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## Comparison of the performance of synthetic maize varieties created based on either genetic distance or general combining ability of the parents

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### Abstract

Synthetic varieties are grown by farmers and used by breeders to select new inbred lines. In countries unable to market hybrids, use of synthetics leads to yield improvements over landraces. Synthetics are derived from intercrossing inbred lines known to possess high general combining ability (GCA) as measured via crossing with testers and phenotyping for yield in multiple environments. Genetic similarity (GS) between lines measured by molecular markers may efficiently estimate GCA. Although the prediction of specific combining ability (SCA) of lines via GS has not been successful, it may have potential to predict the suitability of lines to form a synthetic variety. As this has not been reported, the objective of this research was to compare the performance of four synthetic maize varieties developed using GS calculated between parents using SSR markers with the performance of synthetics developed using GCA based on yield. Synthetics were phenotyped for yield and other agronomic traits in replicated field trials in several environments. The two synthetics formed based on low GS (0.34 and 0.33) performed better than all other synthetics in yield and most agronomic traits. The synthetics formed based on high GS (0.77 and 0.53), performed worst for nearly all traits. The GCA-based synthetics were generally intermediate for all traits. Response of synthetics to environmental variation and efficiencies gained via use of molecular markers in synthetic formation is discussed.

**Keywords:** maize, General Combining Ability (GCA), Genetic Similarity (GS), synthetic varieties

### Introduction

Synthetic varieties were first suggested by Hayes and Garber (1919) and defined by Lonquist (1961) as open pollinated varieties (OPVs) derived from the intercrossing of selfed plants or lines known to possess high general combining ability (GCA), and subsequently maintained by routine mass selection procedures from isolated plantings. When OPVs instead of inbred lines are intercrossed, resulting populations are called composites (Hallauer and Miranda, 1988). Synthetics can be used by either farmers for commercial production or breeders as source populations from which to select new lines. For most developing countries, OPVs are still the preferred choice for planting over hybrids, due to the ease with which they can be developed, maintained and produced in large quantities (Pandey et al, 1984). The level of heterosis is reduced during pollination generations, and seed must be repurchased approximately every five years. In countries with inadequate infrastructure to market hybrids, use of synthetics leads to major yield improvements (Setimela et al, 2006; Pixley and

Bänziger, 2004). Open pollinated varieties developed by modern plant breeding were historically used as commercial cultivars by farmers, and replaced the older landraces that had been selected directly by the farmers. Later, the best OPVs were selected as source populations for further plant improvement and development of synthetics and modern hybrids. In the 1920s, nearly one thousand cultivars available in the United States Corn Belt were selfed in an attempt to develop useful inbred lines (Troyer, 1999). These were often intercrossed to create synthetics. One of the most popular, Iowa Stiff Stalk Synthetic (BSSS), was developed by GF Sprague in the early 1930's by intermating 16 inbreds (Hallauer and Miranda, 1988), and is considered an excellent source population for the selection of inbred lines with high combining ability with other elite inbred lines.

General combining ability is used to designate the average performance of a line in hybrid combinations (Sprague and Tatum, 1942), but has little meaning unless the genetic backgrounds of the lines included in

the hybrids, and the environment in which the hybrids are tested, are specified (Henderson, 1952). Selecting inbred lines based on their GCA in defined crosses is used to develop synthetics and improve yield when selection is directly on yield (a trait of very low heritability) has a limited effect. This suggests that while the inheritance of combining ability is a quantitative trait governed by many genes, each has a larger individual effect than would genes that contribute directly to yield. Lines with high GCA presumably have a larger proportion of favorable yield genes which differ from other lines measured with respect to their specific favorable gene complex (Lonnquist, 1951). Measurements of GCA can be obtained from multiple top crosses made between a line and various elite inbreds, and these crosses are phenotyped for

yield in multiple locations and years. Although specific combining ability (SCA) is more important from the standpoint of obtaining maximum yields in hybrid crosses, GCA is highly important in developing high yielding synthetics. Line performance in top crosses has been shown to be relatively constant after the S1 generation (Jenkins, 1935; Sprague, 1946) and consequently little or nothing would be gained by additional selfing where the production of synthetic varieties is the goal (Lonnquist, 1949).

The use of genetic distances based on molecular markers between pairs of maize inbred lines for predicting their hybrid performance has been suggested because it would be more efficient than the traditional method of field testing of multiple crosses. Although correlations between hybrid performance and marker

**Table 1** - Description of each of the synthetics, including identification of the lines that were intercrossed to form each synthetic. A description of each parental line can be found in [Supplementary Table 1](#). The number of locations where diallel or test crosses were evaluated is given. The range and average genetic similarities (GS) between pairs of parents calculated on SSR markers is given. Locations of field trials in which each synthetic was phenotyped is indicated in [Supplemental Table 2](#).

Synthetic 1	Six lines (15, 28, 32, 37, 40 and 41) selected from a 10 x 10 Diallel of yellow maize lines were intermated to form Synthetic 1. The Diallel was evaluated in replicated trials in 6 locations of Colombia during 2002 and 2003. GS ranged between 0.33-0.62, with a mean of 0.37
Synthetic 2	Eight lines (15, 17, 21, 23, 25, 26, 27 and 28) selected from a 19 x 19 Diallel of acid soil tolerant maize inbreds from CIMMYT population SA3 were intermated to form Synthetic 2. The Diallel was evaluated in replicated trials in 7 locations of Colombia during 2002 and 2003. GS ranged between 0.32-0.50, with a mean of 0.37
Synthetic 3	Seven lines (32, 33, 36, 37, 38, 39 and 42) selected from a 19 x 19 Diallel of acid soil tolerant maize inbreds from CIMMYT population SA4 were intermated to form Synthetic 3. The Diallel was evaluated in replicated trials in 6 locations of Colombia during 2002 and 2003. GS ranged between 0.33-0.47, with a mean of 0.37
Synthetic 4	Six lines (3, 4, 5, 6, 7 and 9) selected from a 14 x 14 Diallel of elite acid soil tolerant maize inbreds from CIMMYT were intermated to form Synthetic 4. The Diallel was evaluated in replicated trials in 7 locations of Colombia during 2002 and 2003. GS ranged between 0.32-0.42, with a mean of 0.36
Synthetic 5	Seven lines (60, 61, 62, 63, 65, 67 and 69) from CIMMYT population SA3 selected from a test cross trial including 143 inbreds and 2 testers were intermated to form Synthetic 5. The trial was evaluated in replicated trials in 5 locations of Colombia during 2004. GS ranged between 0.27-0.72, with a mean of 0.34
Synthetic 6	Seven lines (43, 44, 45, 46, 64, 66 and 68) from CIMMYT Global Maize Program selected from a test cross trial including 143 inbreds and 2 testers were intermated to form Synthetic 6. The trial was evaluated in replicated trials in 5 locations of Colombia during 2004. GS ranged between 0.37-0.49, with a mean of 0.40
Synthetic 7	Six lines (44, 49, 50, 53, 54 and 55) with tropical/sub-tropical background, selected from a test cross trial including 72 inbreds and 2 testers were intermated to form Synthetic 7. The trial was evaluated in replicated trials in 5 locations of Colombia during 2004. GS ranged between 0.33-0.38, with a mean of 0.34
Synthetic 8	Six lines (15, 45, 56, 57, 58 and 59) with tropical and sub-tropical background, selected from a test cross trial including 72 inbreds and 2 testers were intermated to form Synthetic 8. The trial was evaluated in replicated trials in 4 locations of Colombia during 2004. GS ranged between 0.33-0.48, with a mean of 0.38
Synthetic 9	Six QPM lines (70, 71, 72, 73, 74 and 79) selected from a test cross trial including 88 inbreds and 2 testers were intermated to form Synthetic 9. The trial was evaluated in replicated trials in 5 locations of Colombia during 2004. GS ranged between 0.34-0.62, with a mean of 0.45
Synthetic 10	Eight lines (1, 2, 3, 12, 13, 14, 16, and 31) from a cluster in Figure 1 were intermated to form Synthetic 10. GS ranged between 0.75 and 0.88, with an average of 0.77
Synthetic 11	Nine lines (49, 72, 73, 74, 75, 76, 77, 78, and 79) from other cluster in Figure 1 were intermated to form Synthetic 11. GS ranged from 0.42 and 0.72, with an average GS of 0.53
Synthetic 12	Nine lines (1, 5, 25, 33, 36, 38, 48, 49, and 64) from dendrogram in Figure 1 were intermated to form Synthetic 12. GS ranged from 0.26 to 0.46, with an average of 0.34
Synthetic 13	Nine lines (7, 8, 20, 43, 44, 46, 47, 52 and 53) from dendrogram in Figure 1 were intermated to form Synthetic 13. GS ranged from 0.26 to 0.46, with an average of 0.34

based genetic distances have been found in some crop species, this method has had limited success in maize, where a poor correlation is generally seen, especially at higher genetic distances (Godshalk et al, 1990; Melchinger et al, 1990; Reif, 2003b). While the relatively higher correlations between lower genetic distances and lower hybrid performance is not useful for predicting the highest yielding crosses, it would allow the closely related crosses to be avoided. Because this information can generally be gained via pedigree analysis, the use of genetic distance to predict heterosis due to specific combining ability between any two given inbred lines has not been useful. Molecular markers have been effective in assigning maize germplasm to groups that may be useful in heterotic patterns (Yuan et al, 2001; Reif et al, 2003a; Dhilwayo et al, 2009). In contrast to the prediction of SCA, the use of genetic distance between maize inbred lines to predict GCA, and thus the suitability of these lines to form a synthetic variety, may meet with more success. The use of genetic distance, as estimated with molecular markers in the selection of parents for a synthetic maize variety, has not been reported.

The objective of this research was to compare the performance of four synthetic maize varieties developed using genetic distance between the parents as measured by SSR markers with the performance of nine synthetics developed using calculations of GCA based on yield and other agronomic traits.

## Materials and Methods

### *Parental materials and field evaluations*

Thirteen synthetics were formed for this study (Table 1). A description of each of the inbred lines used as the parents of the synthetic populations can be found in Supplementary Table 1. Nine of the synthetics were created by choosing parental inbred lines with high GCA, as measured in a diallel or testcross trial. The parents of the remaining four synthetics were selected based on the genetic distance of parental inbred lines as measured by SSR markers (see below). The parents of Synthetics 10 and 11 were chosen from the more similar lines, ensuring lower genetic distance among lines within these synthetics. The parents of Synthetics 12 and 13 were chosen to represent the least similar lines, thus ensuring maximum genetic distance among lines within these synthetics.

### *Synthetic formation and evaluation*

All synthetics were created using a diallel mating design. Paired rows consisting of 22 plants each were used in generating each cross ( $F_1$ ). At least 20 ears per cross were chosen and 50 seeds from each ear were saved. Two-row plots from each  $F_1$  were planted in the field and plant-to-plant crosses between  $F_{1s}$  were done to obtain the  $F_2$  and reduce the heterotic effects present in the  $F_{1s}$ . A balanced composite of  $F_2$  seeds from 10 ears of each  $F_1$  was saved and used in trial evaluations. To calculate GCA, diallel and line

x tester trials were used. For diallel trials, an  $\alpha$  lattice experimental design with 2 replications per location was used. Line x tester trials were arranged in a randomized complete block design with 2 replications per location in a split-plot arrangement where lines were the main plots and testers the sub-plots. Synthetic evaluation was done using an  $\alpha$  lattice experimental design with three replications per location.

Locations represented different environments in Colombia where maize is cultivated (Supplementary Table 2). La Catalina (location of trial 6, planted in 2010) and Paraguaicito (trials 2 and 7, planted in 2009 and 2010, respectively) are located in the coffee growing area and represent a mid-altitude maize environment. Although they are high potential maize production areas, some disease problems are frequent mainly due to high rainfall. Palmira (location of trials 1 and 8, planted in 2009 and 2010, respectively) is located in a lowland tropical environment with high potential maize production and low disease pressure. Menegua (trial 3, 2010) and three locations in Villavencio (trials 4, 5, and 9 planted in 2010) represented tropical acid soils. Menegua is a representative location of the Colombian Savannas, while Villavencio represents the piedmont environment. Trial 9 was planted in 60% aluminum saturation, while trials 4 and 5 were planted in 70% aluminum saturation and were planted two months apart to gather information on crop rotation in relation to planting date.

### *SSR analysis*

The 79 inbred parental lines and six CIMMYT maize lines (CMLs), which served as control lines in the study, were grown in trays in the greenhouse. Leaf samples were collected from 10-day old plants and eight to 10 plants were bulked for DNA extraction, according to CIMMYT protocols (CIMMYT, 2005). A set of 43 SSR markers was selected for uniform coverage of the genome and repeat units of three or greater from the maize genetics and genomics database (MaizeGDB, <http://www.maizegdb.org>) and were used to analyze the lines. The primers were synthesized by Research Genetics, Inc. (Huntsville, AL, USA). The protocols for SSR amplification and allele detection via polyacrylamide gels were as described in George et al (2004). Primers amplifying unrepeatable bands or those that were difficult to score were removed from the analysis, leaving a subset of 30 markers. Bands were scored as presence/absence polymorphisms and a matrix of binary data was analyzed with NTSYSpc (Rohlf, 1998), using the Jaccard genetic similarity coefficient to calculate a matrix similarities between every pair of genotypes in the study. Cluster analysis was done using the unweighted pair-group method with arithmetic average (UPGMA) method and the relationships among the lines were visualized using a dendrogram. The cophenetic coefficient was computed and tested using the Mantel matrix correspondence test (MXCOMP program) to determine if the dendrogram obtained was a good fit

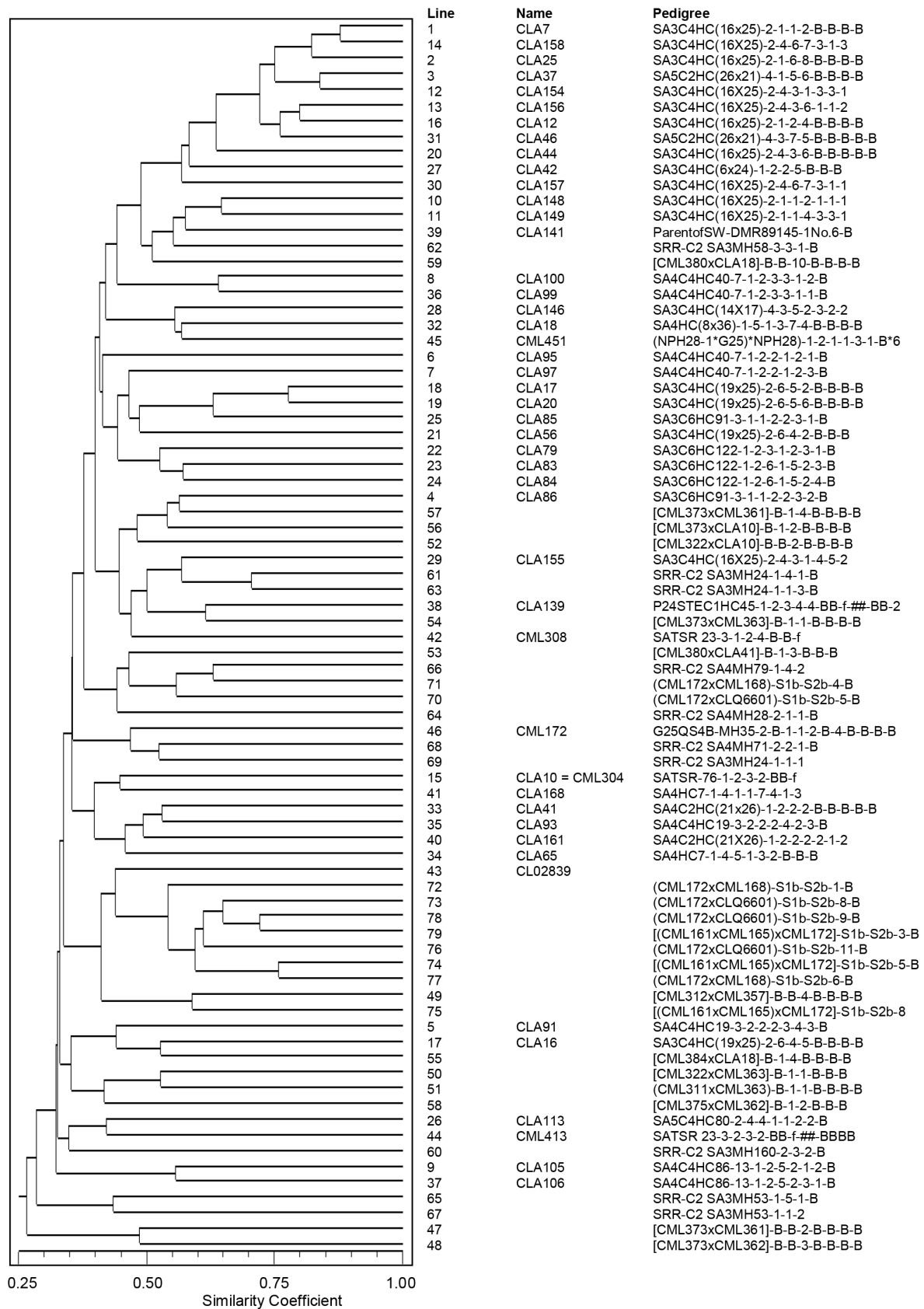


Figure 1 - Dendrogram of 79 yellow lines with varying tolerance to AI based on 30 SSR markers.

to the similarity matrix. Bootstrap values were generated using WinBoot with a setting of 1,000 iterations (Yap and Nelson, 1996).

#### Analysis of field data

Field data were collected from all experiments on a plot basis for number of plants per plot, days to anthesis (number of days from planting to 50% of plants shedding pollen), days to silking (number of days from planting to 50% of plants with visible silks), plant and ear height (distance from the soil surface to the ligule of the flag leaf and to the highest ear-bearing node, respectively), root lodging (percentage of plants with less than 45° inclination between the soil surface and the vertical plant), stalk lodging (percentage of plants with broken stalks below the highest ear-bearing node) grain yield (converted to Mg ha<sup>-1</sup>, adjusted to 150 g kg<sup>-1</sup> grain moisture), grain moisture at harvest (g kg<sup>-1</sup>), ear aspect (1 = good, 5 = bad), and percentage of rotten ears per plot. The stability of synthetics across environments was studied by the non-parametric index  $s^{(2)}$  as proposed by Huehn (1990a, 1990b) and Nassar and Huehn (1987). The GxE interaction for yield was studied using biplots and the GGE model from Yan and Tinker (2005). Repeatability on mean basis was calculated for all the evaluated traits for all the synthetics, for synthetics formed based on GS, and for synthetics based on GCA, using the following formula:

$$R^2 = \frac{\hat{\sigma}_G^2}{\hat{\sigma}_G^2 + \frac{\hat{\sigma}_{G \times E}^2}{e} + \frac{\hat{\sigma}^2}{e \cdot r}}$$

where  $R^2$  = repeatability,  $e$  = number of locations, and  $r$  = number of replications.

## Results and Discussion

### Diversity within the synthetics

The four synthetic populations formed based on genetic similarities (GS) (two having high similarities, two having low) were chosen from the dendrogram shown in Figure 1. Synthetic 10 was formed by intermating the most similar lines in the study; the minimum similarity between any pair of the parents was 0.75, and most similarities between pairs of parents reached 0.88, with a mean of 0.77 overall. These lines from the CIMMYT Global Maize Program in Colombia were all derived from two full sib families from population SA3 (Table 1 and Supplementary Table 1), and thus high similarities were expected. Synthetic 11, also chosen between parents with a high similarity, was formed from a single cluster of the dendrogram in Figure 1, and the minimum similarity between the most different lines was slightly less than 0.42, and the most similar pair of lines was 0.72. An average similarity between all pairs of parents was 0.53. These lines were developed by the CIMMYT Global Maize Program and all but one have CML172 in their pedigrees (most also included CML161, 165, 168

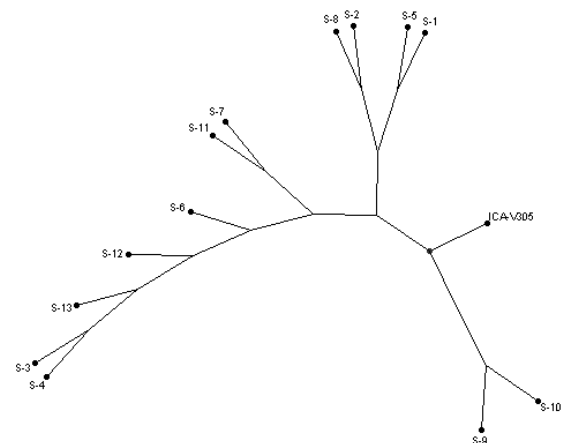


Figure 2 - Relationships between the synthetics evaluated in this study and the check variety as calculated from the Gower's distance between populations

or CLQ6601). The exception was a cross between CML312 x CML357, but it was related according to marker information. Although more diversity was found based on the markers in Synthetic 11 than Synthetic 10, it is still the second most similar group in the study. Synthetics 12 and 13 were chosen from the most distantly related lines in the study. Minimum similarity in these groups was 0.25, maximum similarity was only 0.40, and an average of 0.34.

Synthetics 1 – 9 were developed from lines based on GCA, but GS was also calculated between pairs of parents for each of these synthetics. Because all were chosen based on good GCA, high GS values were not expected, and all 9 synthetics had GS values between 0.34 and 0.45. The values were very consistent between synthetics, and seven had values between 0.34 and 0.38. Choosing synthetics based on GCA seems a good guarantee of reproducibly generating populations with a fairly high level of genetic diversity within the population; however, only two (Synthetic 5 and 7, with average GS = 0.34) matched the highest levels of diversity seen in the synthetics chosen based on marker information.

Figure 2 shows the results of the cluster analysis of the synthetics and one check variety (ICA V305). The synthetics do not cluster together based on original source breeding population, and this may be because each of the source populations share some ancestors and thus are interrelated by pedigree. Some clustering appears to occur if the diversity within the synthetics was low, in addition to being related by pedigree, but populations with high levels of diversity within them are the least related and most isolated according to the dendrogram. Repeatability was estimated, and was greater within the groups of synthetics based on GS between parents than within the groups based on GCA (Supplementary Table 3).

### Field performance of the synthetics

Synthetics and check mean values and standard

**Table 2** - Average performance over locations of each synthetic variety for all measured phenotypes in the study.

Synthetic	GS	Yield (t/ha)	ASI (days)	Epp (No.)	Ant (days)	Silk (days)	Pht (cm)	Eht (cm)	Rlod (%)	Slod (%)	Mois (%)	Rotear (%)	Easp (scale)
S1	0.37	4.89c	-0.79ab	0.93ab	58.4de	59.3de	222ab	103bcd	1.22ab	2.13abc	18.3ab	4.6abc	2.8abc
S2	0.37	5.09c	-1.12ab	0.99a	58.2e	59.3de	223ab	102bcd	0.45ab	1.77abc	18.3ab	4.7abc	2.5bcd
S3	0.37	5.31bc	-0.52a	0.90ab	58.4de	58.9e	226ab	106abc	0.26b	1.52abc	18.1abc	4.2c	2.6bcd
S4	0.36	5.50bc	-0.60ab	0.95a	58.6cde	59.2de	224ab	105abcd	0.33b	1.32abc	17.7bc	4.3bc	2.5bcd
S5	0.34	5.048c	-0.68ab	0.92ab	57.9e	58.6e	219ab	99bcd	0.89ab	2.32ab	18.0abc	6.5a	2.7bcd
S6	0.4	5.99ab	-0.58a	0.94a	58.3de	58.9e	226ab	102bcd	0.48ab	1.18abc	19.2a	4.5abc	2.4cd
S7	0.34	5.29bc	-0.95ab	0.93a	59.4bcd	60.3cd	230ab	104abcd	0.41b	0.83c	18.9ab	5.3abc	2.6bcd
S8	0.38	5.13c	-1.02ab	0.94a	59.7bc	60.8bc	216b	94de	0.37b	1.47abc	18.4ab	5.8abc	2.6bcd
S9	0.45	3.56d	-1.27ab	0.92ab	60.4ab	61.6ab	217b	98cde	1.04ab	0.99bc	19.3a	4.5abc	2.9ab
S10	0.77	3.07d	-1.47ab	0.80b	57.7e	59.1de	198c	86e	1.59a	2.08abc	16.9c	6.4ab	3.2a
S11	0.53	5.47bc	-0.81ab	0.94a	59.8b	60.6bc	233a	104bcd	0.81ab	0.98bc	18.8ab	5.5abc	2.5cd
S12	0.34	6.39a	-0.51a	0.96a	58.3de	58.8e	228ab	105abcd	0.19b	2.36a	18.7ab	5.3abc	2.6bcd
S13	0.33	6.67a	-0.71ab	0.94a	58.3de	59.0e	231ab	110ab	0.30b	0.94c	18.7ab	4.7abc	2.4cd
ICAV-305 (check)	6.37a	-1.72b	0.93ab	61.1a	62.7a	234a	117a	0.59ab	1.90abc	18.6ab	4.4abc	2.3d	
ASE		0.17	0.24	0.03	0.3	0.31	4	3	0.25	0.3	0.3	0.5	0.1

GS: Genetic Similarity; ASI: Anthesis Silk Interval; Epp: Number of ears per plant; Ant: Time from planting to anthesis; Silk: Time from planting to silk; Pht: Plant height; Eht: Ear height; Rlod: Root lodging; Slod: Stalk lodging; Mois: Grain moisture at harvest; Rotear: Ear rot Easp: Ear aspect. ASE: Average Standard Error

deviations for all phenotyped variables over all environments are shown in [Table 2](#). The two synthetics (12 and 13) formed based on lower genetic marker similarity (GS = 0.34 and 0.33, respectively) out-ranked all other synthetics in yield, and in addition, Synthetic 12 had the lowest ASI and highest number of ears per plant. Synthetics 12 and 13 were in the earliest maturing group and 12 was in the tallest group, but had a fairly low ear height (which is good for the stability of the plant and to prevent lodging). Synthetic 13 ranked among the better populations for ASI, ears per plant, plant and ear height. On the other hand, Synthetic 10, formed based on a high genetic similarity (0.77), was the lowest yielding population, having less than half the yield of Synthetics 12 and 13. Synthetic 10 was shorter and displayed the most asynchronous flowering, which would indicate possible low drought tolerance ([Bolaños and Edmeades, 1996](#); [Monneveux et al, 2006](#)). Synthetic 11, with intermediate levels of marker diversity (GS = 0.53), performed poorly or, at best, average in all traits as well.

The nine synthetic populations formed based on combining ability included lines with the highest GCA for each group. Synthetics 7 and 8 were formed with lines selected based on the evaluation of 144 top crosses resulting from hybridizing 72 lines to two testers (LP45 and CLO3613). Synthetic 7 was formed based on evaluation of top crosses in five environments, and Synthetic 8 formed based on evaluation of top crosses in four environments (one location in the coffee growing area was not considered). There are no lines in common between these two synthetics ([Supplementary Table 1](#)). Average GCA of lines used to form Synthetic 7 was 0.98 t/ha, and Synthetic 8 was 0.63 t/ha. In addition, average GS in Synthetic 7 (0.34) was lower than that of Synthetic 8 (0.38). However, average grain yield of Synthetics 7 and 8 was similar (5.286 and 5.126 t/ha, respectively) ([Table 2](#)). The differences in GCA and GS may have been too small to see differences in grain yield. To form Syn-

thetic 9, lines with an average GCA of 1.58 t/ha were crossed, and this resulted in an average grain yield of only 3.56 t/ha; most other evaluated traits performed as badly as Synthetic 10. This result may be because the testers used to generate the top crosses (CML 161 and CML 165), are too closely related to the parents of the synthetic and because all the lines forming this synthetic includes a common line (CML 172). The average GS in Synthetic 9 (0.45) was the highest among the synthetics formed based on GCA. [Henderson \(1952\)](#) cautioned that GCA estimates are specific to the population of genotypes used to form the crosses and the population of environments where crosses are evaluated. Although GCA appeared higher when the top crosses for Synthetic 9 was evaluated (alone), in the joint evaluation of all the synthetics in this study, yield was lower.

Synthetics 5 and 7 had the lowest GS of synthetics chosen based on GCA and performed fairly well, but did not yield as well as the two synthetics (12 and 13) with the lowest GS (chosen based on GS), even though the GS values calculated within all four synthetics was the same (0.34). Although the sample size in this study is too small to conclude that markers are inherently better at forming synthetics, the trend noted here is encouraging. Two of the three synthetics with the highest GS values (Synthetics 9, 10) performed the worst in most variables measured, especially grain yield.

The GxE interaction for yield is presented in the biplots in [Figures 3a and 3b](#). [Figure 3a](#) is a graph depicting which synthetic performs the best in which environment and comparing environments. Similar performance of synthetics in different environments shows possible “mega-environments” or similar growing conditions experienced by the plants in different locations. Three possible mega-environments are seen. Mega-environment 1 (ME-1) is defined mainly by environment 6 (L6), and also containing L2, L7 and L8; L2, L6, and L7 are environments of middle

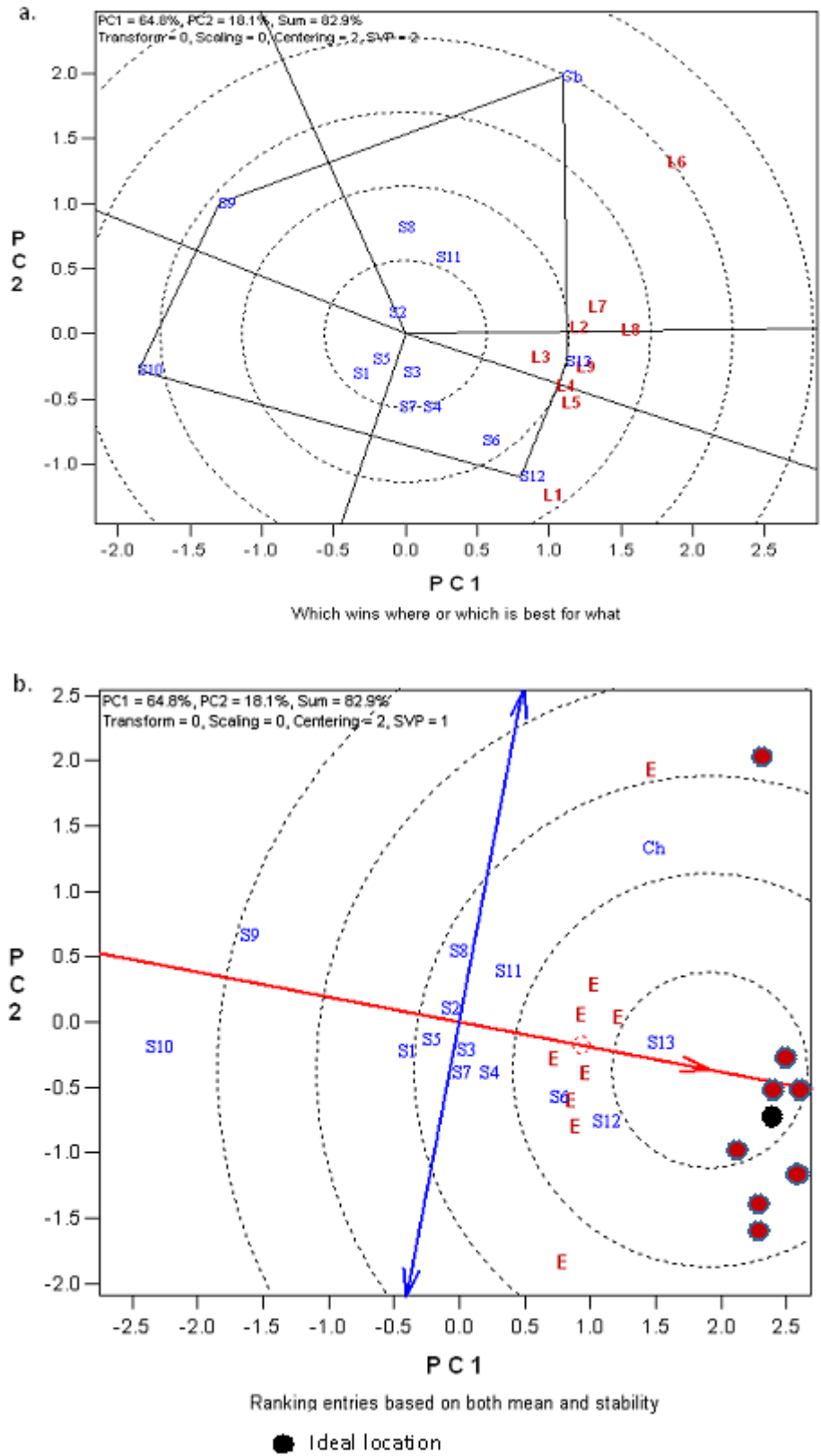


Figure 3 - The GxE interaction for yield represented as biplots. **3a** depicts “which wins where”, or which synthetic performs the best in which environment. **3b** is a stability and productivity graphic.

**Table 3** - Phenotypic values averaged over all synthetics for each site where they were grown. ‡Abbreviations for all traits and statistical values are given in the footnote.

site <sup>†</sup>	Yield (t/ha)	ASI (days)	Epp (No.)	Ant (days)	Silk (days)	Pht (cm)	Eht (cm)	Rlod (%)	Slod (%)	Mois (%)	Rotear (%)	Eeasp (scale)
1	6.72ab	-0.49a	0.93ab	58.3de	58.8de	227bc	111bc	0.62b	0.12d	18.7b	1.5d	2.2c
2	4.50cd	-3.84b	0.88bc	63.0b	66.9b	250b	120ab	0.39b	2.88ab	18.7b	8.2b	2.1c
3	5.07c	-0.69a	0.91bc	52.8f	53.5f	197cd	91cd	0.60b	4.17a	13.5c	2.4cd	2.1c
4	4.78cd	-0.35a	0.96ab	57.6de	57.9de	184d	87cd	0.02b	1.48bcd	19.6b	3.0cd	3.0ab
5	4.53cd	-0.03a	0.76c	57.1de	57.1e	178d	81d	0.52b	1.95bcd	19.6b	3.1cd	3.0ab
6	8.02a	-1.28a	1.08a	61.6bc	62.8c	311a	144a	0.64b	0.78bcd	19.6b	5.6bc	2.5bc
7	3.63d	-3.14b	0.91bc	68.9a	72.0a	257b	119ab	0.13b	0.35cd	23.8a	13.1a	3.6a
8	7.70a	-0.16a	0.91bc	59.8cd	60.0d	246b	124ab	0.42b	0.75cd	17.6b	2.0cd	2.1c
9	5.63bc	-0.32a	0.96ab	56.1e	56.4e	186d	80d	2.42a	2.47abc	15.0c	5.7bc	2.4bc
P ≤ F	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
ASE	0.282	0.288	0.035	0.604	0.567	8.062	5.192	0.317	0.443	0.499	0.841	0.149

<sup>†</sup> Site locations and years: (1) Palmira 2009; (2) Paraguaicito 2009; (3) Menegua 2010; (4 and 5) Villavicencio 2010, aluminum saturation 70%; (6) La Catalina 2010; (7) Paraguaicito 2010; (8) Palmira 2010; (9) Villavicencio 2010, aluminum saturation 60%.

ASE: the Average Standard Error. ASI: Anthesis Silk Interval; Epp: Number of ears per plant; Ant: Time from planting to anthesis; Silk: Time from planting to silk; Pht: Plant height; Eht: Ear height; Rlod: Root lodging; Slod: Stalk lodging; Mois: grain moisture at harvest; Rotear: Ear rot; Eeasp: Ear aspect.

altitude, high yield potential and showing high effect of ear rot (Table 3). In ME-1, the check population (Ch) is the associated genotype, or the genotype performing better under these conditions. ME-2 is defined by L3, L4, L5 and L9 (the environments showing high values of soil acidity), where the associated genotype S13 performed well, indicating a possible source of resistance to acidity and aluminum saturation. Finally, ME-3 is defined by environment L1 (low altitude, high potential and no diseases) to which the synthetics S12 and S6 are associated. L1, L6 and L8 were the most productive environments, as can be observed in Table 3. Figure 3b is a stability and productivity graph and shows that S13, followed by S6 and S12, were the most stable and productive synthetics over different environments. By comparison, the check population shows high yield but some instability. S13 was the nearest to the “ideal” genotype and seems to be associated to the environment L9 (acid soils) in the biplot analysis (Figure 3a and 3b).

#### Efficiencies gained in the use of markers to form synthetic maize varieties

Several studies have been published comparing conventional phenotyping and field selection and marker assisted selection (Dreher et al, 2003; Abalo et al, 2009; Asea and Vivek, 2010). However, their focus was on selecting for qualitative or highly heritable quantitative traits, where MAS is used to select a small number of markers in order to backcross, pyramid, or otherwise move discrete fragments of chromosomes between lines. During this process, many offspring are genotyped for these few markers. The calculation of genetic similarities would require more markers, but on a limited number of inbred parents to be tested. This is much more similar to the process of whole genome selection, which has begun to be used in plant improvement (Bernardo and Yu, 2007; Varshney et al, 2005). The ability to select potential synthetic populations via markers rather than the very time consuming process of calculating GCA via field crosses to many other lines would increase time and

monetary efficiency. Furthermore, many more potential combinations can be tested with markers, but not with field crosses, where diallel crosses limit the possible number of parents tested jointly. Each line added to the study increases the number of possible crosses exponentially.

This study offers an encouraging procedure of a fast and efficient method to predict GCA and create synthetics and other maize OPVs that show good performance for traits that tend to be influenced by heterosis (including yield). However, the small number of samples studied here, and the limited genetic backgrounds represented, require that further investigation continues in order to confirm the hypothesis. Furthermore, the manner in which the synthetics were chosen based on the marker data caused these synthetics to have a wide range of GS values; a more consistent choice of pairs leading to a lower GS and a lower range of GS may have been a better strategy for creating higher yields and should be tested in the future. These results should encourage maize breeders, particularly those working in areas where synthetics are still grown on a large scale, to test markers in the production of their own maize synthetic varieties, and determine if the correlation between genetic marker similarities and general combining ability in maize is a general and repeatable phenomenon.

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Supplementary Table 1. Description of inbred lines used as parents of synthetics.

**Synthetic 1**

<b>Line</b>	<b>Name</b>	<b>Pedigree</b>	<b>GCA</b>
15	CLA10	SINT.AM.TSR76-1-2-3-2-BBB-f	1.12
28	CLA146	SA3C4HC(14X17)-4-3-5-2-3-2-2	0.14
32	CLA18	SA4HC(8x36)-1-5-1-3-7-4-B-B-B-B	0.17
37	CLA106	SA4C4HC86-13-1-2-5-2-3-1-B	0.47
40	CLA161	SA4C2HC(21X26)-1-2-2-2-2-1-2	0.28
41	CLA168	SA4HC7-1-4-1-1-7-4-1-3	0.38

**Synthetic 2**

<b>Line</b>	<b>Name</b>	<b>Pedigree</b>	<b>GCA</b>
15	CLA10	SINT.AM.TSR76-1-2-3-2-BBB-f	1.12
17	CLA16	SA3C4HC(19x25)-2-6-4-5-B-B-B-B	0.53
21	CLA56	SA3C4HC(19x25)-2-6-4-2-B-B-B	0.37
23	CLA83	SA3C6HC122-1-2-6-1-5-2-3-B	0.39
25	CLA85	SA3C6HC91-3-1-1-2-2-3-1-B	0.08
26	CLA113	SA5C4HC80-2-4-4-1-1-2-2-B	1.14
27	CLA42	SA3C4HC(6x24)-1-2-2-5-B-B-B	0.93
28	CLA146	SA3C4HC(14X17)-4-3-5-2-3-2-2	0.65

**Synthetic 3**

<b>Line</b>	<b>Name</b>	<b>Pedigree</b>	<b>GCA</b>
32	CLA18	SA4HC(8x36)-1-5-1-3-7-4-B-B-B-B	-0.06
33	CLA41	SA4C2HC(21x26)-1-2-2-2-B-B-B-B-B	0.12
36	CLA99	SA4C4HC40-7-1-2-3-3-1-1-B	-0.04
37	CLA106	SA4C4HC86-13-1-2-5-2-3-1-B	-0.06
38	CLA139	P24STEC1HC45-1-2-3-4-4-BB-f-##-BB-2	0.64
39	CLA141	ParentofSW-DMR89145-1No.6-B	-0.03
42	CML308	SINT.AM.TSR-23-3-1-2-4-BB-F	0.03

**Synthetic 4**

<b>Line</b>	<b>Name</b>	<b>Pedigree</b>	<b>GCA</b>
3	CLA37	SA5C2HC(26x21)-4-1-5-6-B-B-B-B	-0.26
4	CLA86	SA3C6HC91-3-1-1-2-2-3-2-B	0.94
5	CLA91	SA4C4HC19-3-2-2-2-3-4-3-B	0.96
6	CLA95	SA4C4HC40-7-1-2-2-1-2-1-B	1.23

7	CLA97	SA4C4HC40-7-1-2-2-1-2-3-B	0.87
9	CLA105	SA4C4HC86-13-1-2-5-2-1-2-B	1.3

### Synthetic 5

Line	Name	Pedigree	GCA
60		SRR-C2 SA3MH160-2-3-2-B	0.68
61		SRR-C2 SA3MH24-1-4-1-B	0.75
62		SRR-C2 SA3MH58-3-3-1-B	0.36
63		SRR-C2 SA3MH24-1-1-3-B	0.25
65		SRR-C2 SA3MH53-1-5-1-B	0.66
67		SRR-C2 SA3MH53-1-1-2	0.61
69		SRR-C2 SA3MH24-1-1-1	0.51

### Synthetic 6

Line	Name	Pedigree	GCA
43	CL02839	Ac8328BNC6-166-1-1-1-B*9-B	0.69
44	CML413	Sint Am.TSR-23-3-2-3-2-BB-f-# #-BBBB	0.32
45	CML451	[NPH28-1*G25)*NPH28]-1-2-1-1-3-1-B*6	0.37
46	CML172	G25QS4B-MH35-2-B-1-1-2-B-4-B-B-B-B	0.31
64		SRR-C2 SA4MH28-2-1-1-B	0.78
66		SRR-C2 SA4MH79-1-4-2	0.31
68		SRR-C2 SA4MH71-2-2-1-B	0.32

### Synthetic 7

Line	Name	Pedigree	GCA
44	CML413	Sint Am.TSR-23-3-2-3-2-BB-f-# #-BBBB	0.32
49		[CML312xCML357]-B-B-4-B-B-B-B	1.59
50		[CML322xCML363]-B-1-1-B-B-B	1.35
53		[CML380xCLA41]-B-1-3-B-B-B	0.96
54		[CML373xCML363]-B-1-1-B-B-B-B	0.85
55		[CML384xCLA18]-B-1-4-B-B-B-B	0.8

### Synthetic 8

Line	Name	Pedigree	GCA
15	CLA10	SINT.AM.TSR76-1-2-3-2-BBB-f	0.75
45	CML451	[NPH28-1*G25)*NPH28]-1-2-1-1-3-1-B*6	0.37
56		[CML373xCLA10]-B-1-2-B-B-B-B	0.8
57		[CML373xCML361]-B-1-4-B-B-B-B	0.7

58	[CML375xCML362]-B-1-2-B-B-B	0.65
59	[CML380xCLA18]-B-B-10-B-B-B-B	0.54

### Synthetic 9

Line	Name	Pedigree	GCA
70		(CML172xCLQ6601)-S1b-S2b-5-B	1.58
71		(CML172xCML168)-S1b-S2b-4-B	2.22
72		(CML172xCML168)-S1b-S2b-1-B	1.49
73		(CML172xCLQ6601)-S1b-S2b-8-B	1.93
74		[(CML161xCML165)xCML172]-S1b-S2b-	0.75
79		[(CML161xCML165)xCML172]-S1b-S2b-	1.48

### Synthetic 10

Line	Name	Pedigree
1	CLA7	SA3C4HC(16x25)-2-1-1-2-B-B-B-B
2	CLA25	SA3C4HC(16x25)-2-1-6-8-B-B-B-B
3	CLA37	SA5C2HC(26x21)-4-1-5-6-B-B-B-B
12	CLA154	SA3C4HC(16X25)-2-4-3-1-3-3-1
13	CLA156	SA3C4HC(16X25)-2-4-3-6-1-1-2
14	CLA158	SA3C4HC(16X25)-2-4-6-7-3-1-3
16	CLA12	SA3C4HC(16x25)-2-1-2-4-B-B-B-B
31	CLA46	SA5C2HC(26x21)-4-3-7-5-B-B-B-B-B

### Synthetic 11

Line	Name	Pedigree
49		[CML312xCML357]-B-B-4-B-B-B-B
72		(CML172xCML168)-S1b-S2b-1-B
73		(CML172xCLQ6601)-S1b-S2b-8-B
74		[(CML161xCML165)xCML172]-S1b-S2b-5-B
75		[(CML161xCML165)xCML172]-S1b-S2b-8
76		(CML172xCLQ6601)-S1b-S2b-11-B
77		(CML172xCML168)-S1b-S2b-6-B
78		(CML172xCLQ6601)-S1b-S2b-9-B
79		[(CML161xCML165)xCML172]-S1b-S2b-3-B

### Synthetic 12

Line	Name	Pedigree
1	CLA7	SA3C4HC(16x25)-2-1-1-2-B-B-B-B

5	CLA91	SA4C4HC19-3-2-2-2-3-4-3-B
25	CLA85	SA3C6HC91-3-1-1-2-2-3-1-B
33	CLA41	SA4C2HC(21x26)-1-2-2-2-B-B-B-B-B
36	CLA99	SA4C4HC40-7-1-2-3-3-1-1-B
38	CLA139	P24STEC1HC45-1-2-3-4-4-BB-f-##-BB-2
47		[CML373xCML361]-B-B-2-B-B-B-B
49		[CML312xCML357]-B-B-4-B-B-B-B
64		SRR-C2 SA4MH28-2-1-1-B

### Synthetic 13

Line	Name	Pedigree
7	CLA97	SA4C4HC40-7-1-2-2-1-2-3-B
8	CLA100	SA4C4HC40-7-1-2-3-3-1-2-B
20	CLA44	SA3C4HC(16x25)-2-4-3-6-B-B-B-B-B
43	CL02839	Ac8328BNC6-166-1-1-1-B*9-B
44	CML413	Sint Am.TSR-23-3-2-3-2-BB-f-# #-BBBB
46	CML172	G25QS4B-MH35-2-B-1-1-2-B-4-B-B-B-B
47		[CML373xCML361]-B-B-2-B-B-B-B
52		[CML322xCLA10]-B-B-2-B-B-B-B
53		[CML380xCLA41]-B-1-3-B-B-B

Supplementary Table 2. Locations and brief description of the field sites in Colombia where the synthetics were phenotyped.

Location (trial)	Longitude	Latitude	Altitude (masl)	Temperature range (°C)		Precip. (mm)	Environment
				Min	Max		
La Catalina (6)	75°44' W	4°45' N	1321	17.3	27.1	2062	Mid-altitude high potential maize production; some diseases are frequent due to high rainfall.
Menegua (3)	72°53' W	4°06' N	181	20	31.8	2821	Acid soils environment typical of the savannas.
Palmira (1,8)	76°18' W	3°32' N	1000	18.2	29.7	1100	Lowland tropical high potential maize production, little pressure from diseases.
Paraguaicito (2,7)	75°45' W	4°23' N	1285	16.9	28.1	2118	Mid-altitude high potential maize production; some diseases are frequent due to high rainfall.
Villavicencio (4, 5, and 9)	72°28' W	4°03' N	467	20.5	30.7	4145	Acid soils environment typical of the piedmont.

Supplementary Table 3. Repeatability ( $R^2$ ) estimates on mean basis for all traits measured in the study.

	Yield (t/ha)	ASI (days)	Epp (No.)	Ant (days)	Silk (days)	Pht (cm)	Eht (cm)	Rlod (%)	Slod (%)	Mois (%)
$R^2$ (%) all <sup>†</sup>	92.4	56.6	26.0	91.7	93.2	82.0	87.1	44.8	65.1	48.4
$R^2$ (%) GS	97.2	67.1	81.5	94.0	84.1	95.3	93.6	50.4	69.1	82.3
$R^2$ (%) GCA	88.8	15.0	0.0	85.8	90.9	35.2	57.4	46.4	66.5	26.3

<sup>†</sup> Including check

ASI: Anthesis Silk Interval; Epp: Number of ears per plant; Ant = Time from planting to anthesis; Silk: Time from planting to silk; Pht: Plant height; Eht: Ear height; Rlod: Root lodging; Slod: Stalk lodging; Mois: Grain moisture at harvest;

Rotear: Ear rot; Easp: Ear aspect (1=good, 5=bad).