PRODUCTIVITY OF THREE MAIZE HYBRIDS UNDER DIFFERENT PROPORTIONS OF MALE STERILE AND FERTILE SEEDS

Margarita Tadeo Robledo, Alejandro Espinosa Calderón, J. Jesús García Zavala, Ricardo Lobato Ortiz, Noel O. Gómez Montiel, Mauro Sierra Macías, Roberto Valdivia-Bernal, Antonio Turrent Fernández and Benjamín Zamudio González

SUMMARY

The objective of this study was to find the yield performance of three hybrids of maize, including Tsiri Puma, in their male sterile and fertile versions, and also to determine the best mixing proportions of male sterile and fertile seeds, at two planting dates. Thus, two experiments were established at the Facultad de Estudios Superiores Cuautitlán *(FESC-UNAM) in the spring-summer 2012 season, and a randomized complete block design with three replications was used. The sources of variation were planting dates, hybrids, male sterile and fertile seed proportions (0-100; 20-80; 40-60, 60-40, 80-20; 100-0) and their interactions. The yield was not significantly different between planting dates, but the comparison of means between genotypes detected that hybrid Tsiri Puma 1 (8989kg·ha^{-1}) had a statistically bigger yield than H-47 AE (8190kg·ha^{-1}). The comparison of means (Tukey, 0.05) between the proportions of male sterile and fertile seed, considering the average of the three genotypes and the two planting dates, defined two groups of significance, where the two best yielding were those of 100% male sterile:0% fertile (9441kg·ha^{-1}) and 80% male sterile:20% fertile (9390kg·ha^{-1}) seed proportions. Since the version with only male sterile seed is not a practical feasible option, an appropriate choice is that of 80:20, which optimizes the production schema by needing less labor and requiring only a 20% fraction of fertile seed. The proportion of 100% fertile seed had the lowest yield (8116kg·ha^{-1}) among all seed proportions tested.*

Introduction

Seed production of maize hybrids requires the physical removal of the tassel on female plants in time and in an appropriate way, so as to obtain the planned crossings and thereby to attain quality and genetic identity in the corresponding hybrid (Espinosa *et al*., 2003; Beck and Torres, 2005). This process involves high costs because of the use of too much labor. The manual or mechanical removal of tassels is expensive, requiring from 24 to 50 wages per ha, depending on the uniformity of the female parent, presence of tillers and ease to remove the tassel (Jugenheimer, 1990; Tadeo *et al*., 2003; Martínez *et al*., 2005). As an alternative to this high investment, it could be possible to use some types and sources of gene/cytoplasmic male sterility in maize, since female parents could be used as male-sterile lines, whereupon the detasseling process is reduced, which can also facilitate this activity, allowing a lower price for the hybrid seed (Tadeo *et al*., 2003, 2010, 2013; Martínez *et al.,* 2005).

Male sterility in maize was no longer used during the decade of 1970, because of the susceptibility to leaf blight disease caused by the fungus *Helminthosporium maydis* race T, which caused an epiphyte largely distributed in the corn belt of the USA (Fleming *et al*., 1960; Partas, 1997; Stamp *et al*., 2000; Simmons *et al*., 2001). New sources of male sterility have been used again in various maize breeding programs of different seed companies, the most important of which usually obtain seeds with male sterile system (Liu *et al*., 2002; Weingartner *et al*., 2002; 2004). With the use of new sources of male sterility and with the purpose not to depend on only a single source of sterility, in order to avoid problems of T race, since 1992 the *Facultad de Estudios Superiores Cuautitlán* (FESC-UNAM) in México has worked to incorporate the male sterility character into the basic inbred lines of the maize breeding program developed at UNAM (Tadeo *et al*., 2003, 2010).

Maize inbred lines used to produce hybrids that are increased by using male sterility

KEYWORDS / Detasseling / Hybrids / Male Sterility / Seed Production /
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Margarita Tadeo Robledo. Doctor in Genetics, Colegio de Postgraduados (COLPOS), Mexico. Professor, Universidad Nacional Autónoma de México. e-mail: tadeorobledo@yahoo.com

Alejandro Espinosa Calderón (Correspondence author). Doctor in Genetics, (COLPOS), Mexico. Researcher, Instituto Nacional de Investigaciones Forestales, Agrícolas y Peciuarias (INIFAP), Mexico. Address: Campo Experimental Valle de México. INIFAP. Km 13.5 Carretera Los Reyes-Texcoco. C.P. 56250, Coatlinchan, Texcoco, Estado de México, México. e-mail: espinoale@yahoo.com.mx

J. Jesús García Zavala. Ph.D. in Crop Science, North Carolina State University, USA. Professor, Colpos, Mexico. e-mail: zavala@colpos.mx

Ricardo Lobato Ortiz. Ph.D. in Plant Breeding and Genetics, Cornell University, USA. Professor, Colpos, Mexico. e-mail: rlobato@colpos.mx

Noel O. Gómez Montiel. Doctor in Genetics, COLPOS, Mexico. Researcher, INIFAP, Mexico. e-mail: noelorlando19@hotmail.com

Mauro Sierra Macías. Doctor in Genetics, Universidad de Colima, Mexico. Researcher, INIFAP, Mexico. e-mail: mauro_s55@hotmail.com

Roberto Valdivia-Bernal. Professor, Universidad Autónoma de Nayarit, Mexico. e-mail: beto49_2000@yahoo.com.mx

Antonio Turrent Fernández. Ph.D. in Soil Fertility, Iowa State University, USA. Researcher, Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias (INIFAP), Mexico. e-mail: aturrent37@yahoo. com.mx

Benjamin Zamudio González. Doctor in Edaphology, COLPOS, Mexico. Re-searcher, INIFAP, Mexico. e-mail: bzamudiog@yahoo.com.mx

PRODUCTIVIDAD DE TRES HÍBRIDOS DE MAÍZ BAJO DIFERENTES PROPORCIONES DE SEMILLA ANDROESTÉRIL Y FÉRTIL

Margarita Tadeo Robledo, Alejandro Espinosa Calderón, J. Jesús García Zavala, Ricardo Lobato Ortiz, Noel O. Gómez Montiel, Mauro Sierra Macías, Roberto Valdivia-Bernal Antonio Turrent Fernández y Benjamin Zamudio González

RESUMEN

En este estudio se planteó como objetivo definir la capacidad productiva de tres híbridos de maíz, entre ellos el Tsiri Puma, en sus versiones androestéril y fértil, y determinar la mejor proporción de mezcla de semilla androestéril y fértil, en dos fechas de siembra. Para ello se establecieron dos experimentos en el ciclo primavera-verano 2012 en la Facultad de Estudios Superiores Cuautitlán (FESC-UNAM), empleándose un diseño experimental de bloques completos al azar con tres repeticiones. Las fuentes de variación fueron fechas de siembra, híbridos, proporciones de semilla androestéril y fértil (0-100; 20-80; 40-60; 60-40; 80-20; 100-0) y sus interacciones. El rendimiento no tuvo diferencias significativas entre fechas de siembra, pero la comparación de medias entre genotipos detectó que el híbrido Tsiri Puma 1 (8989kg·ha^{-1}) fue estadísticamente superior en rendimiento a H-47 AE (8190kg·ha^{-1}). La comparación de medias (Tukey 0,05) entre las proporciones de semilla androestéril y fértil, considerando la media de los tres genotipos evaluados y las dos fechas de siembra, definió dos grupos de significancia, donde los dos mejores rendimientos fueron 100% androestéril + 0% fértil (9441kg·ha^{-1}) y 80% androestéril + 20% fértil (9390kg·ha^{-1}). Debido a que la versión con solo semilla androestéril no es prácticamente factible, una opción adecuada es la proporción 80:20, que optimiza el esquema por usar menor cantidad de jornales, pues requiere una fracción de 20% de semilla fértil. La proporción 100% semilla fértil tuvo el menor rendimiento (8116kg·ha^{-1}).

PRODUTIVIDADE DE TRÊS HÍBRIDOS DE MILHO SOB DIFERENTES PROPORÇÕES DE SEMENTE ANDROESTÉRIL E FÉRTIL

Margarita Tadeo Robledo, Alejandro Espinosa Calderón, J. Jesús García Zavala, Ricardo Lobato Ortiz, Noel O. Gómez Montiel, Mauro Sierra Macías, Roberto Valdivia-Bernal Antonio Turrent Fernández e Benjamin Zamudio González

RESUMO

O objetivo de este estudo foi definir a capacidade produtiva de três híbridos de milho, entre eles o Tsiri Puma, em suas versões androestéril e fértil, e determinar a melhor proporção de mistura de semente androestéril e fértil, em duas datas de plantação. Para isto se estabeleceram dois experimentos no ciclo primavera-verão 2012 na Faculdade de Estudos Superiores Cuautitlán (FESC-UNAM), empregando-se um desenho experimental de blocos completos aleatórios com três repetições. As fontes de variação foram datas de plantação, híbridos, proporções de semente androestéril e fértil (0-100; 20-80; 40-60; 60-40; 80-20; 100-0) e suas interações. O rendimento não teve diferenças significativas entre datas de plantação, mas a comparação de médias entre genótipos detectou que o híbrido Tsiri Puma 1 (8989kg·ha^{-1}) foi estatisticamente superior em rendimento a H-47 AE (8190kg·ha^{-1}). A comparação de médias (Tukey 0,05) entre as proporções de semente androestéril e fértil, considerando a média dos três genótipos avaliados e as duas datas de plantação, definiu dois grupos de significância, onde os dois melhores rendimentos foram 100% androestéril + 0% fértil (9441kg·ha^{-1}) e 80% androestéril + 20% fértil (9390kg·ha^{-1}). Devido a que a versão com apenas semente androestéril não é factível na prática, uma opção adequada é a proporção 80:20, que otimiza o esquema por que utiliza menor quantidade de jornadas, já que requer de uma fração de 20% de semente fértil. A proporção 100% semente fértil teve o menor rendimento (8116kg·ha^{-1}).

maintain this feature when commercial seed is produced, so there are two ways to take advantage of male sterility in order to insure that when the seed is delivered to the farmers for commercial planting, the fields of the farmers have fertile plants and/or a combination of fertile plants and male-sterile ones in order to ensure pollination, fertilization and, consequently, grain production. One of the ways to resolve this situation is to use a male parent having a restorative capacity that pollinates to a single-cross or a male sterile inbred line (depending on whether a single cross or a three-way hybrid is going to be increased); this male parent suppresses the male sterility, so the crosses express fertility in the next generation, and there will be up to 100% of fertile plants in the fields of the farmers. The clearest case of this type occurs in sorghum crop (*Sorghum* spp.), where all the hybrid seed is increased in this way (Simeonov, 1995; Allen *et al.*, 2007). The other alternative is to use a fraction of the seed of the hybrid that maintains its male sterile characteristic. This hybrid does not produce pollen. This seed is mixed with a proportion of seed of the same hybrid, but this one is obtained from the cross of fertile progenitors, which produce pollen. In this way, the farmers' fields will contain both male sterile and fertile plants. These fertile plants will pollinate all the plants of the field to ensure grain production (Airy *et al.*, 1987; Espinosa *et al.*, 2009; Tadeo *et al.*, 2010).

Since 1992, FESC-UNAM and the National Institute for Research of Forestry, Agriculture and Animal Science (INIFAP) of Mexico work together with new sources of male sterility. This character has been incorporated to elite inbred lines of both institutions, so maize hybrids now have a high yield performance and it is easy to increase their hybrid seed by the farmers (Tadeo *et al.*, 1997, 2001). Product of these joint investigations is the maize hybrid Tsiri Puma, which was registered in the National Catalogue of Plant Varieties (CNVV). This is the first hybrid with a scheme of male sterility that is released by the FESC-UNAM.

The objective of this research was to know the yield performance of three hybrids of maize, including Tsiri Puma, in their male sterile and fertile versions and, also, to determine the best mixing proportions of male sterile (MS) and fertile (F) seeds, in two planting dates. It is aimed to determine the seed yield of the hybrids by combining six different proportions of mixtures of male sterile and fertile seeds (00-100; 20-80; 40-60; 60-40; 80-20; and 100-00).

Materials and Methods

The study was carried out during the spring-summer 2012 season, at the Almaraz Ranch of the *Facultad de Estudios Superiores Cuautitlán*, field research station 4, UNAM, in plot number 7, municipality of Cuautitlan Izcalli, State of Mexico, at an altitude of 2274m and soil of clay loam texture. Two different planting date experiments were established: one was planted on April 21, 2012 and the other one on June 1, 2012.

Three maize three-way hybrids were planted: Tsiri Puma 1, Tsiri Puma 2 and H-47 AE. Each hybrid was planted using different proportions (mixtures) of seed of their isogenic male sterile and fertile versions. Tsiri Puma was commercially released with the same name; this hybrid and the H-47 were previously registered in The International Union for the Protection of New Varieties of Plants (UPOV). All the hybrid seed was obtained in the of spring-summer season of 2011. Male sterile (100%) and fertile (100%) seeds were produced from single male sterile crosses and fertile ones (the tassels of the female parent were physically removed). Treatments of male sterile and fertile seed proportions were based on previous studies (Espinosa *et al.*, 2009; Tadeo *et al.*, 2010). The hybrid seed was mixed in different proportions, as presented in Table I.

In order to avoid problems with the lack of pollen in the two hybrids in the combination 0% fertile and 100% male sterile, fertile material was planted around the experiments, because it was only intended to test the yield performance of the proportions of fertile and male-sterile plants. The 100% male sterile hybrid would not be commercially used, since fertilization would not take place and there would be no yield.

The experimental plot was a row 5m long, with 0.8m between rows. A randomized complete block design with three replications was used. The population density used was 65,000 plants/ha. A factorial statistical analysis was performed, where the variation factors were: dates of planting (2), hybrids (3), proportions of male sterile and fertile seed (6), and interactions among the variation factors: hybrids, environments and proportions of male sterile and fertile seed. A fertilizer treatment of 80-40-00 was applied at the moment of sowing using ammonium nitrate and triple calcium sulfate. This fertilizer treatment is based on previous studies of the soil where the study was established (Espinosa *et al.*, 2012). For weed control, Gesaprim (atrazine) 2kg/ha, 2-4-d amine 2L/ha and nicosulfuron 1L/ha were applied. The harvest was carried out on December 10, 2012.

Data of the following traits were analyzed: yield, male pollen-shed, plant and ear height, healthy ears (complete, >80% healthy, undamaged by disease), unhealthy ears (>20% rotten or with disease damage), volumetric weight, 200 seeds weight, ear length, rows per ear, seed per row, ear diameter, and seeds per ear. These last seven traits were measured in a sample of five ears per plot. Mean comparison of the traits was made using the Tukey test at the 0.05 probability. The program SAS version 9.0 (SAS, 2002) was used for all the statistical analyses.

Results and Discussion

Mean squares and their statistical significance for each trait analyzed are presented in Table II. For grain yield, the coefficient of variation was 15.7% and the average yield for all entries over the two planting dates was 8,692kg·ha^{-1}. Among proportions of male sterile and fertile seed there were highly significant differences for grain yield but not so between planting dates, among hybrids, and for any interactions (Table II).

Between planting dates there were highly significant differences at the 0.01 level for days to anthesis, plant height, weight of 200 seeds and ear length. On the other hand, the differences between planting dates and among hybrids were significant at the 0.01 level for silking, ear height and weight test. In the other sources of variation, including interactions, no significant differences were found (Table II).

Between planting dates there was significance at the 0.05 level for seeds per row and seeds per ear, while among hybrids there was significance only for seeds per ear. In the other factors of variation, including interactions, no statistical differences (Table II) were detected for these ear traits.

Means comparison for planting dates, considering the average of the three hybrids evaluated under the six different proportions of male sterile and fertile seed, detected no significance for seed yield between the two planting dates (Table III). Seed production values in both dates were very similar; however, the number of days to pollen-sheding and silking in the second period (01 June 2012) were statistically higher with respect to the first planting date (May 21, 2012), probably due to a period of overwhelming at the beginning for the first sowing date. On the other hand, means comparison for traits plant height, ear height, planting dates, volumetric weight, weight of 200 seeds, ear length, seeds per row and seeds per ear indicated that the values obtained on the date May 21, 2012 were statistically superior to the values of the second date, 01 June 2012 (Table III).

With regard to the mean comparison of the three-way hybrids of maize (Table IV), two groups of significance were identified for seed yield; Tsiri Puma 1 and Tsiri Puma 2 were the best hybrids in the first group, while in the second

TABLE I
PROPORTIONS OF MALE STERILE AND FERTILE SEED OF THREE-WAYMAIZE HYBRIDS, TSIRI PUMA 1, TSISRI PUMA 2 AND H-47 AE THAT WERE USED FOR THE EVALUATION OF THEIR YIELD PERFORMANCE

	Genotype	% of seed			Genotype	% of seed	
		MS*	Fertile			Fertile	MS
1	TSIRI PUMA 1	100	0	1	TSIRI PUMA 2	100	0
2	TSIRI PUMA 1	80	20	2	TSIRI PUMA 2	80	20
3	TSIRI PUMA 1	60	40	3	TSIRI PUMA 2	60	40
4	TSIRI PUMA 1	40	60	4	TSIRI PUMA 2	40	60
5	TSIRI PUMA 1	20	80	5	TSIRI PUMA 2	20	80
6	TSIRI PUMA 1	0	100	6	TSIRI PUMA 2	0	100
1	H-47 AE	100	0				
2	H-47 AE	80	20				
3	H-47 AE	60	40				
4	H-47 AE	40	60				
5	H-47 AE	20	80				
6	H-47 AE	0	100				

*MS: male sterile.

TABLE II
MEAN SQUARES AND STATISTICAL SIGNIFICANCE FOR THE TRAITS EVALUATED IN THREE HYBRIDS UNDER SIX PROPORTIONS OF MALE STERILE AND FERTILE SEED IN TWO PLANTING DATES

Trait	PD	H	MS×F	PD×H	PD×MSF	H×MSF	FS×H×MSF	CV (%)	Means
SY	3223443	6872182*	8312351.9**	6199648.	800621.8	5902925.0	674150.6	15.7	8692
PS	551.2**	1.75	2.53	2.28	1.748	2.716	0.809	1.4	78
SD	396.7**	39.4**	7.05	4.00	4.77	2.92	1.75	2.2	79
PH	26320.3**	354.9	456.4	1001.3	103.8	233.2	94.9	6.4	232
EH	17633.3**	5865.4**	97.1	144.7	169.2	159.9	122.6	9.0	122
VW	20694.6**	8019.4**	274.3	12.0	144.6	351.6	322.0	2.0	778
W200S	3050.7**	74.7	21.5	344.0**	27.5	41.3	37.5	7.4	72
EL	19.5**	1.81	0.41	2.92	0.72	0.54	1.12	7.1	14.8
GR	24.0*	6.02	5.6	6.6	3.9	6.3	7.3	5.0	31
SE	13134.0*	5026.8*	590.7	1327.8	1162.2	3597.5	3847.1	8.5	497

*, ** Significant and highly significant values to 0.05 and 0.01 of probability; PD: planting dates, H: hybrids, MSF: male sterile and fertile seed proportions, PD×H: planting dates × hybrids interactions, PD×MSF: planting dates × male sterile and fertile seed proportions interactions, H×MSF: hybrids × male sterile and fertile seed proportions interactions, PD×H×MSF: planting dates × hybrids × male sterile and fertile seed proportions interactions, CV: coefficient of variation, SY: seed yield, PS: pollen-shed, SD: silking date, PH: plant height, EH: ear height, VW: volumetric weight, W200S: weight of 200 seeds, EL: ear length, SR: seeds per row, SE: seeds per ear.

TABLE III
MEANS COMPARISON BETWEEN PLANTING DATES FOR DIFFERENT TRAITS EVALUATED IN THREE MAIZE HYBRIDS CONSIDERING SIX PROPORTIONS OF MALE STERILE AND FERTILE SEED

Variable	2 (06/01/2012) 2012)	1 (05/01/2012)	DSH (0.05)
SY (kg ha^{-1})	8865 a	8519 a	523
PS (days)	80 a	76 b	0.4
SD (days)	81 a	77 b	0.6
PH (cm)	217 b	248 a	6
EH (cm)	110 b	135 a	4
WT (kg hl^{-1})	763 b	791 a	6
W200S (g)	67.1 b	77.7 a	2.0
EL (cm)	14.3 b	15.2 a	0.4
ER	31 b	32 a	0.6
SR	486 b	508 a	16

SY: seed yield, PS: pollen-shed, SD: silking date, PH: plant height, EH: ear height, WT: weight test, W200S: weight of 200 seeds, EL: ear length, ER: Ear rows, SR: seeds per row, DSH: Tukeys's honestly significant difference.

group the hybrid AE H-47 was the best material. Seed yield of Tsiri Puma 1 represented 109.7% of that one of AE H-47, and this is important because AE H-47 has shown good yields in previous studies and could be considered as a check in yield trials (Espinosa *et al.*, 2012; Tadeo *et al.*, 2013).

In this trial, Tsiri Puma 1 had the highest seed yield. This confirms its high productive capacity and the good results observed in other experimental works (Tadeo *et al.*, 2014a. b). Tsiri Puma's yield potential allowed us to register it recently in the National Catalogue of Plant Varieties (CNVV) of México. The similar yield performance between Tsiri Puma 1 and Tsiri Puma 2 could be because in both hybrids the male parent involved in their formation was the same.

Hybrids Tsiri Puma 1 and Tsiri Puma 2 were ready earlier than H-47 AE, which is important because H-47 AE is considered as a variety of intermediate cycle (Espinosa *et al.*, 2012), and hybrids Tsiri Puma 1 and Tsiri Puma 2 have a similar cycle, with the advantages that this fact represents. Means comparison among hybrids, considering the average of planting dates and male sterile and fertile seed proportions, for pollen-shed, plant height, weight test, weight of 200 seeds ear length, seeds per row and seeds per ear, indicated that the three hybrids had similar values for those traits (Table IV).

Hybrids Tsiri Puma 1 and Tsiri Puma 2 showed higher values of volumetric weight with respect to H-47 AE, which could be a positive element for seed quality when used commercially. In the combined analysis of variance, male sterile and fertile seed proportions showed statistical significance for seed yield, indicating that their performance was different; as it has been observed in other studies, the yields obtained in this study were excellent based on the sowing date (Espinosa *et al.*, 2009; Tadeo *et al.*, 2010). Means comparison for male sterile and fertile seed proportions presented two groups of significance (Table IV). In the first one, which had the highest numerical yield, there were five out of the six treatments with proportions and combinations of male sterile and fertile seed. The 100% male sterile and 0% fertile seed proportion had the highest numerical yield, but this combination would not be viable for practical commercial seed production, since a male pollinator is necessary in order to pollinate the female plants.

TABLE IV
MEANS COMPARISON OF THREE HYBRIDS FOR DIFFERENT TRAITS CONSIDERING TWO PLANTING DATES AND SIX PROPORTIONS OF MALE STERILE AND FERTILE SEED

Variable	1 (TSIRI PUMA 1)	2 (TSIRI PUMA 2)	3 (H-47 AE)	DSH (0.05)
SY (kg ha^{-1})	8989 a	8897 ab	8190 b	769
PS (days)	78 a	78 a	78 a	0.6
SD (days)	79 b	79 b	80 a	1
PH (cm)	235 a	229 a	233 a	8
EH (cm)	122 b	110 c	135 a	6
VW (kg hl^{-1})	785 a	787 a	760 b	9
W200S (g)	73.0 a	70.8 a	73.4 a	3.0
EL (cm)	15.0 a	14.8 a	14.5 a	0.6
ER	32 a	31 a	31 a	0.9
SE	505 a	483 a	501 a	24

SY: seed yield; PS: pollen-shed; SD: silking date; PH: plant height; EH: ear height; VW: volumetric weight; W200S: weight of 200 seeds; EL: ear length; SR: seed per row; SE: seeds per ear.

The second group had the lowest yield and corresponds to the 60% male sterile 40% fertile seed proportion, a result that differs from those obtained in previous works, where the lower production was obtained in the treatment of 100% fertile seed (Espinosa *et al.*, 2009; Tadeo *et al.*, 2010).

Five out of the six seed proportions evaluated had similar yields (Table V), and this confirms that in the case of the hybrids evaluated in this study, as in other materials, the combination of male sterile and fertile seed could be done in different proportions with some assurance that the results of productivity in commercial production fields are acceptable (Espinosa *et al.*, 2009; Tadeo *et al.*, 2010). This is in accord with results obtained in previous work with other materials, where the evaluation of seed combinations indicated that the proportions of 33% and 66% of male sterile seed and 67% and 34% of fertile seed, respectively, or proportions of 90% male sterile and 10% of fertile seed and indicates that yield is similar when using the male sterility in different proportions, and in other ~~times~~ studies??? it could be increased. This type of proportions is used by the most important seed companies in the world. Moreover, the combination of male sterile and fertile seed could maintain the genetic quality of the seed hybrid, as it was noted in previous works (Weingartner *et al.*, 2004; Martínez *et al.,* 2005; Espinosa *et al.*, 2009).

Conclusions

Maize hybrids Tsiri Puma 1 and Tsiri Puma 2 had statistically a similar average yield under the six different proportions of fertile and male-sterile seed evaluated in two planting dates.

There was no difference in seed yield among the three maize hybrids, as there was no effect of the two planting dates in which they were evaluated, under six proportions of male sterile and fertile seed.

In the yield trial of six proportions of male sterile and fertile seed, five proportions showed similar yield performance. By considering the average of the three hybrids and the two planting dates, among the different proportions of seed the best mix of seed in practical terms would be the one of 80% male sterile and 20% fertile seed, because it is more convenient to have a highest proportion of plants with male sterile that will not need detasseling during seed production.

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TABLE V
MEANS COMPARISON AMONG PROPORTIONS OF MALE STERILE AND FERTILE SEED FOR DIFFERENT TRAITS CONSIDERING THREE HYBRIDS AND TWO PLANTING DATES

	Seed proportion MS-Fertile		SY ($ton{\cdot}ha^{-1}$)	PS	SD	PH (cm)	EH (cm)	VW (Kg/Ha)	W200S (g)	EL (cm)	SR	SE
1	100	0	9441 a	78 a	79 a	226 a	120 a	775 a	71 a	14.9 a	31 a	491 a
2	80	20	9390 a	78 a	79 a	228 a	124 a	782 a	72 a	14.9 a	32 a	506 a
3	60	40	7850 b	78 a	79 a	233 a	119 a	772 a	72 a	14.5 a	31 a	497 a
4	40	60	8346 ab	78 a	79 a	233 a	125 a	779 a	73 a	14.9 a	32 a	490 a
5	20	80	9011 ab	78 a	79 a	234 a	124 a	782 a	74 a	14.7 a	31 a	496 a
6	0	100	8116 ab	77 a	80 a	240 a	123 a	777 a	72 a	14.8	31 a	498 a
	DSH (0.05)		1331	1.1	1.7	15	11	16	5	1.0	1.5	41

MS: male sterile, SY: seed yield; PS: pollen-shed; SD: silking date; PH: plant height; EH: ear height; VW: volumetric weight; W200S: weight of 200 seeds; EL: ear length; SR: seeds per row; SE: seeds per ear.

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