the Consortium, with the purpose of improving the productivity of maize (*Zea mays* L.) and increasing the size and competitiveness of the seed-producing sector in Mexico and other Latin American countries [1,2]. The Consortium's activities include, among others, research, development, and training in seed production technology [2].

It was estimated that 5% of the hybrid maize seed sown in Mexico came from national seed companies in 2009 [3]. The participation of national companies in the market increased from 24% in 2011, to 31% in 2016; sales grew by 75% in that period, and the seed companies that participated in MasAgro represented 85% of national company sales in 2016 [2], which showed the impact of the MasAgro Program in Mexico's maize seed sector. However, the potential impact of this program may be greater since in Mexico 38,235 tons of certified maize seed were produced in the autumn-winter (A-W) 2018–2019 cycle and 46,682 tons

in the spring-summer (S-S) cycle in 2019 [4–6]. With this amount of seed, 60% of the 7.2 million hectares planted with maize in Mexico could have been supplied [7].

It was estimated that seeds of improved varieties and maize hybrids are sown on 2.3 million hectares in rainfed conditions and there are an additional 3.7 million hectares where new users of hybrids could be found [2]. The native maize planting areas will be preserved as part of the traditions and customs of the native people and because, in many cases, the hybrid grain does not have the organoleptic characteristics or suitable adaptation for those regions.

In the lowland tropics of Mexico, specifically in eight states in the south and southeast of the country (Guerrero, Chiapas, Oaxaca, Campeche, Veracruz, Tabasco, Yucatán, and Quintana Roo), 19% of the national maize grain was produced in 37% of the harvested area in 2019, with the average yield of 1.99 t/ha, while the national average yield was 3.80 t/ha [7]. The lower yield in this region is mainly attributed to abiotic factors such as water deficit and biotic factors such as the Tar Spot Complex disease, in addition to the little use of improved varieties and inefficient crop management practices. A similar situation occurs in countries of Mesoamerica and northern South America. However, the maize hybrids developed by CIMMYT have shown resilience to these limitations and are an alternative for seed and grain producers [8]. To achieve the expected impact, it is necessary that the seed of these hybrids be produced and distributed to farmers in these areas.

The commercial production of hybrid maize seed requires technical information on agronomic management; optimal adaptation areas; appropriate sowing dates, flowering differential, and female:male row ratio; correct way of detasseling; proper planting density; conventional fertilization; and acceptable response to biofertilizers, among other practices that allow obtaining the maximum yield of high-quality seed. It is also necessary to know the environmental requirements of the parents for their development. For example, temperature affects productivity, as it influences the growth rate and phenological development of plants [9–11]. Cultural practices such as sowing date influence maize yield through the growing degree days (GDD) of the crop [12].

The three-way cross hybrids (TWH) selected for this research have stood out for their grain yield and adaptation to tropical environments in Mexico and potentially in Mesoamerica and some countries in South America. These hybrids and their progenitors are registered in the National Catalog of Plant Varieties [13] and some Mexican companies already produce and sell certified seed [4–6]. However, few national companies have the technical and physical infrastructure to carry out research and implement the seed production technology for these hybrids. Consequently, most companies apply a generic management package for all hybrids, which results in a decline in the seed yield potential of each particular hybrid.

Given the need to generate a research model for seed production technology useful for developing seed companies, the present research was proposed with the objective of evaluating the progenitors of six tropical TWH for seed production purposes and generating planting recommendations for the best synchronization of flowering among their parents.

2. Materials and Methods

2.1. Genetic Materials and Experimental Locations

Ten parents of six three-way cross hybrids adapted to tropical lowlands were evaluated. The female parents were five single crosses (FSC or females hereinafter) and the male parents were five inbred lines (ML or males hereinafter). In all cases, the inbreeding of the males and the parents of the females was higher than seven selfed-generations (>S7) (Table 1). The experiments (Exps. hereinafter) were established at the CIMMYT's Experimental Station close to Agua Fría, Puebla, Mexico (20°27'15.30" N; 97°38'29.51" W), at 110 m elevation.

Table 1. Female single crosses, male inbred lines, three-way cross hybrid (TWH) denomination, and year of release.

Female Single Crosses	Male Inbred Line	Pedigree	TWH	Year of Release
T13/T14	T10	T13/T14//T10	CLTHW13002	2013
T4/T6	T10	T4/T6//T10	CLTHW14003	2014
T32/T10	T27	T32/T10//T27	CLTHW15001	2016
T7/T1	T11	T7/T1//T11	CLTHY13002	2013
T7/T1	T31	T7/T1//T31	CLTHY15013	2016
T34/T31	T7	T34/T31//T7	CLTHY15031	2016

2.2. Details of Experiments

Genotypes were evaluated in three groups of experiments. The purpose of the first and second group was to develop TWH parental seed production technology, with the same planting density for males and with three different planting densities for females. The objective of the third group of experiments was to generate information on the floral phenology of the parents, for which both were sown at the same planting density and sowing dates, which would allow specifying the strategies to achieve greater synchronization between the male flowering of ML and the female flowering of FSC that increases the hybrid seed yield of the TWH.

In the first group (Exps. 1, 2, and 3), four males (T7, T11, T27, and T31) were evaluated on three sowing dates at a planting density of 71,111 plants/ha (24 plants/row), while the five males (including T10) were evaluated only in Exp. 1. Inbred line T10 was not evaluated in the other experiments because of an error in the manual filling of the seed envelopes, which was not detected until the advanced stages of the development of the plants. The sowing dates were 23 November 2017 (SD1), 29 November 2018 (SD3), and 5 December 2018 (SD4) for Exps. 1, 2, and 3, respectively (Table 2).

Table 2. Characteristics of the three groups of experiments: crop growth seasons, experiment number, planting dates, and parents of three-way-cross hybrids.

Parents	Group 1			Group 2			Group 3		
	2018A		2019A	2018A		2019A	2018A		2019A
	Exp. 1	Exp. 2	Exp. 3	Exp. 4	Exp. 5	Exp. 6	Exp. 7	Exp. 8	Exp. 9
	SD1	SD3	SD4	SD2	SD3	SD4	SD1	SD3	SD4
T7	X	X	X						
T10	X								
T11	X	X	X						
T27	X	X	X						
T31	X	X	X						
T13/T14				X	X	X	X	X	X
T32/T10				X	X	X	X	X	X
T4/T6				X	X	X	X	X	X
T7/T1				X	X	X	X	X	X
T34/T31					X	X		X	X

Sowing dates: 23 November 2017 (SD1), 16 December 2017 (SD2), 29 November 2018 (SD3), and 5 December 2018 (SD4). X indicates an experiment in which each parent participated.

In the second group of experiments, the five females were evaluated in two experiments (Exps. 5 and 6), and only four females in Exp. 4, in which T34/T31 was not included. These experiments were planted in three population densities: PD1, 82,667; PD2, 93,333; and PD3, 101,333 plants/ha (31, 35, and 38 plants/row, respectively), on each of three sowing dates: SD2: 16 December 2017, SD3: 29 November 2018, and SD4: 5 December 2018, for Exps. 4, 5, and 6 (Table 2).

These same females were evaluated in a third group of three experiments (Exps. 7, 8, and 9) at the same planting density, 71,111 plants/ha (24 plants/row), and on the same planting dates (SD1, SD3, and SD4) as the males (Table 2).

In all the experiments, a randomized complete block design with factorial arrangement and three repetitions was used. In the first and third group of experiments, the genotypes were the main plot and the sowing dates subplots; in the second group, planting densities were included as a sub-subplot. The experimental plot consisted of four rows (13.5 m²). Data were collected from the two central rows.

In both crop growth seasons, the experiments were sown side by side at a distance of 5 m. Sowing was manual and irrigation and fertilizer were applied by a drip-irrigation system. Agrochemicals were applied following standard practices for the experiment station.

2.3. Data Collection and Analysis

On each parent, data collected included number of days from sowing to male and female flowering at 0% (first plant flowering), 10%, 50%, and 90% of male plants shedding pollen (days to anthesis, DTA) or female plants with visible stigmas (days to silking, DTS); duration of male flowering or pollen shedding period (PP) based on the difference DTA 90%–DTA 0%; and duration of female flowering or silk exposed period (SP) based on the difference DTS 90%–DTS 0%.

Per plot were collected from five plants the distance from the ground to the uppermost tip of the ears where silks extrude (DS) from the upper female ear and plant height as the distance from the tip of the tassel to the ground (distance to tassel, DT).

The experiments were harvested when grain moisture averaged 18% across all entries (moisture monitored periodically using the outer two rows of the plot). All ears of the two central rows of each plot were harvested, shelled, and weighed to obtain seed yield per plot (SYP) in kg/plot. Exps. 1 and 7 were harvested on 23 April 2018; Exps. 2, 4, and 8 on 25 April 2019; and Exps. 3, 6, and 9 on 13 May 2019.

Seed yield per hectare (SY) in kg/ha was calculated at 12% moisture content using the following formula: $SY = \frac{SYP \times 10,000}{\text{plot area in m}^2} \times \frac{100 - \% \text{ moisture}}{88}$. The seed yield of the female single crosses was adjusted to a 6:2 female to male row ratio [14]; hence, their effective seed yield (ESY) in kg/ha was calculated as 75% of SY.

Temperature data were recorded at a weather station (Davis Vantage Pro 2) located 260 m (straight line) from the experiments. These data were used to calculate growing degree days (GDD) according to the formula $GDD = (T_{\max} + T_{\min})/2 - T_{\text{base}}$, where T_{\max} and T_{\min} = daily maximum temperature (if temperature was greater than 30 °C, the value of 30 was recorded) and daily minimum temperature (if the temperature was less than 10 °C, the value of 10 was assigned), respectively; and T_{base} = base temperature, 10 °C [15,16].

A residual analysis was conducted, thus facilitating assessment of the homogeneity of variances.

For male inbred lines, an analysis of variance (ANOVA) for Exp. 1 was performed in which the five males participated, along with an ANOVA combining Exps. 1, 2, and 3 with four males in which the source of sowing date variation was incorporated. In the case of female single crosses, an ANOVA included the five females, three planting densities, and two sowing dates combining Exps. 5 and 6; also, another ANOVA was performed with four females for Exps. 4, 5, and 6. In the same way, we proceeded with the experiments in which the same sowing density (Exps. 8 and 9; and Exps. 7, 8, and 9), respectively, was used.

The analysis of variance was performed using Proc GLM of SAS (version 9.4), and the means were compared using Tukey ($p \leq 0.05$).

3. Results and Discussion

3.1. Parental Seed Management

3.1.1. Male Inbred Lines

The results of the ANOVA combined for Exps. 1, 2, and 3 (with four males and three sowing dates) indicated that the two sources of variation effects were significant ($p < 0.01$) for all variables except for pollen shedding period (PP). The effect of SD \times ML interaction was not significant ($p < 0.01$) for the variables related to the phenology of male flowering, but it was for the distance to tassel (DT) and seed yield (SY) ($p < 0.01$) (Table 3).

Table 3. Mean squares of the combined analysis of variance of Exps. 1, 2, and 3 involving data on four male inbred lines (ML) and three sowing dates (SD).

Source	df	DTA (days)				PP (days)	DT (cm)	SY (kg/ha)
		0%	10%	50%	90%			
SD	2	173.58 **	214.08 **	205.44 **	213.53 **	2.09 ns	320.58 **	2,698,813.36 **
ML	3	45.73 **	47.33 **	53.29 **	45.29 **	1.76 ns	2944.89 **	8,199,353.07 **
SD \times ML	6	2.29 ns	2.31 ns	2.93 ns	3.01 ns	0.60 ns	260.25 **	1,263,451.10 **
CV (%)		1.45	1.42	1.45	1.35	19.07	3.28	7.54

Days to anthesis (DTA) at 0 (beginning), 10%, 50%, and 90%; Pollen shedding period (PP); Distance to tassel (DT); Seed yield (SY). ** Significance ($p \leq 0.01$); ns: not significant; Coefficient of variation (CV %).

Advancing the sowing date by 6 and 12 calendar days in the growing cycle 2017A vis-à-vis 2018A (SD1 vs. SD3 and SD1 vs. SD4) delayed DTA at the beginning of flowering in Exp. 1 by 7 days and by 5 days with respect to Exps. 2 and 3, respectively. In the 2018A cycle, even though the difference in sowing dates between SD3 and SD4 was 6 days, the difference in DTA was only 2 days (Table 4).

Table 4. Means for three sowing dates and four MLs from Exps. 1, 2, and 3.

Concept and (n)	DTA (days)				PP (days)	DT (cm)	SY (kg/ha)
	0%	10%	50%	90%			
Sowing Dates							
SD1: Exp. 1 (12)	88 a	89 a	91 a	93 a	4.0	214 a	4704 b
SD3: Exp. 2 (12)	81 c	81 c	83 c	85 c	4.4	204 b	5605 a
SD4: Exp. 3 (12)	83 b	83 b	85 b	87 b	4.8	209 ab	4899 b
Male lines							
T31 (9)	86 a	87 a	89 a	90 a	4.3	201 b	6193 a
T27 (9)	85 a	86 a	88 a	90 a	4.4	236 a	5282 b
T11 (9)	82 b	83 b	84 b	86 b	3.9	199 b	4918 b
T7 (9)	82 b	82 b	84 b	86 b	5.0	199 b	3885 c

(n): Number of means included in the analysis; Days to anthesis (DTA) at 0 (beginning), 10%, 50%, and 90%; Pollen shedding period (PP); Distance to tassel (DT); Seed yield (SY). Values with the same letter in each column and within each type of classification are not different (Tukey test, $p \leq 0.05$).

Between sowing dates, it stands out that in both the ANOVA (Table 3) and the comparison of means (Table 4), a parallel was observed in the responses of the sources of variation for the variables 0% (beginning), 10%, 50%, and 90% of DTA, so variables 10% and 50% of DTA are not included in the subsequent tables. The four ML showed on average 17% more SY in SD3 than the average of the other two sowing dates.

Male inbred line T31 was the one with the highest SY for the average of the three experiments, although it flowered later than T7 and T11, but with a similar distance to the spike as the other two. The inbred line with the highest height of the tassel was T27 and it surpassed the other three (which averaged 200 cm) by 35 cm. In general, these male lines with tassels at 2 m height guarantee that pollen will spill onto the stigmas of any female

parent included in the present study. The male flowering of T27 was similar to that of T31, with a seed yield similar to that of T11 and higher than that of T7 (Table 4).

T10 participated only with the other four male inbred lines in Exp. 1. In the ANOVA (Table A1), it is observed that the effect of genotypes was significant for seed yield and the beginning and end of flowering ($p \leq 0.01$) but not for distance to tassel and duration of flowering ($p > 0.05$). T10 seed yield (8.0 t/ha) was superior to that of the other male inbred lines, whose yield varied from 3057 to 6786 kg/ha. Furthermore, T10 was the earliest, T7 and T11 had intermediate flowering, and T27 and T31 were the latest. The pollen shedding period was similar among the males (Table 5).

Table 5. Means of male lines in Exp. 1.

ML and (n)	DTA (days)		PP (days)	DT (cm)	SY (kg/ha)
	0%	90%			
T27 (3)	90 a	93 a	4.7	229	4303 c
T31 (3)	90 a	93 a	4.7	216	6786 b
T11 (3)	87 ab	89 b	4.0	208	4672 c
T7 (3)	85 bc	88 bc	6.0	203	3057 d
T10 (3)	83 c	86 c	4.7	204	8001 a

(n): Number of means included in the analysis; Days to anthesis (DTA) at 0 (beginning) and 90%; Pollen shedding period (PP); Distance to tassel (DT); Seed yield (SY). Values with the same letter in each column and within each type of classification are not different (Tukey test, $p \leq 0.05$).

When comparing Tables 4 and 5 results, some differences are observed in the magnitude of the seed yield, but not in their order (i.e., T27, T11, and T7 were the male inbred lines with the lowest SY). The same happened for DTA: both early and late male inbred lines kept the same order. The number of days from the beginning of pollen shedding to 90% of plants flowering was 4 to 5, estimating 7 days for the total pollen shedding duration for these male inbred lines. Westgate et al. [17] indicate that pollen release can take 5 or 6 days, although MacRobert et al. [18] point out that, when the days of very low pollen emission are included, pollen shedding and stigma emission can occur over a period of 7 to 14 days. In CIMMYT, in evaluations of tropical inbred lines over the past seven years, the pollen shedding period ranged from 4 to 7 days (unpublished data), which is considered to be ample time to pollinate female single crosses.

3.1.2. Female Single Crosses

The five females were included in the two sowing dates in the 2019A cycle (SD3: Exps. 5 and 8; SD4: Exps. 6 and 9). Similar to the results for the male inbred lines regarding the ANOVAs and comparisons of means, a parallel was observed in the responses of the sources of variation for the variables of 0 (beginning), 10%, 50%, and 90% of DTS, so in the following tables the variables 10% and 50% are not included. Table 6 shows that the main sources of variation, SD, FSC, and PD, were significant for all variables ($p < 0.01$), except in the ESY for SD, but not for SP in the factors FSC and PD ($p > 0.05$). Most of the first- and second-order interactions were not significant. In Exps. 8 and 9, the results were similar except that there were no differences for SD between sowing dates nor SD \times FSC interaction in any variable (Table 7).

The mean yield of the five females was similar for both sowing dates; however, on the second date, the beginning of female flowering was 4 days later, with 1 more day of stigma exposure, while stigma height did not show agronomic differences between the sowing dates. Females T34/T31, T4/T6, and T7/T1 were on average 5 days later and 16% lower in ESY than the average of T32/T10 and T13/T14 (Tables 8 and 9).

Table 6. Mean squares of the combined analysis of variance (ANOVA) of Exps. 5 and 6.

Source	df	DTS (days)		SP (days)	DS (cm)	ESY (kg/ha)
		0%	90%			
Sowing dates (SD)	1	435.60 **	273.88 **	18.68 **	448.90 **	67,623.21 ns
FSC	4	118.46 **	111.04 **	0.88 ns	3887.54 **	9,741,608.46 **
Plant densities (PD)	2	5.28 *	25.43 **	8.04 **	698.63 **	4,443,690.0 **
SD × FSC	4	4.77 *	1.35 ns	1.84 *	26.37 ns	1,330,460.24 **
PD × FSC	8	1.10 ns	0.99 ns	0.67 ns	67.19 ns	860,668.61 **
SD × PD × FSC	8	0.73 ns	0.27 ns	0.40 ns	24.12 ns	395,147.11 ns
CV (%)		1.68	1.25	16.93	6.45	8.08

Days to silking (DTS) at 0 (beginning) and 90%; Silking period (SP); Distance to stigmas (DS); Effective seed yield (ESY). * Significance ($p \leq 0.05$); ** Significance ($p \leq 0.01$); ns: not significant; Coefficient of variation (CV %).

Table 7. Mean squares of the combined analysis of variance (ANOVA) of Exps. 8 and 9.

Source	df	DTS (days)		SP (days)	DS (cm)	ESY (kg/ha)
		0%	90%			
Sowing dates (SD)	1	70.53 **	30.00 **	8.53 **	24.30 ns	76,104.03 ns
FSC	4	53.67 **	41.92 **	1.25 ns	1402.88 **	1,962,139.80 **
SD × FSC	4	0.37 ns	0.58 ns	0.80 ns	27.05 ns	430,160.37 ns
CV (%)		1.41	1.02	21.27	6.69	7.96

Days to silking (DTS) at 0 (beginning) and 90%; Silking period (SP); Distance to stigmas (DS); Effective seed yield (ESY). ** Significance ($p \leq 0.01$); ns: not significant; Coefficient of variation (CV %).

Table 8. Means by sowing dates and planting densities of female single crosses in Exps. 5 and 6.

Concept and (n)	DTS (days)		SP (days)	DS (cm)	ESY (kg/ha)
	0%	90%			
Sowing dates					
SD 3: Exp. 5 (45)	74 b	79 b	5.3 a	125 a	6533
SD 4: Exp. 6 (45)	78 a	83 a	4.4 b	121 b	6478
Planting densities					
82,667 pt/ha (30)	76 b	80 c	4.4 b	125 a	6687 a
93,333 pt/ha (30)	76 ab	81 b	4.7 b	126 a	6767 a
101,333 pt/ha (30)	76 a	82 a	5.4 a	117 b	6064 b
Female single crosses					
T4/T6 (18)	78 a	83 a	4.9	124 b	6103 b
T7/T1 (18)	77 a	82 a	5.0	136 a	6094 b
T34/T31 (18)	78 a	83 a	4.6	98 c	5761 b
T13/T14 (18)	74 b	78 b	4.7	127 b	7103 a
T32/T10 (18)	73 b	78 b	5.1	130 ab	7466 a

(n): Number of means included in the analysis; Days to silking (DTS) at 0 (beginning) and 90%; Silking period (SP); Distance to stigmas (DS); Effective seed yield (ESY). Values with the same letter in each column and within each type of classification are not different (Tukey test, $p \leq 0.05$).

The ANOVA of the response of the four females that were included in Exps. 4, 5, and 6 (Table A2) confirms the significant effect ($p < 0.01$) of the three main sources of variation for all variables, except for SD in ESY and PD at the start of DTS (0%), and the absence of significance of most of the interactions between such sources of variation. In the ANOVA of Exps. 7, 8, and 9, there were no differences for SP nor for SD × FSC interaction in any of the variables (Table A3).

Comparing the data from Tables 8 and 10, it is observed that, by including in the analysis the information from Exp. 4 sown on 16 December 2017 (SD2), and considering the four common females at the three sowing dates, the statistical differences between sowing dates are of little magnitude and, therefore, have little agronomic importance in terms of seed production. Regarding the differences between planting densities, the highest

ESY occurred in the lowest densities and, although the differences between 82,667 and 93,333 pt/ha are not considered relevant from the agronomic point of view, at 101,333 pt/ha, ESY declined 8% with respect to the lower density. It is recommended for future work to evaluate different topological arrangements by modifying the distance between rows and between female plants.

Table 9. Means by sowing dates for female single crosses in Exps. 8 and 9.

Concept and (n)	DTS (days)		SP (days)	DS (cm)	ESY (kg/ha)
	0%	90%			
Sowing dates					
SD3: Exp. 8 (15)	74 b	80 b	5.2 a	123	6274
SD4: Exp. 9 (15)	78 a	82 a	4.1 b	122	6374
Female single crosses					
T4/T6 (6)	78 a	83 a	4.7	128 ab	6228 abc
T7/T1 (6)	78 a	82 a	4.0	137 a	6012 bc
T34/T31 (6)	79 a	83 a	4.5	98 c	5591 c
T13/T14 (6)	74 b	79 b	5.0	119 b	6820 ab
T32/T10 (6)	72 c	77 c	5.2	131 ab	6970 a

(n): Number of means included in the analysis; Days to silking (DTS) at 0 (beginning) and 90%; Silking period (SP); Distance to stigmas; (DS); Effective seed yield (ESY). Values with the same letter in each column and within each type of classification are not different (Tukey test, $p \leq 0.05$).

Table 10. Means by sowing dates and planting densities of four female single crosses in Exps. 4, 5, and 6.

Concept and (n)	DTS (days)		SP (days)	DS (cm)	ESY (kg/ha)
	0%	90%			
Sowing dates					
SD2: Exp. 4 (36)	75 b	80 b	4.8 b	158 a	6433
SD3: Exp. 5 (36)	73 c	79 c	5.5 a	131 b	6680
SD4: Exp. 6 (36)	78 a	82 a	4.4 b	127 b	6703
Planting densities					
82,667 pt/ha (30)	75	80 b	4.4 b	140 a	6826 a
93,333 pt/ha (30)	76	81 a	5.0 a	141 a	6703 a
101,333 pt/ha (30)	76	81 a	5.3 a	136 b	6287 b
Female single crosses					
T4/T6 (27)	78 a	83 a	5.0 ab	135 b	5935 b
T7/T1 (27)	77 a	82 a	5.1 a	145 a	6019 b
T13/T14 (27)	74 b	78 b	4.4 b	135 b	7188 a
T32/T10 (27)	73 b	78 b	5.0 ab	141 a	7280 a

(n): Number of means included in the analysis; Days to silking (DTS) at 0 (beginning) and 90%; Silking period (SP); Distance to stigmas; (DS); Effective seed yield (ESY). Values with the same letter in each column and within each type of classification are not different (Tukey test, $p \leq 0.05$).

Interestingly, the ESY of the later flowering T4/T6 and T7/T1 females was 21% lower than the average of the earlier flowering T32/T10 and T13/T14, when in general the earliest flowering maize genotypes yield less than the later ones. In this regard, Jiang et al. [19] found that late hybrids in Texas yielded more than early hybrids in sowings from mid-May to early June (warmer). On the contrary, due to climate change an irregular distribution and a reduced amount of precipitation as well as a short crop cycle are more frequent, so it is not uncommon for early maize hybrids to outperform late hybrids in the central highlands of Mexico [20].

Unlike the results from Group 2 of experiments, in Group 3, there is more than a week difference in DTS between the experiment sown in 2017 and those sown in 2018, but they agree that the early flowering females yielded more than the late ones (Table 11).

Table 11. Means by sowing dates and female single crosses in Exps. 7, 8, and 9.

Concept and (n)	DTS (days)		SP (days)	DS (cm)	ESY (kg/ha)
	0%	90%			
Sowing dates					
SD1: Exp. 7 (12)	83 a	88 a	5.3	141 a	7864 a
SD3: Exp. 5 (12)	74 c	79 c	4.2	129 b	6376 b
SD4: Exp. 6 (12)	77 b	81 b	5.0	128 b	6639 b
Female single crosses					
T4/T6 (9)	81 a	86 a	4.7	131 ab	6511 b
T7/T1 (9)	80 a	84 b	4.2	140 a	6326 b
T13/T14 (9)	76 b	81 c	5.1	126 b	7435 a
T32/T10 (9)	75 b	80 d	5.2	134 ab	7567 a

(n): Number of means included in the analysis; Days to silking (DTS) at 0 (beginning) and 90%; Silking period (SP); Distance to stigmas; (DS); Effective seed yield (ESY). Values with the same letter in each column and within each type of classification are not different (Tukey test, $p \leq 0.05$).

3.1.3. Relationship between Sowing Dates, Growing Degree Days, Floral Phenology, and Seed Yield

Multiple factors influence the differential response of genotypes to environmental changes inherent to sowing dates. Among them, temperature stands out, especially that which occurs in the critical periods of flowering and grain filling. Male inbred line T31 and the females sown in SD1 required an average of one more week for the beginning of DTA and DTS, respectively (Table 4, Table 5, and Table 11) and required a lower number of GDD in comparison with the other sowing dates (Figure 1 and Table A4). Jiang et al. [19] found that, at later sowing dates (prevalence of colder temperatures during the growing season), hybrids required fewer GDD to reach DTS and were taller; however, contrary to this study, they produced less grain yield.

Figure 2 shows that the GDD accumulation rate during the crop cycle of Exp. 4 sown on 16 December 2017 (SD2) resembled more the rates of the experiments sown on 29 November (SD3) and 5 December 2018 (SD4) than Exps. 1 and 7 (11.19 °C/day) sown on 23 November 2017 (SD1). It is also observed that, during the first 30 days after sowing (DAS), the accumulation of GDD was similar in all the experiments, but in the subsequent 30 days the accumulation of GDD in the experiments sown on SD1 and SD2 was lower than in those sown on SD3 and SD4, because, during that period in 2018, the temperature was lower than in 2019 (Figure A1). Finally, from 60 to 120 DAS, in the experiments planted on SD1, the plants developed under a lower temperature than in the other experiments, which maintained a similar behavior among them (Figure A1).

Temperature affects the rate of the physiological processes, for example, high temperatures during two weeks prior to flowering increase the rate of foliar senescence (Zaidi et al. 2016) [21]. In this study, the cooler temperatures before flowering in SD1 delayed the DTA of the males and DTS of the females, regardless of being late or early flowering, but did not modify the PP and SP, respectively (4–6 days).

Studies in different latitudes found that the optimum maximum temperature for maize flowering varies between 29 and 37.3 °C [22–24]. Between the beginning of flowering and physiological maturity (80 to 100 DAS), plants sown on SD1 developed with a mean difference between the maximum and minimum daily temperatures of 16 °C while in the other experiments the mean difference was 11 °C. During this period, the females grew under warm daytime temperatures and cool nights (Figure A1) that could stimulate production and accumulation of photoassimilates (biomass), and this could be responsible for the highest ESY in Exp. 7. In Venezuela, the areas suitable for growing maize with a

daily temperature range of 13.5 °C (20.5–34.0 °C) were determined [25], which guarantees the cool nights essential for adequate flowering and grain filling [26]. On the other hand, high night temperatures during pollination and grain filling reduce maize yield due to increased respiration and decreased rates of net dry matter accumulation [27], which might explain the lower ESY that was obtained for SD2, 3, and 4. It has been reported that temperatures during flowering and the early stages of grain filling for tropical lowland maize have an optimal threshold between 23 °C at night and 34 °C during the day [28]. It is therefore necessary to establish optimal sowing dates (windows) for specific locations in the tropics, where the ESY of the females and/or the SY of the males can be maximized depending on the case.

The number of GDD required to complete the growth period of any maize genotype varies according to the environmental conditions [29,30] throughout the phenological stages of the crop [11]. Therefore, it is not only necessary to analyze the GDD from sowing to flowering, but during the entire crop cycle, for a better use of cultural practices that decrease the risk of too early or too late sowing [31].

Based on the foregoing, our results indicate that it is convenient to sow single female crosses in November, so that the vegetative and reproductive phases will develop in the months of cool temperatures from December until the end of February; otherwise, late sowings in December, whose flowering will occur in March, increase the risk of poor pollination due to pollen sterilization [32] so grain filling will be incomplete [16]. In contrast, in temperate zones, late sowings may imply a sudden drop in temperature and solar radiation during grain filling that are associated with low yield [19,33].

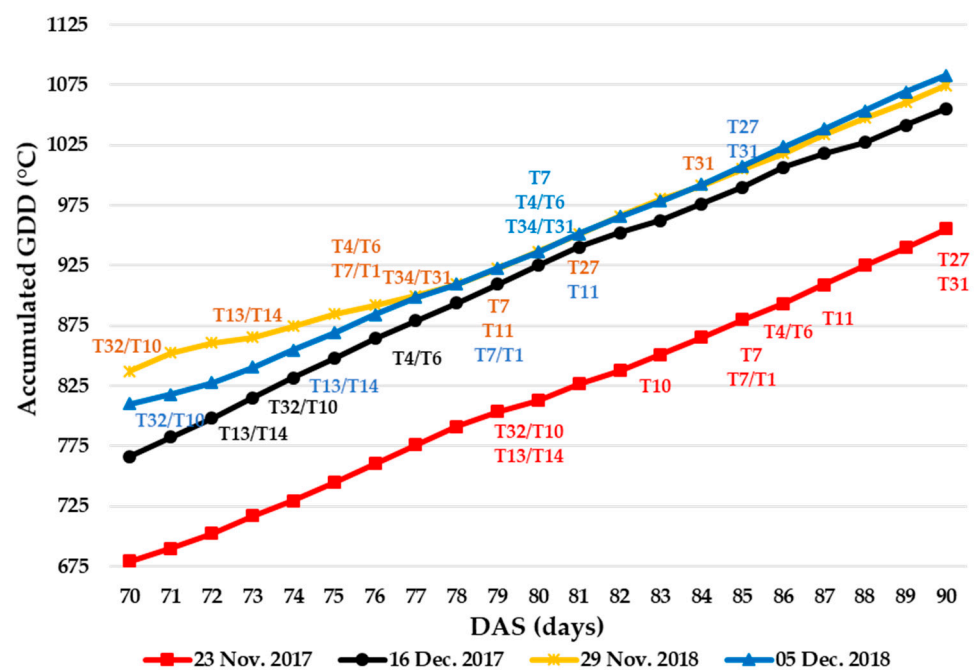


Figure 1. Accumulated growing degree days (GDD) and days after sowing (DAS) until flowering by the parents of the three-way cross hybrids on four sowing dates (SD): SD1 (Red, Exps. 1 and 7), SD2 (Black, Exp. 4), SD3 (Yellow, Exps. 2, 5, and 8), and SD4 (Blue, Exps. 3, 6, and 9).

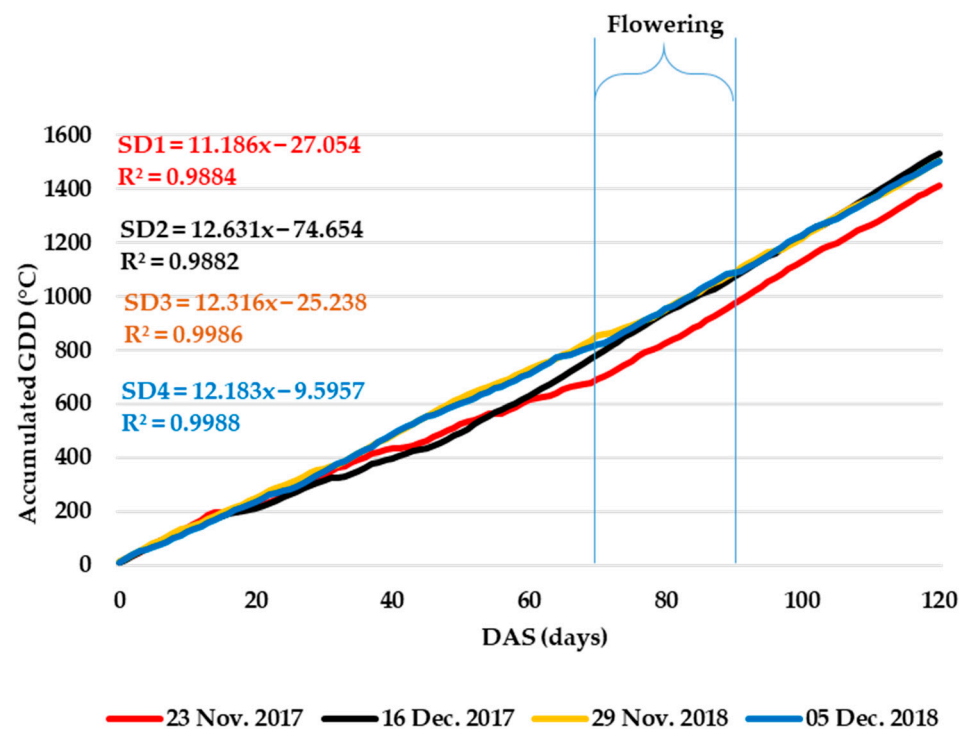


Figure 2. Accumulated growing degree days (GDD) and days after sowing (DAS) during the crop growth cycle by the parents of the three-way cross hybrids on four sowing dates (SD): SD1 (Red, Exps. 1 and 7), SD2 (Black, Exp. 4), SD3 (Yellow, Exps. 2, 5, and 8), and SD4 (Blue, Exps. 3, 6, and 9).

Controversy exists around the best model to calculate GGD in maize. When comparing eight models, those that did not consider supra-optimal temperatures (outside the range of 10–26 °C) and those that considered calendar days were not very precise; the most accurate was the one that involved the “Crop Heat Unit” function, which is estimated by separated functions for daytime and nighttime temperatures [34]. Thus, it is worthwhile in future studies to evaluate other thermal functions as well as to estimate the phenological phases of the TWH parents. It is also advisable to evaluate the option of continuing to use the GDD method (10–30) but with base temperatures that generate greater precision. It is concluded that, in order to estimate the sowing dates that optimize the periods of plant growth with the most adequate thermal requirements, it is necessary to complement the recommendations of calendar days with information on the thermal requirements of both parents.

3.2. Hybrid Seed Production

3.2.1. Floral Synchronization in Hybrid Seed Production

The period between male and female flowering is vital because pollination, fertilization, and eventually grain filling and grain yield depend on it [35,36]. That is valid in commercial sowings for the purpose of obtaining grains or in hybrid seed production. In these cases, technical recommendations often only indicate the days to flowering of the parents and, if applicable, differential plantings between females and males based on the number of days [20,37–40]. Phenological characterization of parents based on GDD allows defining with greater precision the optimal sowing date to achieve floral synchrony [16,41]. In only a few publications on maize seed production, both days to flowering and the GDD necessary to reach this phenological stage are indicated. Similarly, agronomic recommendations for parental management such as localities, planting density, and sowing dates are scarce [16,42]. Private seed companies keep this information secret as part of their confidentiality policies.

Stigmas are receptive by approximately 5 days after the beginning of female flowering and they senesce 8 days later if they are not pollinated [43], whereas pollen release can last 5 to 6 days [17]. This information coincides with the results of the present study, since both the mean period of pollen release of the male inbred lines as well as the period of exposure of stigmas of the female single crosses were 4 to 5 days (Tables 4, 5, 10 and 11, respectively). However, males, because of inbreeding, show less vigor than females, in which heterosis is present [16]. Therefore, it is recommended to sow males on at least two consecutive sowing dates, so that the pollination period of the males completely encompasses the receptivity period of the females [18].

In the present study, the increase in the plant density of the FSC did not significantly increase seed yield, so the specific technology of seed production of the six three-way cross hybrids follows, based on the results of the experiments in Groups 1 and 3.

CLTHW13002 and CLTHW14003

Figure 3 shows the trend of the male flowering of T10 (red) and female flowering of T13/T14 (ocher) and T4/T6 (green) with data from Exps. 1 and 7, respectively. A single figure was generated since T10 is a common ML and participated only in Exp. 1.

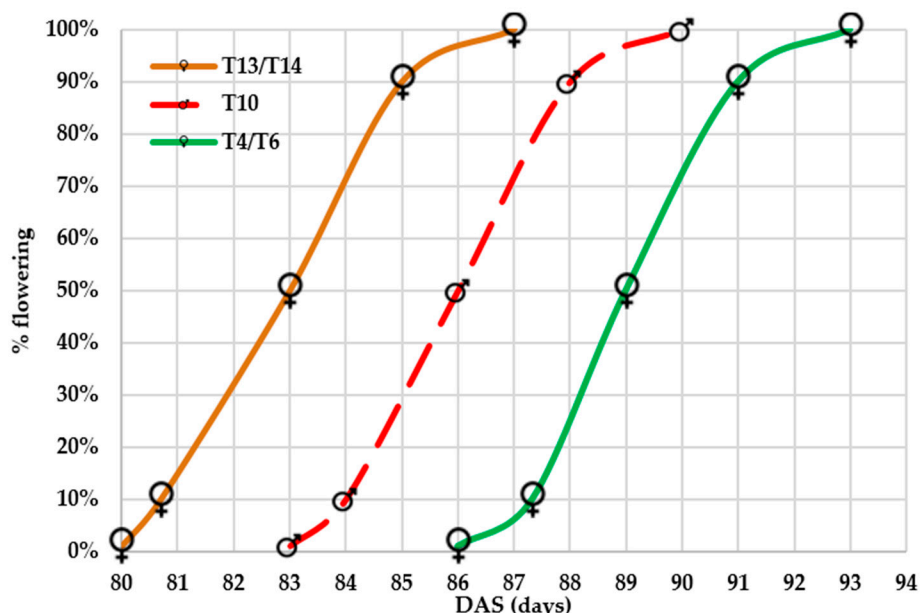


Figure 3. Days to silking of T13/T14 and T4/T6 in Exp. 7 and days to anthesis of T10 in Exp. 1.

Even though T10 is the earliest of the males, the T13/T14 female is even 3 days earlier. For the studied location, in the autumn-winter cycle, to produce hybrid seed of CLTHW13002 in a 6:2 sowing row ratio, it is recommended to first sow one row of T10 and 3 days later (nearby 35 GDD) six rows of T13/T14 and the other row of T10. In this way, throughout the SP of T13/T14 (761–827 GDD), T10 will release pollen decreasing the risk of contamination with external pollen at the beginning of flowering of the female. Unlike T13/T4, T4/T6 was consistently later (827–894 GDD); therefore, to produce CLTHW14003, the six rows of T4/T6 must be sown first and 4 days later (47 GDD) the two T10 rows.

CLTHW15001

In this case, there are 10 days difference between the female flowering of T32/T10 (745–813 GDD) and the male flowering of T27 (880–925 GDD) (Figure 4). This combination involves the earliest female with one of the two latest males in the study, so T27 must be sown on two planting dates, first one row and the second row 5 days later (61 GDD). The six rows of T32/T10 should be sown 10 days after the males' first date (126 GDD). A second

date of males is sown, anticipating a lag with the first date due to some unforeseen rain during those 10 days until T32/T10 is sown.

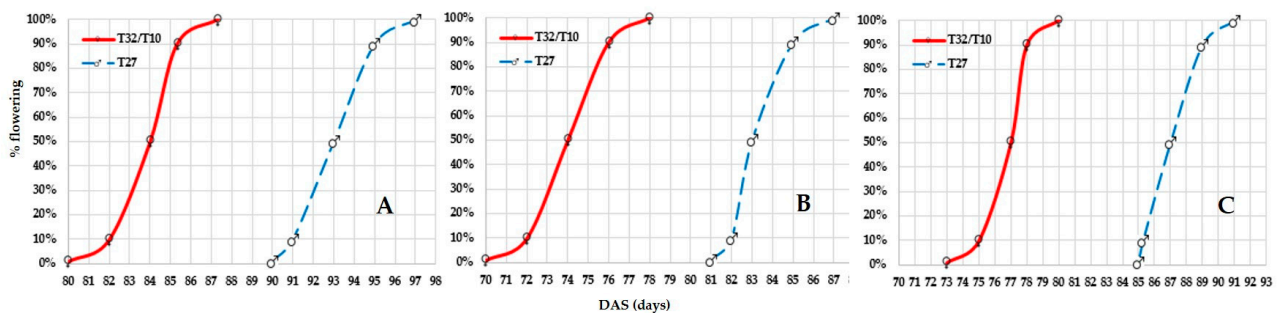


Figure 4. Days to silking of T32/T10 and days to anthesis of T27 in Exps. 7 and 1 (A), Exps. 8 and 2 (B), and Exps. 9 and 3 (C).

CLTHY13002 and CLTHY15013

Figure 5 shows a 2-day span between T7/T1 female flowering and T11 male flowering. In the seed production of CLTHY13002, to ensure complete coverage with pollen of T11 (880-925 GDD) during the female flowering of T7/T1 (813-867 GDD), a row of T11 should be sown first and 2 or 3 days later (35 GDD) six rows of T7/T1 plus another row of T11.

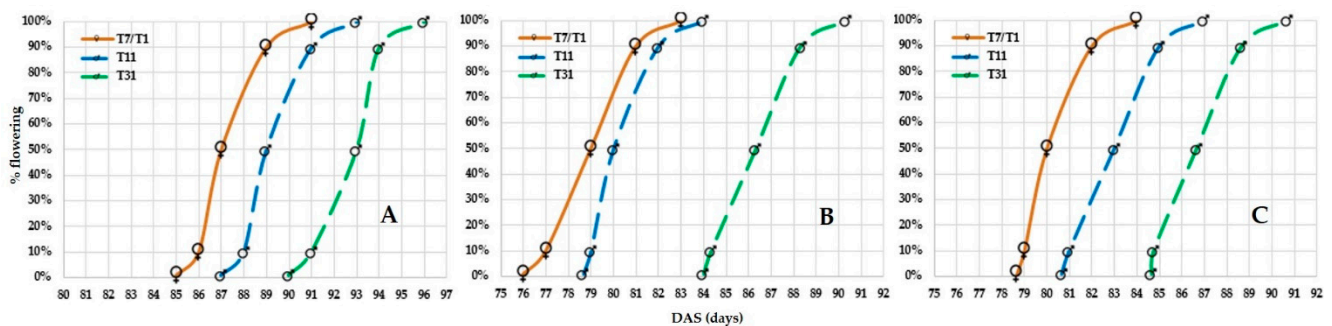


Figure 5. Days to silking of T7/T1 and days to anthesis of T11 and T31 in Exps. 7 and 1 (A), Exps. 8 and 2 (B), and Exps. 9 and 3 (C).

Unlike the previous case, T31 was the second latest male (956 GDD); therefore, in seed production of CLTHY15013, the two rows of T31 should be sown first and 5 days later (75 GDD) the six rows of T7/T1. However, in Figure 5B, an 8-day span between T7/T1 and T31 is observed as opposed to the 5 days in Figure 5A,C. Thus, in this particular case, the GDD effect may affect T31 in different magnitude in relation to T11 and T7/T1, regardless the three parents were sown on SD3. Because of this inconsistency, it is advisable that T31 be sown preventively on two planting dates: first one row of T31 and 3 days later (35 GDD) the second row of T31, while the six rows of T7/T1 have to be sown 8 days later (100 GDD) than the first row of T31 to have pollen presence for as long as possible during T7/T1 female flowering.

CLTHY15031

T34/T31 and T7 showed 1-day difference between their female and male flowering, respectively (Figure 6), so they can be sown on the same day. However, to avoid contamination with foreign pollen in the first days of female T34/T31 flowering, it is recommended to sow one row of T7 first and 2 to 3 days later (35 GDD) both the six rows of T34/T31 and the remaining row of T7.

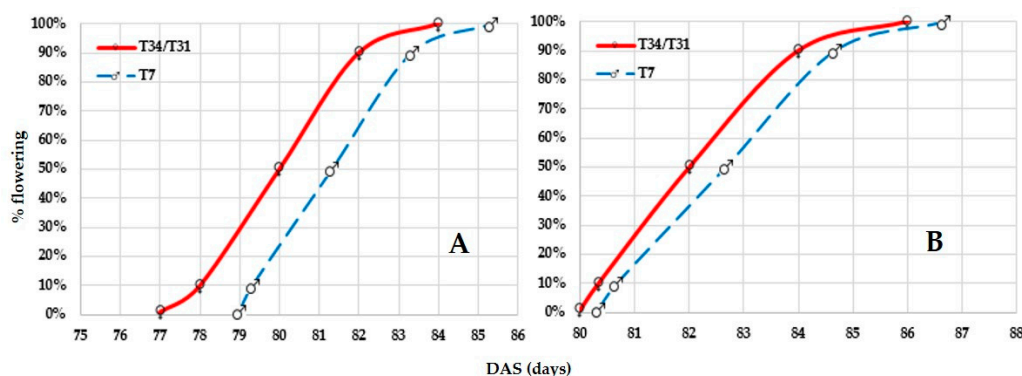


Figure 6. Days to silking of T34/T31 and days to anthesis of T7 (ML) in Exps. 8 and 2 (A) and Exps. 9 and 3 (B).

In all cases for which a second sowing date for the males is recommended, it is assumed that machinery is available to do the sowing, or, in the case of rains after the first sowing, staff are available to planting.

If the coincidence between female flowering of FSC and male flowering of ML is regarded as the sole criterion for seed production, it is clear that simultaneous sowing of both parents represents the least complexity in the management of seed production plots, and a greater interval in the sowing of the parents would be of greater complexity. Therefore, the ranking from the least to greatest risk in seed production of these six hybrids are as follows: CLTHY15031, CLTHW14003, CLTHY13002, CLTHW13002, CLTHY15013, and CLTHW15001.

When comparing the seed production complexity of these six hybrids against three-way cross public hybrids adapted to the tropical lowlands of Mexico, hybrid H-520 stands out, for which the simultaneous sowing of its parents is recommended [44]. A more complex example is H-563, for which for the autumn-winter crop season it is recommended to sow the male first and the female 11 days after [38]. In these two hybrids, a 4:2 female:male rows ratio is also recommended, which is generally the ratio used for seed production of single-cross hybrids, for which the female is an inbred line that does not have the same vigor as an FSC [45]. So, it is assumed that the males of these two hybrids (H-520 and H-563) do not have the ability to pollinate the females in a 6:2 ratio. In the present study, the ESY of the females was estimated for the ratio 6:2, based on previous research whose data are not presented. Furthermore, it is necessary to adjust the seed production technology of these hybrids, with field results obtained in isolated fields where females are detasseled in the two planting row ratios.

3.2.2. Effective Seed Yield (ESY) for FSC and Seed Yield (SY) for ML

The following analysis considers the seed producer's point of view, based on the expected yield of TWH in a 6:2 ratio (75% of the plot area), and the performance of male inbred lines when increased in isolated plots (100% of the area).

In Exp. 1, T10 superiority was shown in terms of SY; additionally, T10 confers both the FSC and the TWH, resistance to the fungal disease tar spot complex (TSC), produced by the synergistic interaction of three fungi: *Phyllachora maydis*, *Monographella maydis*, and *Coniothyrium phyllachorae* [46].

T32/T10 and T13/T14 females obtained the highest yields in all the experiments. The first option for TWH seed producers looking for both high-yielding FSC and ML is to produce CLTHW13002. The second option is to produce CLTHW15001, whose female will produce similar seed yield but that of the male (T27) will be 86% lower than T10 (Exp. 1).

Among the parents of yellow-grain hybrids, T7/T1 produced from 6% to 8% more than T34/T31 while T31 obtained 26% and 60% more seed than T11 and T7, respectively. Consequently, CLTHY15013, CLTHY13002, and CLTHY15031 are suggested as the first, second, and third options, respectively, among yellow-grain hybrids.

Individual information of the parents responses provided in this study is useful for seed producers' (public or private sector) with their own maize breeding programs that wish to access public germplasm to be crossed with their own parents. However, it is necessary to quantify the heterotic effects derived from combining parents for public use from CIMMYT with their own genotypes, which implies carrying out combining ability research, among others.

4. Conclusions

CLTHW13002 (T13/T14//T10) is the best option for seed producers looking for both high-yielding female single cross and flowering synchrony of parents. In the literature review, scarce information is found on sowing recommendations for three-way-cross tropical maize hybrid seed production.

From an agronomic point of view, planting density did not affect female flowering days, stigma height, or effective seed yield of female single crosses, so the lowest planting density should be used to save seed and inputs. Growing degree days affected parental phenology traits of the three-way-cross hybrids in the same way, so the recommendations on differential planting dates between parents are similar for each hybrid on the different sowing dates. Sowings in November favor the presence of days with lower temperatures during flowering and hot days and cool nights during grain filling, which is associated with a higher effective seed yield of female single crosses.

It is necessary to monitor the accumulated GDD to detect on time temperature changes that may modify days to flowering, the detasseling schedule, as well as the grain-filling period duration, and therefore effective seed yield improvement. Our methodology allows the scientific community, farmers, and companies that are interested in seed production of maize hybrids to rank the difficulty level in producing seed of three-way-cross maize hybrids according to the effective seed yield of the females and/or the differential in flowering between both parents.

The information generated in this study will enhance the use of CIMMYT's tropical parental single crosses as females and inbred lines as males, by breeders and seed producers working in both the private and public sector in the IMIC-LatAm influence region, but also in areas with similar environmental characteristics around the world.

For hybrids to be adopted by farmers, hybrid combinations must first be accepted by seed producers. Maize breeders have to incorporate seed production technology criteria during parents selection process, so that seed production will be feasible and profitable.

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Appendix A

Table A1. Mean squares of the analysis of variance of Exp. 1 involving five male lines.

Source	df	DTA (days)		PP (days)	DT (cm)	SY (kg/ha)
		0%	90%			
ML	4	24.23 **	22.43 **	1.07 ns	1231 ns	11,927,702.95 **
CV (%)		1.38	1.18	25.52	2.55	8.46

Days to anthesis (DTA) at 0 (beginning) and 90%; Pollen period (PP); Distance to tassel (DT); Seed yield (SY). ** Significance ($p \leq 0.01$); ns: not significant; Coefficient of variation (CV %).

Table A2. Mean squares of the combined analysis of variance (ANOVA) of Exps. 4, 5, and 6.

Source	df	DTS (days)		SP (days)	DS (cm)	ESY (kg/ha)
		0%	90%			
Sowing dates (SD)	2	203.25 **	118.11 **	11.86 **	10239.73 **	807,769.90 ns
FSC	3	160.07 **	184.99 **	2.40 *	598.10 **	14,291,233.74 **
Plant densities (PD)	2	2.58 ns	20.53 **	8.69 **	305.01 **	2,868,422.79 **
SD × FSC	6	2.58 ns	2.21 ns	1.44 ns	67.90 ns	1,209,763.53 **
PD × FSC	6	0.77 ns	0.44 ns	1.57 ns	73.77 ns	1,037,679.49 *
SD × FSC × PD	12	0.92 ns	1.02 ns	0.42 ns	31.14 ns	345,271.62 ns
CV (%)		1.52	1.36	17.87	5.45	9.26

Days to silking (DTS) at 0 (beginning) and 90%; Silking period (SP); Distance to stigmas (DS); Effective seed yield (ESY). * Significance ($p \leq 0.05$); ** Significance ($p \leq 0.01$); ns: not significant; Coefficient of variation (CV %).

Table A3. Mean squares of the combined analysis of variance (ANOVA) of Exps. 7, 8, and 9.

Source	df	DTS (days)		SP (days)	DS (cm)	ESY (kg/ha)
		0%	90%			
Sowing dates (SD)	2	241.19 **	242.03 **	3.86 ns	645.08 **	7,571,699.53 **
FSC	3	86.99 **	66.78 **	1.88 ns	321.81 **	3,592,101.07 **
SD × FSC	6	1.49 ns	1.36 ns	0.27 ns	43.56 ns	439,581.49 ns
CV (%)		1.53	1.00	23.1	5.55	7.68

Days to silking (DTS) at 0 (beginning) and 90%; Silking period (SP); Distance to stigmas (DS); Effective seed yield (ESY). ** Significance ($p \leq 0.01$); ns: not significant; Coefficient of variation (CV %).

Table A4. Daily and accumulated growing degree days ($^{\circ}\text{C}$) from sowing dates to flowering.

DAS	23 November 2017		16 December 2017		29 November 2018		5 December 2018	
	GDD day	GDD accu.	GDD day	GDD accu.	GDD day	GDD accu.	GDD day	GDD accu.
70	6.5	679.5	16.0	766.5	15.0	837.0	1.2	810.0
71	10.5	690.0	16.0	782.5	15.5	852.5	3.9	818.0
72	12.5	702.5	15.5	798.0	8.0	860.5	4.2	827.5
73	14.5	717.0	17.0	815.0	5.0	865.5	2.9	840.5
74	12.5	729.5	16.5	831.5	9.0	874.5	5.5	854.5
75	15.0	744.5	16.5	848.0	10.0	884.5	14.5	869.0
76	16.0	760.5	16.5	864.5	7.5	892.0	15.0	884.0
77	15.5	776.0	14.5	879.0	8.0	900.0	14.5	898.5
78	15.0	791.0	15.0	894.0	9.5	909.5	11.0	909.5
79	12.5	803.5	15.5	909.5	13.0	922.5	13.5	923.0
80	9.5	813.0	15.5	925.0	14.0	936.5	13.0	936.0
81	13.5	826.5	15.5	940.5	14.5	951.0	15.5	951.5
82	11.0	837.5	12.0	952.5	15.0	966.0	14.0	965.5
83	13.5	851.0	10.0	962.5	14.5	980.5	13.0	978.5
84	14.5	865.5	13.5	976.0	11.0	991.5	14.0	992.5
85	14.5	880.0	14.0	990.0	13.5	1005.0	15.0	1007.5
86	13.5	893.5	16.5	1006.5	13.0	1018.0	16.0	1023.5
87	15.5	909.0	11.5	1018.0	15.5	1033.5	15.0	1038.5
88	16.0	925.0	9.5	1027.5	14.0	1047.5	15.0	1053.5
89	15.0	940.0	14.0	1041.5	13.0	1060.5	15.5	1069.0
90	15.5	955.5	14.0	1055.5	14.0	1074.5	14.0	1083.0

DAS: Days after sowing, GDD: Growing Degree Days ($^{\circ}\text{C}$).

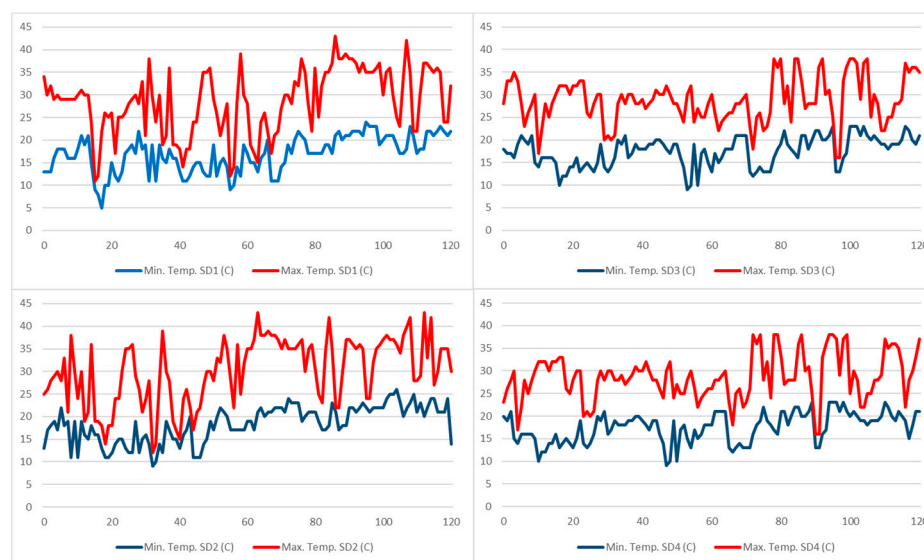


Figure A1. Maximum and minimum temperatures during ML and FSC cycle from sowing dates SD1 (23 November 2017), SD2 (16 December 2017), SD3 (29 November 2018), and SD4 (5 December 2018) to 120 days after sowing.

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