







## Article

# Development of Seed Production Technology of CIMMYT Tropical Single Cross Maize Hybrids

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**Abstract:** Medium and small seed companies require information on maize parental seed production to make rational choices on what germplasm to introduce into their seed and breeding pipelines. The objective of this study was to generate public data on the appropriate hybrid seed production information of five female lines (T21, T26, T28, T33 and T38) and one line (T10) as a male hybrid parent. The effect of female and male sowing date and sowing density on hybrid seed production characteristics including flowering time, flowering duration, distance between tassel and stigmas and effective seed yield was determined. Recommendations to stagger male and female sowing to achieve flowering synchrony for the hybrid parents were complemented with data of seedling growth stages. The results were similar for T21, T26, T28, and T38; sowing the female and T10 on a row proportion of 4 females:1 male, and on a second date, when the coleoptiles of the female plants are halfway to emerge, sowing the missing male row to complete the 4:2 ratio. T33 is a late flowering line, therefore it is desirable to sow T33 first and T10 five days later, or when the T33 coleoptile begins to emerge. Plant densities did not cause differences in most plant characteristics. Line T21 showed good female parental traits. While this study provides a knowledge framework for seed production technology for these single cross hybrids, data specific to seed production regions need to be generated by seed companies to define the best regimes for hybrid production.

**Keywords:** maize; female inbred lines; hybridization; seed production; heat units

## 1. Introduction

The world maize trade in 2019/2020 is forecast to reach nearly 167 million tons, almost unchanged from the previous season [1]. In Mexico, the 2019 maize output is officially estimated at 26 million tons, caused by seasonal rainfall deficits and a reduction in the area planted [2].

The increase in world maize production in the last two decades is associated with the intensification in the use of maize hybrid seed. In Mexico, during the first half of 2019, close to 42,000 tons of maize seed of 289 varieties were qualified [3], which is equivalent to 60% of the certified maize seed of the year 2017 (70,265 t), which covered 46% of the cultivated area of this crop [4].

In 2011, Secretary of Agriculture, Livestock, Rural Development, Fisheries and Food (SAGARPA by its acronym in Spanish) now known as Secretariat of Agriculture and Rural Development (SADER) and the International Maize and Wheat Improvement Center (CIMMYT) implemented the Sustainable Modernization of Traditional Agriculture (MasAgro) Project. The objectives of the project included

promoting the competitiveness of the national seed sector to increase maize production through collaborative research on genetic improvement to develop hybrids with high yield potential and stability in rainfed conditions.

The MasAgro project has resulted in an increased use of CIMMYT lines and derived hybrids by medium and small seed companies who in many cases do not have the capacity to conduct their own seed production research, and therefore need that information to be provided to them. According to surveys conducted at CIMMYT, some seed companies collaborating in the MasAgro project increased their seed sales by more than 40% between 2015 and 2018; in 2019 they sold more than one million bags of seed (CIMMYT Socio-Economics Program, data not published). During 2019, 69 seed companies participated in the MasAgro project whose key benefit to seed companies is access to CIMMYT lines for both breeding and hybrid seed production.

The effective management of parental seed balances the costs of production and effectiveness of maintaining sufficient stocks of viable female and male hybrid parents. Within this context, a suitable crop management of female lines is paramount to the business case for an efficient hybrid seed production, whose success depends on several factors: management, weather, and the suitability of the parents to hybrid seed production. Maize traits that affect suitability of inbred parents for hybrid seed production include seed yield, agronomic traits, and male and female parents flowering synchrony. In addition, female parents must have a high yield of marketable parental seed, a single stem, medium to small tassel with good exertion that facilitates detasseling, good cob coverage and a high proportion of flat and large seeds [5].

It is necessary to generate and/or take advantage of the seed production technology already available while maintaining high seed quality and reasonable seed prices [6].

Commercial hybrid seed production requires technical information of crop field management, such as: adaptation areas, sowing dates, flowering synchrony, female to male rows ratio, detasseling methods, plant density, conventional fertilization, and response to biofertilizers, in addition to other factors that allow for maximum yields of seed of high genetic purity of each parent.

For intellectual property reasons, most publicly available information on hybrid maize seed production is from public institutions. For example, in Mexico, at the National Research Institute in Forestry, Agriculture and Livestock (INIFAP), seed production technology for nine outstanding Quality Protein Maize (QPM) was generated, indicating seed yields higher than 3.5 t/ha [7]. In Mexico, some private seed companies that are commercializing hybrids from MasAgro Project have conducted their own seed production research, but the knowledge derived from this research is not available in the public domain nor do they have a reference to validate their own results; therefore, it is necessary that CIMMYT develops the corresponding single crosses seed production technology and make it public. The objective of this study was to conduct seed production research focusing on sowing date and plant density in order to provide public recommendations for producing hybrid seed from germplasm released by CIMMYT's tropical breeding program.

## 2. Materials and Methods

### 2.1. Genetic Materials and Experimental Locations

Six inbred lines (>F7) adapted to tropical lowlands were used in this study. Five of these lines are currently used as female parents of five single cross hybrids released by the tropical maize breeding program at CIMMYT (Table 1). The inbred line T10, used as a male for all five hybrids, has good resistance to Tar Spot Complex (TSC), a disease caused by the synergistic interaction of three fungi: *Phyllachora maydis*, *Monographella maydis* and *Coniothyrium phyllachorae* [8]. Experiments (hereafter Exps.) were established at CIMMYT's experiment station near Agua Fria, Puebla (20.45° N; 97.64° W), 110 m elevation.

**Table 1.** Parental lines, their single cross hybrids, and year of evaluation in the MasAgro Network.

Female Inbred Lines	Male Inbred Line	Pedigree	Single-Cross Hybrids	Year of Release
T21	T10	T21/T10	CLTHW14001	2015
T33		T33/T10	CLTHW15002	2016
T26		T26/T10	CLTHW15005	2016
T28		T28/T10	CLTHW15007	2016
T38		T38/T10	CLTHW15109	2017

## 2.2. Details of Experiments

Two experiments were established at CIMMYT's experiment station. The first experiment (hereafter Exp.) evaluated all lines for seed production traits at three sowing dates, 71,111 plants/ha (24 plants/row). The sowing dates were: 23 November 2017, 29 November 2018 and 5 December 2018. The second experiment evaluated the female and male lines at three plant densities: D1, 82,667; D2, 93,333 and D3, 101,333 plants/ha (31, 35 and 38 plants/row, respectively). The sowing dates used for the second experiment were: 16 December 2017, 29 November 2018 and 5 December 2018. T10 only participated in Exps. 1.1 and 2.3 due to a manual error at seed preparation during envelopes labeling in the rest of experiments. Therefore, plants of T10 did not correspond to this line in the field. This error was detected during plants development (Table 2). Experimental design for both experiments was a randomized factorial with three replications. In the first experiment, inbred lines were the main plot and sowing dates subplots, and in the second experiment densities was included as sub-sub-plots. Each plot comprised 4 rows of 4.5 m with 0.75 m between rows. Data for each plot were collected on the two central rows only.

**Table 2.** First and second experiments, crop growth seasons, experiment number, sowing dates and lines involved.

Exp. 1			Exp. 2		
2018A	2019A		2018A	2019A	
Exp. 1.1 23 Nov. 2017	Exp. 1.2 29 Nov. 2018	Exp. 1.3 05 Dec. 2018	Exp. 2.1 16 Dec. 2017	Exp. 2.2 29 Nov. 2018	Exp. 2.3 05 Dec. 2018
T10					T10
T21	T21	T21	T21	T21	T21
T26	T26	T26	T26	T26	T26
T28	T28	T28	T28	T28	T28
T33	T33	T33	T33		
	T38	T38		T38	T38

The experiments were hand-planted. Irrigation and fertilizer were applied using a drip-irrigation system. Insecticides, herbicides, fungicides, fertilizers, and irrigation regime were applied following standard practices for the experiment station. In both crop growth seasons, experiments were sown side by side at a distance of 5 m.

## 2.3. Data Collection and Analysis

Data collected on each plot included: days from sowing to male and female flowering at 0% (first plant flowering), 10, 50, 90 and 100% of plants of the male or female line releasing pollen (days to anthesis, DTA) or plants with visible stigmas (days to silking, DTS).

Data on plant height in cm from the ground to the tip of the tassel (DT) and the distance from the ground to the uppermost tip of the ears where silks extrude (DS) of the upper female ear were collected from five plants per plot.

The experiments were harvested when grain moisture averaged 18% across all entries (moisture monitored periodically using plot outer two rows). 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. 1.1 and 2.1 were harvested on 23 April 2018; Exps. 1.2 and 2.2 on 25 and 26 April 2019; and Exps. 1.3 and 2.3 on 13 and 14 May 2019, respectively.

The seed yield per hectare (SY) in kg/ha was calculated at 12% moisture using the following formula:  $SY = (SYP \times 10,000) / (\text{plot area in m}^2) \times (100 - \% \text{ moisture}) / 88$ .

Finally, the seed yield of the female lines was adjusted to a 4:2 female to male rows ratio [9], hence their effective seed yield (ESY) in kg/ha was calculated as 66.7% of SY.

Temperature data were recorded at a weather station located 260 m from the experiments. These data were used to calculate Heat Unit (HU), according to the formula:  $HU = (T_{\max} + T_{\min}) / 2 - T_{\text{base}}$ ; where:  $T_{\max}$  and  $T_{\min}$  = daily maximum and minimum temperature, respectively ( $^{\circ}\text{C}$ );  $T_{\text{base}}$  = base temperature,  $10^{\circ}\text{C}$  [10].

After primary curation of raw data to remove outliers a residual analysis was conducted, facilitating assessment of homogeneity of variances.

For each experiment a combined analysis of variance (ANOVA) was performed. For the first experiment the sources of variation were inbred lines, sowing dates and their interaction. In the second experiment, sowing densities and their interactions with lines were incorporated.

The analysis of variance was performed using Proc GLM of SAS (version 9.4), and the means were compared using the least significant difference test (LSD) ( $p \leq 0.05$ ). For T10, comparisons were made by orthogonal contrasts between the data of Exps. 1.1 and 2.3 and among plant densities in Exp. 2.3.

### 3. Results and Discussion

#### 3.1. Parental Seed Management

##### 3.1.1. Male Inbred Line T10

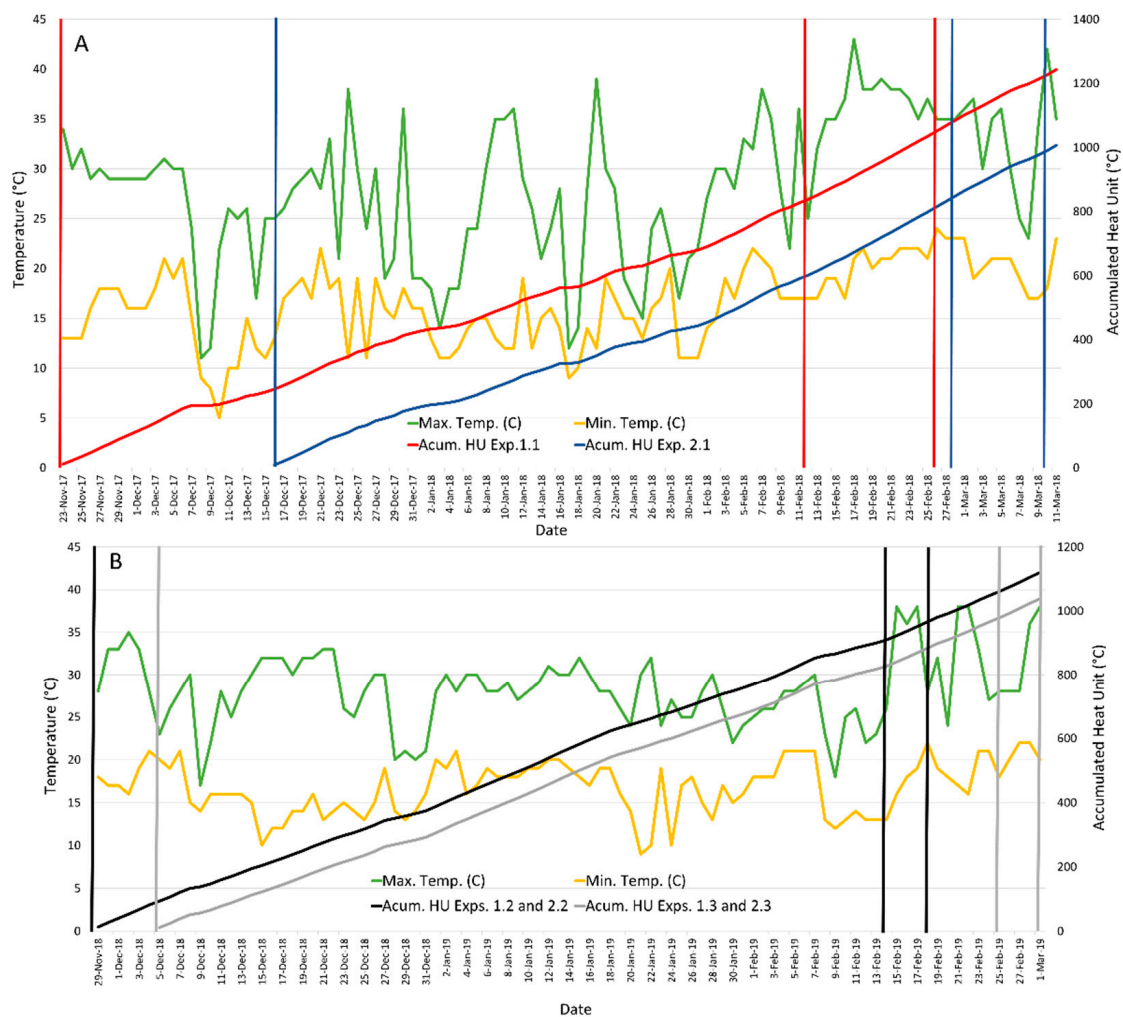
The inbred line T10 in Exp. 1.1, sown on November 23, 2017 in a single plant density started male flowering at 83 days (Table 3) when it had accumulated 851 HU (Figure 1A, Table A1). In Exp. 2.3, planted on December 5, 2018, mean male flowering began four days earlier at 79 days (Table 3) after accumulation of 923 HU (Figure 1B, Table A1). Between those two sowing dates, the differences in the number of days to reach the various phenological stages of flowering were between 4 to 5 days (Figure 1A red lines, Figure 1B gray lines, and Table 3). To reach 90% DTA in Exp. 1.1, T10 accumulated 925 HU and 978.5 HU on average in Exp. 2.3. One would expect to initiate flowering after the accumulation of a similar number of heat units, so the difference in the number of heat units observed here may reflect the daily resolution used to calculate this variable. A more detailed calculation using hourly measurements may provide a more accurate representation of the heat accumulation by plants over the growing season allowing for more precise comparisons of plant phenology in thermal time units. Other related parameters that contribute to HU are photoperiod, vernalization, sunshine hours, photo thermal units and heliothermal units [11].

The heat units required to reach the physiological maturity of the grain in maize are influenced by sowing date and consequently, differences therein in temperature regime experienced by growing plants [12]. In a series of data recorded in Karaj, Iran, from 2002 to 2010, an average of 624 HU (derived from  $^{\circ}\text{F}$ ) accumulated for pollen release and 672 HU for female flowering was obtained [13]. In addition to temperature, other environmental stresses such as water deficit can influence flowering. In our experiments, all plots were subjected to the same irrigation regimes allowing direct comparison of genotypes.

In Exp. 2.3, plant density significantly ( $p < 0.01$ ) influenced the initiation of male flowering only at the highest plant density, despite this, the difference in initiation did not result in a significant difference in male flowering stages (Table 3). Additionally, differences of one to two days in DTA between plant densities are not considered of agronomic importance for hybrid seed production [14].

In Exp. 1.1, at 71,111 plants/ha, the distance to the tassel of T10 was 14 cm longer and the seed yield on the experimental unit was 795 kg/ha greater than the mean of the three higher plant densities

in Exp. 2.3. However, in Exp. 2.3, no significant influence of plant density was found on either distance to tassel or SY (Table 3). The range of densities evaluated in Exp. 2.3 may not have encompassed a broad enough range to fully elucidate a relationship between plant density, plant height and seed yield in line T10, nonetheless, it is more likely that sowing date has a stronger influence than plant density on these plant traits. Based on the results obtained for this male line, there is no benefit in terms of yield of sowing at higher plant densities, therefore it is more cost effective to grow at lower plant density for parental seed production.



**Figure 1.** Maximum and minimum temperatures and accumulated Heat Units (HU) at the International Maize and Wheat Improvement Center (CIMMYT) Tropical Experimental Station. Vertical lines indicate sowing dates and flowering periods: (A): Red, Exp. 1.1; blue, Exp. 2.1; (B): Black, Exps. 1.2 and 2.2; gray, Exps. 1.3 and 2.3.



**Table 3.** Means and orthogonal contrasts of agronomic traits of the male line T10 for sowing dates and plant densities.

Concept & (n)	DTA (days)				DT (cm)	SY (kg/ha)
	0%	10%	50%	90%		
Sowing dates						
Mean Exp. 1.1 (3)	83	84	86	88	204	8001
Mean Exp. 2.3 (9)	79	79	81	83	190	7206
Difference Exp. 1.1 vs. 2.3	4 *	5 *	5 *	5 *	14 *	795 *
Plant densities						
D1 82,667 pt/ha (3)	78	79	81	83	186	7011
D2 93,333 pt/ha (3)	78	79	81	83	194	7452
D3 101,333 pt/ha (3)	80	80	82	84	190	7158
D1 vs. D2	0	0	0	0	8	441
D1 vs. D3	2 *	1	1	1	4	147
D2 vs. D3	2 *	1	1	1	4	294
Male line						
Averages T10 (12)	80	81	83	85	193	7407

(n): Number of means included in the analysis; Days to anthesis (DTA) at 0 (beginning), 10, 50, 90 and 100%; DT: Distance to the tassel; SY: Seed yield. \* Significance ( $p \leq 0.05$ ), according to Student's t-test.

### 3.1.2. Female Inbred Lines

In the combined ANOVA of Exp. 1, the sowing date had a significant impact on all variables with the exception of distance to silks (Table 4). Lines showed significant differences for days to 10% silking, distance to silks and effective seed yield. Interactions between sowing dates and lines were significant only for 50 and 90% of female flowering traits (Table 4).

**Table 4.** Mean squares of the combined analysis of variance (ANOVA) of Experiment 1.

Source	df	DTS (Days)				DS (cm)	ESY (kg/ha)
		0%	10%	50%	90%		
Dates	2	110.71 **	142.13 **	144.18 **	172.76 **	56.46 ns	1125080.04 *
Lines	4	8.91 ns	10.54 *	9.15 ns	7.94 ns	514.02 *	2344102.13 **
Dates $\times$ lines	7	2.42 ns	2.53 ns	3.85 *	4.42 **	65.99 ns	122992.65 ns
CV (%)		1.53	1.44	1.43	1.23	7.23	7.82

Days to silking (DTS) at 0 (beginning), 10, 50, 90 and 100%; DS: Distance to stigmas; ESY: Effective seed yield. \* Significance ( $p \leq 0.05$ ); \*\* Significance ( $p \leq 0.01$ ); ns: not significant; CV (%): Coefficient of variation.

For Exp. 2 (Table 5), there were significant differences ( $p \leq 0.05$ ) in all traits for sowing dates. For lines and plant densities, all traits showed significant differences ( $p \leq 0.05$ ), except for distance to stigmas. Interactions between lines and plant densities were not significant with the exception of 90% DTS. Interactions between sowing date and line were significant for all variables except the start of flowering. Interactions between sowing date and plant density were significant for all variables except effective seed yield. No significant interactions were observed between combinations of sowing date, line and plant density for any trait.

In the 2017–2018 season, the Exps. 1.1 and 2.1 were sown 24 days apart, but there were only 17 days of difference for the mean date of beginning of flowering (Figure 1A). The mean flowering of lines in Exp. 1.1 began at 86 days (Table 6), while in Exp. 2.1 the mean onset was at 79 days (Table 7). This difference was reflected in the shorter duration to reach 900 HU; in Exp. 1.1, it took 86 days to reach 900 HU compared with only 79 days in Exp. 2.1 due largely to the lower daily temperatures at the start of Exp. 1.1 (Figure 1A, Table A1). T33 was the last line to begin flowering in both Exps. 1.1 and 2.1, indicating a genetic requirement of this genotype for additional HU compared with other lines.

The thermal regulation of flowering was under strong genetic control and was therefore genotype dependent [15], making assessment using heat units a more robust measure to compare contrast lines to be evaluated or used in hybrid production. In this regard, in Nayarit, Mexico, ranges from 807.51 to 818.75 HU were found in different genotypes evaluated across 15 years by INIFAP in spring and summer seasons [16].

**Table 5.** Mean squares of the combined ANOVA of Experiment 2.

Source	df	DTS (days)				DS (cm)	ESY (kg/ha)
		0%	10%	50%	90%		
Sowing dates (SD)	2	241.37 **	206.22 **	205.00 **	194.00 **	4173.49 *	667064.46 *
Lines (L)	4	21.65 *	32.14 **	27.80 *	23.67 *	1200.31 ns	7507367.3 **
Plant densities (PD)	2	24.87 **	24.19 **	30.06 **	32.66 **	39.41 ns	468768.26 *
PD × L	8	1.31 ns	1.04 ns	1.32 ns	2.80 *	31.17 ns	164074.32 ns
SD × L	5	2.47 ns	3.10 *	3.63 **	3.30 *	509.33 **	447710.28 **
SD × PD	4	25.15 **	25.87 **	26.19 **	25.84 **	191.89 **	199473.09 ns
SD × PD × L	10	1.99 ns	1.63 ns	1.56 ns	1.63 ns	55.58 ns	177789.01 ns
CV (%)		1.36	1.31	1.30	1.34	7.25	8.29

Days to silking (DTS) at 0 (beginning), 10, 50, 90 and 100%; DS: Distance to stigmas; ESY: Effective seed yield; \* Significance ( $p \leq 0.05$ ); \*\* Significance ( $p \leq 0.01$ ); ns: Not significant; CV (%): Coefficient of variation.

**Table 6.** Means by sowing dates of female lines in Experiment 1.

Concept and (n)	DTS (days)				DS (cm)	ESY (kg/ha)
	0%	10%	50%	90%		
Sowing dates						
Exp. 1.1; 23 Nov. 2017 (15)	86 a	87 a	89 a	92 a	93	3783 a
Exp. 1.2; 29 Nov. 2018 (15)	80 c	81 b	82 b	84 b	92	3614 a
Exp. 1.3; 05 Dec. 2018 (15)	82 b	82 b	84 b	86 b	89	3158 b
Female lines						
T21 (9)	82	83 b	85	87	105 a	3994 a
T26 (9)	82	83 b	84	87	86 b	4009 a
T28 (9)	82	83 b	84	87	87 b	3499 b
T33 (9)	84	85 a	87	89	89 b	2845 c
T38 (6)	81	81 b	83	85	89 b	2976 c

(n): Number of means included in the analysis; Days to silking (DTS) at 0 (beginning), 10, 50, 90 and 100%; DS: Distance to stigmas; ESY: Effective seed yield. Values with the same letter in each column and within each type of classification are not different (LSD,  $p \leq 0.05$ ).

In the 2018–2019 season, both Exps. 1 and 2 were planted simultaneously across two sowing dates. Between the experiments sown on 29 November 2018 (Exps. 1.2 and 2.2) and on 5 December 2018 (Exps. 1.3 and 2.3), there were seven days of difference between sowing dates. For Exps. 1.2 and 1.3, the difference in the mean beginning of flowering was two days (Table 6) and between Exps. 2.2 and 2.3, planted at higher densities, the mean difference in the start of flowering was five days (Table 7). In the last two experiments of Exp. 2, the later flowering line T33 was not included, which may have lowered the observed average flowering data in Exps. 2.2 and 2.3 compared with 2.1. Contrary to what happened in 2017, lines that were first sown in 2018 (Table A1, Figure 1B, black lines) grew at a higher temperature in the first days after sowing than those sown a week later (gray lines) and flowered in fewer days than those planted later. In the experiments where T33 was not included, the mean onset of female flowering was at 78 days in Exp. 2.1 and 83 days in Exp. 2.3. Interestingly, in Exp. 2.1, the plants accumulated 909.5 HU at 78 days, while in Exp. 2, at 83 days, plants accumulated 979 HU (Figure 1B, Table A1).

Based on these results, seed producers should assess the hybrid effective seed yield (ESY) for each particular female line they wish to produce, as ESY varies significantly (ranging from 2845 to

4009 kg/ha in Exp. 1). In addition, while we observed higher seed yields on average under higher plant densities (Exp. 2) the differences were not statistically significant, and due to a higher cost of production under higher densities (Table 7), lower plant densities would be favorable from a business point of view.

**Table 7.** Means by sowing dates, plant densities of female lines in Experiment 2.

Concept and (n)	DTS (days)				DS (cm)	ESY (kg/ha)
	0%	10%	50%	90%		
Sowing dates						
Exp. 2.1; 16 Dec. 2017 (36)	79 b	80 b	82 b	84 b	110 a	3877 a
Exp. 2.2; 29 Nov. 2018 (36)	78 b	79 c	81 c	83 c	90 b	3936 a
Exp. 2.3; 05 Dec. 2018 (36)	83 a	83 a	85 a	87 a	88 b	3727 b
Plant densities						
82,667 pt/ha (39)	80 a	81 a	83 b	85 b	96	3699 a
93.333 pt/ha (39)	79 b	80 b	82 c	84 c	95	3911 a
101333 pt/ha (39)	81 a	82 a	83 a	86 a	97	3929 a
Female lines						
T21 (27)	80 b	81 b	82 b	84 b	107	4345 a
T26 (27)	80 ab	81 b	82 b	85 b	92	4198 a
T28 (27)	80 b	80 b	82 b	84 b	91	3832 b
T33 (9)	82 a	83 a	85 a	87 a	107	2786 d
T38 (18)	80 ab	81 b	83 ab	85 ab	86	3125 c

(n): Number of means included in the analysis; Days to silking (DTS) at 0 (beginning), 10, 50, 90 and 100%; DS: Distance to stigmas; ESY: Effective seed yield. Values with the same letter in each column and within each type of classification are not different (LSD,  $p \leq 0.05$ ).

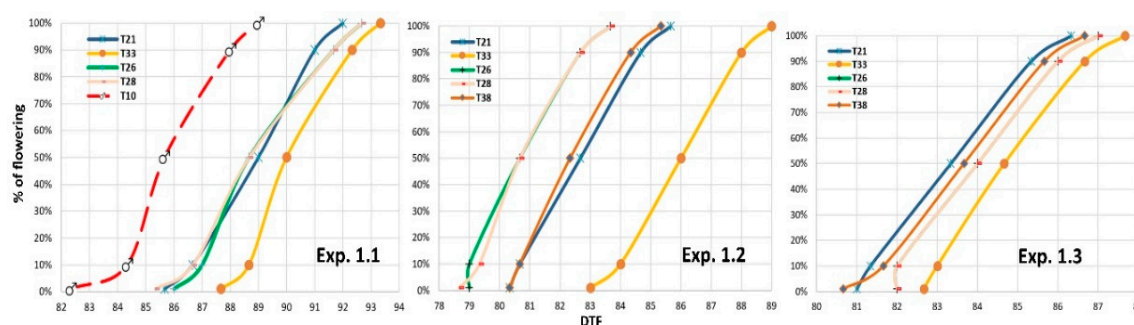
### 3.2. Hybrid Seed Production

#### 3.2.1. Floral Synchronization for Hybrid Seed Production

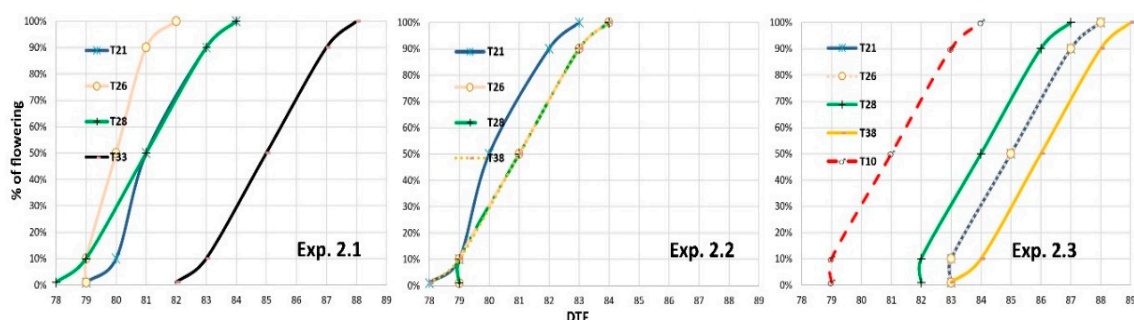
The results from analysis of the flowering pattern of the male parent and female lines indicate that it is necessary to apply differential sowing dates to match the floral synchronization of the parents of some hybrid combinations (Table 1). The greater the difference in male and female sowing dates, the greater the potential investment needed in agronomical management practices (e.g., fertilizer, irrigation and pesticide applications) [17]. Therefore, the synchronization of flowering is paramount in hybrid seed production; therefore, production costs need to be weighed against the potential value of the hybrid in the market place when considering the best hybrid combinations for production and marketing.

This study provides insights on sowing intervals required between the parental lines of the five single cross hybrids, to achieve floral synchronization. T33 was the latest flowering female in Exps. 1.1, 1.2, 1.3 (Figure 2) and Exp. 2.1 (Figure 3), while the other four female lines have similar flowering dates. Consequently, to achieve a good floral match between T33 and T10, it is recommended to sow T33 first and the male five days later, or when the T33 coleoptile begins to emerge. In order to guarantee that in the seed production field there will be pollen of T10 during the entire female flowering period of T33, a second sowing date of T10 is desirable. This being the case, this sowing should occur when the T33 seedlings have completely emerged and before the first leaf is completely unfolded (V1). If T10 and T33 lines were simultaneously sown, anthesis would start before stigmas are exposed, therefore hybrid seed would not be produced or seeds would only be developed at the base of the cob and a larger proportion of round and large seeds would be harvested [14].





**Figure 2.** Days to flowering (DTF) of lines T21, T26, T28, T33 and T38 (females) and T10 (male) in Exps. 1.1, 1.2 and 1.3.



**Figure 3.** Average days to flowering (DTF) of lines T21, T26, T28, T33 and T38 (females) and T10 (male) in Exps. 2.1, 2.2 and 2.3.

In Exp. 2 (Table 7), the effect of plant densities on female flowering days was of low agronomic impact. Previous studies did not obtain differences of more than one day of male and female flowering comparing 62,500 and 82,500 plants/ha [18]. Consequently, based on similar analysis to female line management, the sowing recommendation for lines T21, T26, T28 and T38 as female hybrid parents is to plant at lower densities to maximize profitability of hybrid seed production. Any lack of floral synchronization would reduce seed yield and expose the female parent to be “contaminated” with foreign pollen [14]. Thus, for a female:male row ratio of 4:2, we recommend, for the hybrids described here, sowing four female rows and one of the two male rows at one date and later sowing the second male row thus increasing the probability of the synchronization of flowering. The second male row should be planted when the coleoptiles of the female plants are halfway to being fully emerged.

The sowing interval recommendations of the second sowing date of the T10 male line are made under the assumption that machinery for sowing is available; or in the case of rains after the first sowing, personnel are available to do manual seeding.

T10 is the only one inbred line resistant to TSC in the CIMMYT's tropical maize breeding program and this resistance is transmitted to their hybrids; therefore, it is the common male. Even though the breeder continues searching for earlier flowering females, at present, the ones that best combine are the five evaluated in this study.

In case both parents have to be sown in the same date, day to flowering of female must be accelerated to match the flowering of T10. In this regard, some directions are as follows: (1) soak the female seed in water for 12 to 24 h, before sowing, in order to accelerate germination and consequently flowering by one or two days; (2) start the detasseling of the females and/or trim the surrounding husk leaves of the young ear to accelerate the emergence of stigmas by two or three days [14]; (3) when the female is vigorous as is the case of T21, female and male lines can be sown at different sowing depths, since it is estimated that for every 2 cm of sowing depth there will be a delay in the emergence of one day [19]. If, on the contrary, it is desired to delay the flowering of the male line T10, the burning of

leaves at a juvenile stage will significantly delay male flowering initiation with minimal reductions in seed weight. The costs and practicalities of this approach would need to be assessed carefully [20].

Some female parents have tassels that are physically difficult to eliminate, others break easily or begin to produce pollen before emerging completely. Understanding the male flowering of female parents is important not only for breeding seed production but when considering hybrid seed production costs and efficiencies [14]. To enable manual or mechanical detasseling, it is suggested to select female lines such as T21, with large tassels and a high amount of fiber in the peduncle [21].

### 3.2.2. Female Height of Stigmas and Male Height Tassels

Differences for stigma height were significant ( $p \leq 0.05$ ) between sowing dates, but not between lines nor between plant densities. Lines T21 and T33 were the ones that, on average, exposed their stigmas at a greater distance from the ground and exceeded the other lines by 20 cm (Table 7). All female lines showed a difference greater than or close to one meter between their stigmata and the line T10 tassel (Table 8), which favors an efficient dispersion of pollen to stigmata due to the gravity effect. The T21 line, with tassels at 230 cm distance from the ground, exceeded the mean distance of the other lines by 13 cm, but was nonetheless still easily accessible for manual detasseling.

**Table 8.** Differences in centimeters between height of stigmas of lines T21, T26, T28, T33 and T38 and the tassel of line T10.

Experiment/Plant Density (plants/ha)	T21	T26	T28	T33	T38
Exp. 1.1: 71,111	97	121	118	109	
Exp. 2.3: 82,667	93	100	101		100
Exp. 2.3: 93,333	100	109	103		104
Exp. 2.3: 101,333	101	110	98		107
Mean	98	110	105	109	103

Plant height and that of the upper ear are important in a commercial seed production field, because both traits influence the detasseling and pollination efficiencies [18]. The plant height of T10 and the difference between tassel height and silk height of the five female parents is ideal for hybrid production. However, if a line like T10 is used as a female, it may be difficult to achieve good hybrid production due to the challenges associated with detasseling (increasing cost) and in finding a complementary male plant with suitable tassel height.

### 3.2.3. Effective Seed Yield

The effective seed yield varied across sowing date and, to a lesser extent, across plant density (Tables 6 and 7). Lines T21 and T26 had the highest effective seed yield across experiments. The average effective seed yield of lines T21, T26 and T29 exceeds the yield suggested for hybrid seed production field plots in the Central High Valleys of Mexico [6].

Previous studies reported significant differences ( $p \leq 0.05$ ) between plant densities, i.e., at 62,500 plants/ha it was obtained 3787 kg/ha and 4282 kg/ha at 82,500 but the 1000-seed weight decreased by 2.57% [18]. In a study, under higher plants densities, (90,000, 100,000 and 110,000) a positive trend to increase seed yield as plants densities increase (3084, 3138 and 3313 kg/ha, respectively) was found, but the volumetric weight of the seed was not affected (79.53, 79.17 and 78.55) [22]. Our results, in contrast, did not find significant differences in ESY among plant densities. Considering the costs of production within the ranges of densities studied, the appropriate density for commercial hybrid seed production using the lines described here is 82,667 plants/ha. Some seed markets prefer certain seed sizes [14]. The number of seeds per plant, the 1000-seed weight, and seed grading (size, shape and uniformity) were not evaluated in this study; comparisons between those variables and ESY are recommended to be included in future studies.

#### 4. Conclusions

Different sowing dates expose lines to environmental factors (temperature, mainly) that influence the duration of growth in flowering. It is necessary to evaluate germplasm of relevance in the specific geographies and relevant production periods where line management and commercial seed production of single cross hybrids is desired. In our study, the flowering interval between silking in the female lines and anthesis in the male line was influenced by sowing dates. To ensure the floral synchronization of the parental lines, it is necessary to stagger the sowing of two rows of male, one sown with the female and a second planted when the female reached an appropriate phenological stage (half emerged coleoptile in this case). Plant density did not affect the days of flowering or the effective seed yield. Since the cost of production of parental and hybrid seed increases with plant density, it is more efficient and equally effective to produce seeds of both females and male lines under lower densities. It is recommended to evaluate seed grading to meet the needs of the market. Among the five female lines studied, T21 is more profitable for seed producers. Inbred lines with similar characteristics (highest effective seed yield, large tassel, tassels at 230 cm distance from the ground or less and difference between height of upper stigmas and male tassel more than one meter), are recommended for the seed production of single cross hybrids that are tropically adapted. The graphical representation of flowering patterns of both female and male lines contributes to hybrid selection and facilitates the optimization of second male sowing at the appropriate phenological stage of the female for hybrid production.

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#### Appendix A

**Table A1.** Daily and accumulated Heat Units (°C) from sowing dates to flowering.

DAP	23 Nov. 2017		16 Dec. 2017		29 Nov. 2018		05 Dec. 2018	
	HU day	HU acum.	HU day	HU acum.	HU day	HU acum.	HU day	HU acum.
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

DAP: Days after sowing, HU: Heat Units (°C).

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