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Development of single-cross maize hybrids with different parent selection strategies

Desarrollo de híbridos de maíz de cruza simple con diferentes estrategias de selección de progenitores

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ABSTRACT

This study aimed to assess the efficiency of molecular markers compared to two genotypic methods in generating high-yield hybrids and their associated components. 70 hybrid maize were evaluated using a completely randomized design with three replications across two locations in Tamaulipas state, Mexico. A combined analysis of variance and orthogonal contrasts using hybrid means was conducted. Significant differences were observed in all agronomic and yield variables among genotypes and environments. Notably, genotype × environment interaction was observed in variables such as number of rows, ear diameter, and days to male and female flowering. Hybrids yielding over 9.0 t ha^{-1} included P3097, P3092, 30F53, and LEARB9 × UAY113, the latter developed using the molecular marker approach. Orthogonal contrasts revealed differences between the molecular method and *per se* evaluation, as well as the crossbreeding test, in terms of yield, ear diameter, plant, and ear height. Additionally, significance was noted between the *per se* method and crossbreeding in plant and ear height. Microsatellite analysis provided valuable insights to complement traditional hybridization programs.

KEY WORDS: *Zea mays* L., Crossing, Hybridization, Molecular markers, Breeding methods, Genotypic methods.

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RESUMEN

El objetivo del trabajo fue evaluar la eficiencia de los marcadores moleculares en comparación a dos métodos genotécnicos para formar híbridos con altos niveles de rendimientos y sus componentes. Se evaluaron 70 híbridos de maíz, bajo un diseño completamente al azar con tres repeticiones en dos localidades de Tamaulipas. Se realizó un análisis de varianza combinado y contrastes ortogonales con las medias de los híbridos. Existió diferencias en todas las variables agronómicas y de rendimiento en genotipos y ambientes, sin embargo, en interacción genotipo × ambiente fue en número de hileras, diámetro de mazorca, días a floración masculina y femenina. Los híbridos con rendimiento superior a 9.0 t ha^{-1} fueron P3097, P3092, 30F53 y LEARB9 × UAY113; este último obtenido mediante la estrategia de marcadores moleculares. Los contrastes ortogonales mostraron diferencias para el método molecular vs. evaluación *per se* y la prueba de mestizos en rendimiento, diámetro de mazorca, altura de planta y de mazorca; así mismo, se detectó significancia en el método *per se* vs. mestizos en altura de planta y de mazorca. Los microsatélites revelaron información útil para ser utilizados como herramientas auxiliares en los programas tradicionales por hibridación.

PALABRAS CLAVE: *Zea mays* L., Cruzamientos, Hibridación, Marcadores moleculares, Métodos Genotécnicos.

Introduction

In genetic improvement, understanding the genetic components of the lines intended for use as parents in hybrid and commercial variety development is crucial. Within plant breeding, genotypic methods have been developed for the evaluation and selection of promising lines for hybrid formation. Among the most common techniques are evaluating lines individually to identify those with high yield potential, favorable agronomic traits, and strong combining ability (Buenrostro-Robles *et al.,* 2017). Additionally, the general combining ability test involves creating and assessing crossbreeds to determine the additive genetic effects of the lines (Sánchez-Ramírez *et al.,* 2020).

Genotypic methods play a pivotal role in hybrid development programs by providing insights to identify and select materials likely to yield progenies with significant levels of heterosis in yield and its components (Vélez-Torres *et al.,* 2018). However, this process demands significant investment in terms of cost, time, and breeder effort for line evaluation and selection.

Furthermore, thanks to advancements in molecular biology, identification and characterization methods have emerged based on molecular markers, which often overcome

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some limitations of traditional techniques (Azofeita-Delgado, 2006). Molecular markers, being neutral, can be utilized from the earliest phenological stages of seedlings, exhibit polymorphism, facilitate accurate genotype identification, and are devoid of epistatic effects, thereby streamlining the process and reducing the time and effort typically required for directed crosses of progenitors (Miklas *et al.,* 2006). Given this background, the use of microsatellite-type molecular markers or SSRs (simple sequence repeats) is distinguished by their high polymorphism levels, genome-wide distribution, Mendelian inheritance, and codominance (Ni *et al.,* 2002). Consequently, SSRs could serve as an alternative adjunct to traditional breeding methods, offering insights into the genetic constitution of lines that prove valuable in selecting parents for hybrid generation based on their genetic divergence. This facilitates the pairing of lines for crosses according to their genetic distances.

In maize (*Zea mays* L.) breeding programs, several traditional methods have been evaluated with differing degrees of success in hybrid generation (Acevedo-Cortés *et al.,* 2020; Ramírez-Díaz *et al.,* 2019; Rodríguez-Pérez *et al.,* 2020). Additionally, some researchers have explored the use of molecular markers alongside traditional methods to predict the heterotic performance of parent pairs in hybrid formation (Beyene *et al.,* 2019; Crossa *et al.,* 2017; Lariépe *et al.,* 2017; Marcón *et al.,* 2019; Mwangangi *et al.,* 2019; Nyaga *et al.,* 2020). These investigations have demonstrated the efficacy of molecular markers in providing accurate predictions regarding performance parameters.

Numerous studies have assessed the accuracy of both traditional and molecular methods for hybrid generation; however, research institutions dedicated to genetic improvement must consider the economic, temporal, and labor costs associated with their implementation. Despite global advancements in this domain, the use of molecular markers as adjunct tools in genetic improvement programs, particularly for maize hybrid generation, remains understudied in Mexico. Hence, this study aims to compare the efficiency of microsatellite-type molecular markers or SSRs with two traditional genetic methods for maize hybrid generation in northern Tamaulipas. We hypothesize that utilizing SSRs will expedite the production of high-yield hybrids.

Material and Methods

Plant material

For the production of single hybrids, 37 yellow maize lines (S_3 and S_5) were utilized as parental candidates. Among these lines, 27 were sourced from the Campo Experimental Rio Bravo (CERIB), Tamaulipas, under the support of the Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias (INIFAP), designated as LEARB, while the remaining 10 lines originated from the Universidad Autónoma Agraria Antonio Narro (UAAAN), denoted as UAY101, UAY103, UAY104, UAY105, UAY106, UAY108, UAY110, UAY111, UAY113, and UAY114. The hybridization process involved three genotypic methodologies: firstly, leveraging the genetic divergence among the lines estimated using molecular information from SSR markers; secondly, assessing lines individually using the *per se* method; and thirdly, selecting lines based on their general combining

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ability through the formation and evaluation of crosses. The specific techniques for each method are described below.

Formation of hybrids with molecular information

Genomic DNA extraction

In the spring-summer of 2017, DNA extraction was conducted using 100 mg of mesocotyl and coleoptile tissue from three individual eight-day-old seedlings per line. This was achieved using a commercial DNA extraction kit (ChargeSwitch™ gDNA Plant Kit®) with a KingFisher Flex extraction robot (Thermo Scientific®, Waltham, Maryland, USA), following the guidelines of the manufacturer. The concentration and quality of the extracted DNA were assessed via absorbance testing at 260/280 nm using an ultra-low volume spectrophotometer (NanoDrop™ 2000c, Thermo Scientific®, Wilmington, USA).

Genotyping of lines with microsatellites

For each of the 37 lines, three individuals were analyzed using 22 *loci* of simple repeated DNA sequences (Table 1). The microsatellites utilized were sourced from the Maize Genetics and Genomics Database (http://www.maizegdb.org/ssr.php).

Polymerase chain reaction (PCR)

PCR amplification involved an initial denaturation of 4 minutes at 95°C, followed by 24 cycles comprising 1 minute at 95°C (denaturation), 2 minutes at 55°C (alignment), and 2 minutes at 95°C (extension), with a final extension of 1 hour at 72°C. Each reaction contained 2 μL of 10X PCR Buffer (500 mM KCl, 100 mM Tris-HCl, pH 9.0 at 25°C), 0.4 μL of 10 mM dNTPs (2.5 mM each dNTP), 1.2 μL of 25 mM $MgCl_2$, 0.2 μL of Taq DNA polymerase (1 total unit), 2.5 μL of template DNA (10 ng μL^{-1}), 2.0 μL of 4 pM of each primer pair (1 μL of each forward and reverse), and 11.7 μL of distilled water, in duplicate. The PCR was performed using a thermal cycler (Gene AMP PCR® System 9700, Singapore).

Electrophoresis and fragment analysis

PCR products were assessed through vertical electrophoresis (MG33-1063, C.B.S. Scientific® Del Mar California, USA). 8% acrylamide gels (CIMMYT, 2006) were utilized, with the separation of fragments of lower molecular weight (75-278 bp) conducted for 180 minutes at 250 V, and for fragments with higher molecular weight (105-376 bp), the separation was extended to 240 minutes at 250 V. Gel development was performed using $AgNO_3$ (Sigma®, USA) following the methodology of CIMMYT (2006). Subsequently, gels were documented using a MiniBis Pro 16 mm transilluminator (Bio-Imaging Systems®, Jerusalem, Israel).

Table 1. Microsatellite and oligonucleotide *loci* used for microsatellite studies in maize lines.

Locus	BIN	Product size (bp)	Forward // Reverse oligonucleotides
phi127	2.07	105-126	NED-ATATATGCATTGCCTGGAACTGGAAGGA//AATTCAAACACACGCCTCCCGAGTGTGTGT
phi051	7.06	136-154	6-FAM-GCGAAAGCGAACGACAACAATCTT//ACATCGTCAGCAGATTATATTGCAGACCA
phi115	8.03	292-312	HEX-GCTCCGTGTTTCGCGCCTGAA//ACCATCACCTGAATCCATCACA
phi033	9.02	224-270	6-FAM-ATCGAAATGCAGGCGCGATGGTTTTTTCTC//ATCGAGATGTTCTACGCCCTGAAGT
phi072	4.01	134-163	6-FAM-GTGCATGATTAATTATTTCTCCAGCCTT//GACAGCGCGCGCAAATGGATTGAACT
phi093	4.08	272-296	NED-GTGCGTCAGCTTCATCGCGCTACAAG//CCATGCATGCATGCTTGCAATACAATGGATACA
phi024	5.00	354-376	HEX-CTCCGCTTCCACTGTTCCA//TGTCCGCTGCTGCTCTTCTACCCA
phi085	5.06	233-266	6-FAM-AGCAGAACGGCAAGGGCTACT//TTTGGCACACACCACCACGACGA
phi121	8.04	93-105	6-FAM-AGGAAAATGGAGCCGGTGAACCA//TTGGTCTGGACCAAGCACACATACACAC
phi056	1.01	231-278	NED-ACTTGCTTGCCTGCCTGCCGTTAC//CGCACACCACCACTTCCCAGAA
phi064	1.11	75-121	HEX-CGAATTGAAATAGCTGCGAGAACCT//ACAATGAACGGTGGTGGTTATCAACACACGC
phi96100	2.00-2.01	218-300	6-FAM-AGGAGGACCCCAACTCCTG//TTGCACGAGGAGCCATCGTAT
phi101249	?	114-161	NED-TTCCTCCTCCTCCACTGCCTCCTC//AAGAACAGCGAAGCAGAGAAGAGG
phi029	3.04	139-176	NED-TCTTTTTCTTCCTCCACAAGCAGCGAA///TTTCCAGTTGCCACCGACGAAGAAGAACTT
phi073	3.05	186-203	HEX-GTGCGCGAGAGGCTTGACCAA//AAGGGTTGAGGGGGCGAGGAA
phi96342	10.XX	223-256	6-FAM-GTAATCCCACGTCCTCCTATCAGCC//TCCAACTTGAACGAACTCCTC
phi427913	1.XX	117-207	NED-CAAAAGCTAGTCGGGGGGTCA//ATTGTTCGATGATGACACACACTACGC
phi402893	2.00	205-243	HEX-GCCAAGCTCAGGGTCAAG//CACGAGCGTTATTCGCTGCTGT
phi308090	4.01-4.04	190-226	6-FAM-CAGTCTGCCACGAAGCAA//CTGTCGGTTTTTCGGTCGGTCTTCTTCTT
phi330507	5.02-5.06	128-161	NED-GTAAAGTACGATGCGCGCGCCTCCC//CGGGGGGTAGAGAGGAGGAGAGTTGTGTG
phi213398	4.01-4.04	287-320	6-FAM-GTG-GTGACCTAAACTTGGCAGACCC///CAAGAGAGGTACCTGCTGCATGGC
phi159819	6.00-6.08	119-139	6-FAM-GATGGGCCCTAGACCAGCTT//GCCTCTCTCCCCCATCTCTCTCGGT

BIN: position of the allele on the chromosome.

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Analysis of molecular information

Allelic profiles were generated for each line from the direct reading of the gels. Subsequently, Roger's genetic distance matrix modified by Wright (1978) was constructed using the computer program NTSYS (Rohlf, 2009). A cluster analysis based on the genetic distances was performed using the Unweighted Pair-Group Method with Arithmetic Mean (UPGMA) method. Hybrid formation based on molecular information was performed under irrigated conditions during the Spring-Summer of 2018 at Campo Experimental Rio Bravo, Tamaulipas. Planting was conducted in furrows 20 m long per row with a furrow spacing of 0.82 m. At flowering, directed crosses were made between pairs of lines with greater genetic distance, resulting in the generation of 20 single hybrids with molecular information.

Formation of hybrids by *per se* evaluation of lines

Evaluation of the lines was conducted under irrigation conditions during the Spring-Summer of 2018 at the Campo Experimental Río Bravo, Tamaulipas. The trial was established using a randomized complete block experimental design with three replications. Each experimental unit comprised four 5 m long furrows with a furrow spacing of 0.82 m, and an approximate stocking density of 65,000 plants per hectare. At harvest, 40 plants with complete competition were collected from each line to estimate yield and agronomic variables. An ANOVA was performed using SAS® statistical software version 9.4 (SAS Institute Inc., 2011), followed by mean comparison using Tukey's test ($p \leq 0.05$). Based on the *per se* information, eight outstanding lines were selected, which were subsequently used to form 28 single-cross hybrids in the Autumn-Winter of 2019 through directed crosses under irrigated conditions.

Formation, crossbreeds evaluation, and hybrid generation with crossbreed information

Using the general combining ability method, 37 crossbreeds were generated under irrigation conditions during the Spring-Summer of 2018 at CERIB, Rio Bravo, Tamaulipas, with the LRB-3A line from the INIFAP-Río Bravo Experimental Field genetic improvement program used as a tester. Crossbreeds evaluation took place during the Autumn-Winter of 2019 under irrigation conditions using a randomized complete block experimental design with three replications. Each experimental plot comprised four 5 m long furrows with 0.82 m spacing between furrows. At harvest, 40 plants with complete competition were collected from each plot to estimate yield and agronomic variables. Analysis of variance and Tukey's test ($p \leq 0.05$) were conducted using SAS statistical software version 9.4 (SAS Institute Inc., 2011). Based on the crossbreed information, the top eight lines were selected based on yield parameters, which were then used to produce 15 single-cross hybrids through manual plant-to-plant pollination during the Spring-Summer of 2019 under irrigated conditions.

Evaluation of the hybrids generated with the three methodologies

A total of 70 maize hybrids were evaluated. Among these, 63 were experimental hybrids produced by the INIFAP-CERIB genetic program, comprising 20 hybrids with molecular information, 28 with *per se* evaluation, and 15 through crossbreeding. Additionally, 7 commercial hybrids commonly used in the region were included as controls: INIFAP (H-443A, 23 × 19), Syngenta (SYN307), and Pioneer (P3097, P3092, and 30F53). The single-cross hybrids were established under irrigated conditions in the localities of Rio Bravo and Diaz Ordaz in the north of Tamaulipas state during the Autumn-Winter 2020 cycle.

Field experimental design

The field experiments were established using a 10 × 10 lattice experimental design with three replications in each environment. Each experimental unit comprised two rows, each 5 m long, with a distance of 0.8 m between rows. The spacing between plants was 0.20 m, resulting in a plant density of 62,500 plants ha^{-1}.

Evaluated variables

Days for male and female flowering (DFM and DFF), defined as the days when 50% of the plants exhibited dehiscent anthers (male flowering) and receptive stigmas (female flowering), respectively; plant and ear height in centimeters (PH and EH), measured in five representative plants per plot to calculate the average height from the stem base to the flag leaf and from the stem base to the insertion of the ear, respectively; ear diameter and ear length in centimeters (ED and EL), measured in five representative ears per plot at the central part and from the base to the tip of the ear, respectively; row number per ear (RN) in the middle part of the ear; shattering percentage (SPW), calculated as the ratio between grain weight and total ear weight; grain yield (YIELD), estimated based on the field weight of each plot, with adjustments made for shattering percentage, the yield was extrapolated to t ha^{-1} and adjusted to 14% moisture content.

Statistical analysis

To analyze the study variables, a combined analysis of variance was conducted across locations, followed by a comparison of means using Tukey's test ($p \leq 0.05$). The linear additive model used for the combined analysis was as follows:

$$y_{ijk} = \mu + A_j + \beta k_{(j),} + C_i + (CA)_{ij} + e_{ijk}$$

Where: y_{ijk} = observation of the i^{-th} genotype in the j^{-th} block and k^{-th} environment, μ = overall mean, A_j = effect of the k^{-th} environment, $\beta_{k(j)}$ = effect of the j^{-th} block within the k^{-th} environment, C_i = effect of the i^{-th} genotype, $(CA)_{ij}$ = effect of the interaction between the i^{-th} genotype and the k^{-th} environment, e_{ijk} = random effect of the experimental unit error.

20 hybrids were produced based on the greatest genetic distances between parents, 28 hybrids were generated using the *per se* method, and 15 hybrids were formed based on the general combining ability of the lines through crossbreeding. All hybrids underwent an analysis of orthogonal contrasts to compare the three methods and observe the relative efficiency of each in hybrid formation. The statistical software SAS® version 9.4 (SAS Institute Inc., 2011) was utilized to perform the combined analysis of variance and orthogonal contrasts.

Results and Discussion

Analysis of variance

The genotypes exhibited significant differences ($p \leq 0.01$) in grain yield (YIELD), shattering percentage (SPW), number of rows (RN), ear diameter and length (ED and EL), days to male and female flowering (DFM and DFF), and plant and ear height (PH and EH) (Table 2). These variations indicate substantial divergence among the single cross hybrids, given the distinct genetic backgrounds of the parent combinations. Therefore, the results suggest that among the evaluated hybrids in the northern zone of Tamaulipas, at least one genotype demonstrates superiority. Similar findings were reported by Ferdoush *et al*. (2017), who observed a high degree of variation among genotypes in yield parameters using ANOVA. Likewise, Singh *et al*. (2017) identified significant differences in yield traits, indicating extensive genetic variability. Guillén-de la Cruz *et al*. (2009) reported that increasing genetic diversity among parents enhances differences in agronomic and physiological characteristics in their crosses.

Regarding environmental sources of variation, significant statistical differences ($p \leq 0.01$ and 0.05) were observed for YIELD, SPW, NR, ED, EL, DFF, DFM, PH, and EH. Cervantes-Adame *et al*. (2020) associated differences in yield trait expression with genetic and environmental factors such as climate, temperature, precipitation, altitude, and latitude. These results align with a study by Ramírez-Díaz *et al*. (2019) focusing on the selection of lines and crosses with a high combining ability for yield and its components. Concerning genotype-by-environment interaction, statistical differences ($p \leq 0.01$ and 0.05) were found for NR, ED, DFM, and DFF variables, suggesting that hybrids do not maintain consistent yield across evaluation environments. This variability poses challenges for breeders as it may bias the selection of superior genotypes with adaptability to a wider region. Velázquez-Cárdelas *et al*. (2018) emphasized the constant concern among breeders regarding this phenomenon, especially when its magnitude is substantial. It necessitates trials in multiple locations to identify materials with better stability and higher grain yield or the recommendation of new materials for a restricted agricultural area, potentially reducing economic efficiency in seed production. The overall average yield in this study was 6.5 t ha^{-1}, surpassing the Tamaulipas state average of 5.1 t ha^{-1} in 2019 (SIAP, 2020). Thus, at least one experimental cross could be considered for commercial use in the northeastern region of Mexico.

Table 2. Mean squares and significance of analysis of variance in 96 yellow corn hybrids in yield parameters in Diaz Ordaz and Rio Bravo, Tamaulipas, Mexico, 2020.

SV	DF	YIELD	SPW	NR	ED	EL	DFM	DFF	PH	EH
Environment (A)	1	1202.5**	20.7*	307.8**	34.4**	120.6**	5913.7**	7518.4**	182833.7**	84603.6**
Reps/A	4	12.4**	125.4**	0.9	0.09**	30.3**	1.3	1.5	159.2	59.7
Subl/Reps×A	54	1.0	6.4	0.7	0.02	1.7**	1.0*	1.3**	123.5*	70.7**
Genotypes (G)	95	8.5**	17.4**	5.7**	0.2**	7.6**	18.9**	18.4**	369.3**	258.6**
G×A	86	0.9	5.7	0.9*	0.02**	0.9	1.7**	1.6**	90.2	54.7
Error	335	0.7	4.5	0.6	0.01	0.7	0.7	0.7	82.1	41.0
CV (%)		13.2	2.5	5.4	2.8	5.9	1.0	1.1	4.5	9.4
Mean		6.5	85.2	14.1	4.3	14.9	78.1	79.3	197.5	67.4

*, **: different at $p \leq 0.05$ and 0.01, respectively. SV: sources of variation, DF: degrees of freedom, YIELD: grain yield, SPW: shelling percentage, NR: number of rows, ED: ear diameter, EL; ear length, DFM: days to male flowering, DFF: days to female flowering, PH: plant height, EH: ear height, Reps/A: replicates within environment, Subl/Reps×A: subblocks within replicates by environment, G×A: genotype-by-environment interaction, CV: coefficient of variation.

In the means test, the control hybrids Pioneer P3097, P3092, 30F53, and the experimental hybrid LEARB9×UAY113 exhibited statistically similar grain yields of 10.9, 10.6, 9.8, and 9.1 t ha^{-1}, respectively, indicating competitiveness with commercial hybrids in the northern region of Tamaulipas (Table 3). Additionally, the cross LEARB9×UAY113 showed statistically higher yields compared to the hybrid Syn307 (Syngenta) and H-443A (INIFAP), with increases of 300 and 800 kg ha^{-1}, respectively. Notably, H-443A is a genotype released by INIFAP in northeastern Mexico. These results contrast with those of Reyes *et al*. (2009), who reported yields ranging from 4.9 to 8.3 t ha^{-1} and an average of 7.1 t ha^{-1} for the hybrid H-443A, competitive with commercial controls A-7573Y, P30F53, and D-2020Y, which produced 6.8, 6.9, and 7.3 t ha^{-1}, respectively. Among the selected genotypes, experimental hybrids LEARB9×UAY113 and LEARB3×UAY101 were generated using molecular information from pairs of lines with greater genetic distance. Lariépe *et al*. (2017) described that considering genetic distances between parental lines better estimates the potential combining ability of inbred lines when crossed with unrelated lines. Similarly, Marcón *et al*. (2019) concluded that using molecular markers of SSRs type correlates significantly with genetic distances of parents and heterosis in morpho-agronomic characters. Hybrids UAY103×LEARB23, UAY103×LEARB2, and UAY103×LEARB8 were formed using information from the lines *per se*, indicating the significant contribution of progenitor lines to grain yield expression, which could be utilized in releasing single-cross hybrids or generating synthetic varieties within a maize breeding program (Guillén-De la Cruz *et al.,* 2009). However, among hybrids generated with crossbreeding information in this selected group, no outstanding material in yield and/or components was identified.

Regarding the shattering percentage (SPW) variable, all crosses performed similarly compared to commercial hybrids, indicating favorable characteristics for shelling percentage, a crucial factor for achieving good grain yield (Table 3). However, controls surpassed experimental hybrids in row number (NR) and ear diameter (ED), with values of 15.6 and 15.4 for NR, and 4.9, 4.8, and 4.6 for ED, respectively. Noteworthy genotypes for ear length (EL) included P3097, 30F53, LEARB9×UAY113, UAY103×LEARB23, H443A, UAY101×LEARB3, and UAY103×LEARB8. Similar findings have been reported in various studies (Acevedo-Cortés *et al.,* 2020; Rodríguez-Pérez *et al.,* 2020; Sánchez-Ramírez *et al.,* 2020), emphasizing the significance of yield parameters in maize lines and their combinations. For days to male and female flowering, genotypes ranged from 76.8 to 81.8 for DFM and from 77.1 to 82.1 for DFF, while plant and ear height varied from 190.3 to 217 cm in PH and from 61.1 to 82.6 cm in EH. These results underscore wide genetic divergence based on parental line origins in hybrid combinations, along with the influence of climatic and edaphic conditions throughout the phenological stages of crops. This aligns with findings by Velázquez-Cárdelas *et al*. (2018), who evaluated commercial and mestizo maize hybrids formed with germplasm from INIFAP and CIMMYT, observing differences across almost all study variables.

Table 3. Means of 10 yellow maize hybrids generated with molecular information, *per se* line evaluation, and crossbreeding tests for yield parameters in Diaz Ordaz and Rio Bravo, Tamaulipas, Mexico, 2020.

Hybrids	YIELD	SPW	NR	ED	EL	DFM	DFF	PH	EH
P3097	10.9 ^{a}	87.4 $^{a-e}$	13.0 $^{o-y}$	4.6 $^{b-g}$	17.0 $^{a-e}$	79.0 $^{i-r}$	79.6 $^{i-r}$	217.8 ^{a}	76.6 $^{a-c}$
P3092	10.6 ^{ab}	86.6 $^{a-g}$	15.4 $^{a-j}$	4.8 ^{ab}	15.8 $^{b-n}$	81.1 $^{d-h}$	82.1 $^{b-g}$	206.8 $^{a-h}$	82.6 ^{a}
30F53	9.8 ^{abc}	87.8 $^{a-e}$	15.6 $^{a-g}$	4.9 ^{a}	18 ^{ab}	81 $^{d-i}$	81.6 $^{c-i}$	202.18 $^{a-l}$	73.1 $^{a-g}$
LEARB9×UAY113†	9.1 $^{a-d}$	86.8 $^{a-f}$	13.6 $^{h-y}$	4.5 $^{b-i}$	16.2 $^{a-l}$	81.0 $^{d-i}$	81.3 $^{e-k}$	193.1 $^{c-o}$	70.0 $^{a-k}$
SYN307	8.8 $^{b-e}$	88.6 ^{abc}	15.4 $^{a-i}$	4.6 $^{a-e}$	15.6 $^{c-q}$	77.8 $^{n-w}$	78.6 $^{m-v}$	209.1 $^{a-f}$	73.0 $^{a-h}$
UAY103×LEARB23	8.4 $^{c-f}$	85.2 $^{a-k}$	14.4 $^{d-t}$	4.6 $^{b-i}$	16.4 $^{a-i}$	78.1 $^{m-w}$	79.0 $^{l-u}$	203.1 $^{a-k}$	70.1 $^{a-j}$
UAY103×LEARB2	8.4 $^{c-g}$	85.6 $^{a-k}$	14.8 $^{c-o}$	4.6 $^{b-i}$	15.8 $^{b-o}$	78.3 $^{l-v}$	79.1 $^{k-t}$	201.3 $^{a-l}$	61.1 $^{d-n}$
H-443A	8.3 $^{c-h}$	85.4 $^{a-k}$	13.8 $^{g-y}$	4.4 $^{d-q}$	16.1 $^{a-m}$	81.8 $^{c-f}$	82.0 $^{c-h}$	190.3 $^{e-p}$	79.8 ^{abc}
LEARB3×UAY101†	8.2 $^{c-i}$	85.2 $^{a-k}$	14.4 $^{d-t}$	4.5 $^{b-n}$	16.3 $^{a-j}$	80.0 $^{e-m}$	81.6 $^{c-i}$	206.0 $^{a-i}$	71.3 $^{a-j}$
UAY103×LEARB8	8.1 $^{c-j}$	87.9 $^{a-e}$	13.2 $^{m-y}$	4.3 $^{e-u}$	15.9 $^{a-n}$	76.8 $^{q-a}$	77.1 $^{t-a}$	200.6 $^{a-m}$	74.5 $^{a-f}$

†Detected with molecular information. YIED: grain yield, SPW: shattering percentage, NR: number of rows, ED: ear diameter, EL: ear length, DFM: days to male flowering, DFF: days to female flowering, PH: plant height, EH: ear height. Means with equal letters in each column are not statistically different (Tukey, $p \leq 0.05$).

Contrasts between genotypic methods

A contrast represents a linear combination of treatment effects, and if there are k treatments, k^{-1} orthogonal contrasts can be examined (Rebolledo, 2002). Table 4 presents the analysis of

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variance for the experiment, revealing differences ($p \leq 0.01$) among treatments (breeding methods) for the variables grain yield (YIELD), plant height (PH), and ear height (EH), with significance ($p \leq 0.05$) for ear diameter (ED). This suggests that at least one parental selection method differs from the others concerning these variables.

Table 4. Mean squares and significance of the analysis of variance of three methods of parent choice in single cross hybrids of maize in Diaz Ordaz and Rio Bravo, Tamaulipas, Mexico, 2020.

SV	DF	YIELD	SPW	NR	ED	EL	DFM	DFF	PH	EH
Treatments	2	1.1**	3.6	0.9	0.0*	0.0	1.0	1.1	123.9**	18.4**
Blocks	5	10.0**	8.4*	2.6**	0.2**	2.0**	75.4**	62.1**	1370.2**	636.4**
Error		0.1	2.5	0.1	0.0	0.1	1.3	1.3	16.3	2.2
CV (%)		5.2	1.8	2.2	1.5	3.0	1.4	1.1	2.0	2.2

*, **: different at p ≤ 0.05 and 0.01, respectively. SV: sources of variation, DF: degrees of freedom, YIELD: grain yield, SPW: shattering percentage, NR: number of rows, ED: ear diameter, EL: ear length, DFM: days to male flowering, DFF: days to female flowering, PH: plant height, EH: ear height.

Regarding the two contrasts, Contrast 1 (molecular vs. *per se*, crossbred) exhibited differences ($p \leq 0.01$ and 0.05) for grain yield, ear diameter, plant height, and ear height (Table 5). These results indicate that employing molecular markers in a breeding program can serve as a valuable tool for effectively predicting parents for generating outstanding hybrids in these traits. Nyaga *et al*. (2020) suggest that selecting parental lines based on molecular markers and their genetic distances can lead to the development of exceptional hybrids for certain yield parameters. Tomkowiak *et al*. (2020) noted that involving parents with greater genetic distances, as determined through SSRs molecular markers, can enhance the heterosis effect for yield and its components. Additionally, several authors have highlighted that the use of traditional breeding methods assisted by molecular markers improves prediction accuracy in hybrid generation (Crossa *et al.,* 2017; Mwangangi *et al.,* 2019; Technow *et al.,* 2014). On the other hand, Beyene *et al*. (2019) argued that integrating molecular markers into conventional phenotypic selection is a preferable option for accelerating the development and release of new genotypes at a lower cost, time, and effort.

The second contrast (*per se* vs. crossbreeding) revealed differences ($p \leq 0.01$) in ear height and ($p \leq 0.05$) in plant height, with no differences observed in the other study variables. This suggests that in the materials of this study, the use of *per se* selection of lines or the use of crossbreeding does not differ concerning yield traits, but does in terms of their agronomic variables.

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Table 5. Orthogonal contrasts of three breeding methods with molecular information, line evaluation *per se*, and crossbreeding test for the formation of single hybrids in maize, in Diaz Ordaz and Rio Bravo, Tamaulipas, Mexico, 2020.

SV	DF	YIELD	SPW	NR	ED	EL	DFM	DFF	PH	EH
Molecular vs. *per se* and crossbreeds	1	1.9**	3.7	0.0	0.0**	0.0	0.0	1.4	120.8*	0.4*
Per se vs. crossbreeds	1	0.2	3.5	0.1	0.0	0.0	1.9	0.9	127.1*	36.4**

*, **: different at p ≤ 0.05 and 0.01, respectively. SV: sources of variation, GL: degrees of freedom, YIELD: grain yield, SPW: shattering percentage, NR: number of rows, ED: ear diameter, EL: ear length, DFM: days to male flowering, DFF: days to female flowering, PH: plant height, EH: ear height.

Conclusions

The utilization of the studied strategies for parental line selection resulted in varying outcomes in the formation of maize single-cross hybrids, with differences observed in different magnitudes depending on the variable targeted for improvement. A hybrid demonstrating superior performance was identified based on the genetic distance between its parental lines, derived from molecular information, and showed competitiveness with the commercial controls in the region. Microsatellite-type molecular markers exhibited efficiency in predicting the performance of maize single-cross hybrids compared to traditional *per se* and crossbreeding genotyping methods. SSRs can serve as a supportive tool in plant breeding programs for predicting and generating new single hybrids, offering the advantage of saving time and resources as they do not require testing the combinatorial fitness of their progenitors.

Authors' Contributions:

Conceptualization of the work: RHM, ASV. Methodology development: RHM, ASV, CARM. Experimental validation: RHM, CARM. Analysis of results: RHM, ASV, HLS, RLO. Data management: RHM, ASV. Manuscript writing and preparation: RHM, ASV, HLS, RLO, FCG. Drafting, revising, and editing: RHM, CARM, ASV, HLS, RLO, FCG. Project management: ASV, RHM. Fund acquisition: RHM, ASV.

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Conflict of Interest:

The authors declare that they have no conflict of interest.

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