GENOTYPE-ENVIRONMENT INTERACTION ON PRODUCTIVITY AND PROTEIN QUALITY OF SYNTHETIC TROPICAL MAIZE (*Zea mays* L.) VARIETIES

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SUMMARY

*In Mexico, maize (*Zea mays* L.) is the main source of energy and proteins for the population. It is therefore indispensable to generate corn varieties with a high yield potential and a better amino acid profile. The objective was to determine the genotype-environment interaction on the productive capability, physical characteristics, and quality protein maize (QPM) in 16 synthetic tropical maize varieties. These varieties were grown in seven environments of the states of Veracruz and Guerrero, Mexico, in 2012 and 2013; the controls used were normal endosperm (VS-536) and QPM (V-537C), in a completely random block design with three replicates. Physical properties of the kernel were determined and protein, lysine and tryptophan contents of the kernel and endosperm were quantified. Significant differences ($P<0.01$) were found among varieties (V), environments (E), and their interaction (V×E). Cotaxtla 2012B (E1) and Iguala 2012B (E4) were the two environments with the highest yields (4.8 and 5.1t·ha^{-1}, respectively). Among varieties, the highest grain yield was obtained from VS-536 (4.08), 9C (3.96), 10C (3.95) and 11C (3.94t·ha^{-1}) across environments. All the varieties showed a small kernel (≤33g/100 kernels). Lysine and tryptophan in kernel and endosperm of varieties QPM ranged 2.62-3.83 and 2.34-3.25g/100g protein, respectively. Grain yield of varieties 1C, 3C, 4C, 5C, 6C, and 8C was associated to better kernel and endosperm protein quality. The results of the current study indicate the existence of a high variability and the possibility of improving QPM cultivars for the evaluated traits.*

Introduction

Maize (*Zea mays* L.) has an important role in human and animal nutrition in some 25 developing countries worldwide, especially in Africa and Latin America (Prasanna *et al*., 2001), providing on average 37 and 46% of the daily protein and energy requirements for the human body (FAO, 2014). Like other cereals, maize has a low biological value compared against proteins from animal sources; therefore, family nuclei in the rural zones of Mexico usually eat maize products accompanied with

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INTERACCIÓN GENOTIPO-AMBIENTE EN PRODUCTUVIDAD Y CALIDAD PROTEÍNICA DE VARIEDADES SINTÉTICAS TROPICALES DE MAÍZ

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RESUMEN

*En México, el maíz (*Zea mays *L.) es la principal fuente de energía y proteínas de la población, por ello es indispensable generar variedades de maíz con alto potencial de rendimiento y un mejor perfil de aminoácidos. El objetivo fue determinar la interacción genotipo-ambiente en la capacidad productiva, las características físicas y la calidad de la proteína (ACP) en 16 variedades sintéticas tropicales de maíz. Estas variedades se cultivaron en siete ambientes del estado de Veracruz y Guerrero, México en 2012 y 2013; como testigos se usaron las variedades de endospermo normal (VS-536) y de ACP (V-537C), bajo un diseño de bloques completos al azar con tres repeticiones. En la cosecha se tomó una muestra aleatoria de 500 g por variedad y se determinó las propiedades físicas del grano y cuantificó el contenido de proteína, lisina y triptófano en grano y endospermo, por duplicado. Se encontraron diferencias significativas (P≤0,01) entre variedades (V), ambientes (A) y para la interacción (VxA). Cotaxtla 2012B (A1) e Iguala 2012B (A4) fueron los ambientes que presentaron los más altos rendimientos (4,8 y 5,1 t ha^{-1}, respectivamente). Entre variedades, el mayor rendimiento de grano se obtuvo con VS-536 (4,08), 9C (3,96), 10C (3,95) y 11C (3,94 t ha^{-1}) a través de ambientes. Todas las variedades presentaron un grano pequeño (≤33g/100 granos). El contenido de lisina y triptófano en grano y endospermo varió de 2,62 a 3,83 y 2,34 a 3,25g/100g de proteína, respectivamente. El rendimiento de grano de las variedades 1C, 3C, 4C, 5C, 6C y 8C se asoció con una mejor calidad de proteína en grano y endospermo. Los resultados del presente estudio, indican la existencia de alta variabilidad y la posibilidad de mejoramiento de las características de los cultivares de ACP evaluados.*

INTERAÇÃO GENÓTIPO-AMBIENTE EM PRODUTIVIDADE E QUALIDADE PROTÉICA DE VARIEDADES SINTÉTICAS TROPICAIS DE MILHO

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RESUMO

*No México, o milho (*Zea mays *L.) é a principal fonte de energia e proteínas da população, por isto é indispensável gerar variedades de milho com alto potencial de rendimento e um melhor perfil de aminoácidos. O objetivo foi determinar a interação genótipo-ambiente na capacidade produtiva, as características físicas e a qualidade da proteína (ACP) em 16 variedades sintéticas tropicais de milho. Estas variedades se cultivaram em sete ambientes do estado de Veracruz e Guerrero, México em 2012 e 2013; como testemunhas se usaram as variedades de endosperma normal (VS-536) e de ACP (V-537C), sob um desenho de blocos completos aleatórios com três repetições. Na colheita se tomou uma amostra aleatória de 500g por variedade, se determinaram as propriedades físicas do grão e se quantificou o conteúdo de proteína, lisina e triptofano em grão e endosperma, por duplicado. Encontraram-se diferenças significativas (P≤0,01) entre variedades (V), ambientes (A) e para a interação (VxA). Cotaxtla 2012B (A1) e Iguala 2012B (A4) foram os ambientes que apresentaram os mais altos rendimentos (4,8 e 5,1 t ha^{-1}, respectivamente). Entre variedades, o maior rendimento de grão se obteve com VS-536 (4,08), 9C (3,96), 10C (3,95) e 11C (3,94 t ha^{-1}) através de ambientes. Todas as variedades apresentaram um grão pequeno (≤33g/100 grãos). O conteúdo de lisina e triptofano em grão e endosperma variou de 2,62 a 3,83 e 2,34 a 3,25g/100g de proteína, respectivamente. O rendimento de grão das variedades 1C, 3C, 4C, 5C, 6C e 8C se associou com uma melhor qualidade de proteína em grão e endosperma. Os resultados do presente estudo, indicam a existência de alta variabilidade e a possibilidade de melhoramento das características dos cultivares de ACP avaliados.*

beans, which help to somehow balance this protein deficiency, so as to compensate for the maize limitations (Krivanek *et al*., 2007).

The improvement of quality protein maize (QPM) began with the aim of improving the nutritional value of the protein in the maize kernel (Krivanek *et al*., 2007). The commonly used approach is based on manipulating the *opaco-2 (o2)* mutation, a recessive gene that increases lysine and tryptophan levels in maize kernels (Ignjatovic-Micic *et al*., 2010). However, the incorporation of the *o2* gene into high yield cultivars has not been commercially successful due to its pleiotropic effect (soft endosperm, susceptibility to pests in stored grains, uprooting by winds, etc.). Fortunately, these problems have been corrected through the manipulation of three genetic systems: a) the simple recessive allele of the *opaco-2* gene; b) endosperm modifiers contained in *o2o2*, which increase lysine and tryptophan levels; and c) genes that modify the soft endosperm and transform it into hard endosperm (Vasal, 2000; Vivek *et al*., 2008).

The term QPM (quality protein maize) refers to maize with a higher lysine and tryptophan content and a relatively hard endosperm, which makes it resistant to pests during storage (Galicia *et al*., 2011). Thus, the contribution of QPM to human nutrition, especially in poor countries where maize is the staple food, has been well documented (Bressani, 1994; Krivanek *et al*., 2007; Vivek *et al*., 2008; Nuss and Tanumihardjo, 2011). However, in Mexico the use of these varieties has not become general practice, and native maize is grown or varieties with a normal endosperm offered by seed producers (Zepeda-Bautista *et al*., 2009).

The local conversion of different groups of normal lines to QPM began in 2002, within the Maize Program of the Cotaxtla Experimental Field (CECOT, from its Spanish initials), which belongs to the National Institute of Forestry, Agricultural and Livestock

Research (INIFAP), Mexico. Initially, the lines were crossbred with line CML-144 from the International Maize and Wheat Improvement Center (CIMMYT), which acted as the donor of the *o2* characteristic. The crossbreeds self-pollinated and the selected lines were backcrossed to recover the characteristic. After this selection process, in 2008, simple crosses were formed through a diallel scheme to find the specific combinatory aptitude (SCA) and the general combinatory aptitude (GCA) of the participating lines (Andrés-Meza *et al.*, 2011). Using the lines with the highest grain yield and high GCA, 11 maize synthetics were integrated. Under this context, the present goal was to determine the genotype-environment interaction on the productive capability, physical characteristics and protein quality in 16 synthetic tropical maize varieties.

Materials and Methods

Field evaluations were carried out on 16 maize varieties, 11 of which are new experimental synthetic maize varieties, integrated from lines with different levels of inbreeding and selected for their good performance *per se* and high effects of general combinatory aptitude of the component lines (Andrés-Meza *et al.*, 2011). Three are experimental synthetics selected for drought tolerance (TS6, LPSC3, and 3SC), and two were commercial varieties (VS-536 and V-537C), the first of the latter was obtained through genetic recombination of nine inbred lines from the maize programs in Cotaxtla, Veracruz, Iguala, Guerrero, Rio Bravo, Tamaulipas, and Ocotlan, Jalisco, Mexico, while the second one was obtained through genetic recombination of 10 half-sib families from the Poza Rica 8763 population (Table I).

The varieties were sown in seven different environments during the years 2012 and 2013. Five environments were established in the central zone of Veracruz state and the other two in Iguala, Guerrero, Mexico. The planting and harvest times, planting conditions, and fertilization doses are shown in Table II. Total fertilizer P_2O_5, K_2O and one third of N were applied 10 days after planting (dap) and the remaining N (as urea) was applied 30 dap.

The precipitation patterns and amounts differed markedly between the 2012 and 2013 growing seasons. Standard weather data were recorded for each site using the nearest weather observatories set up in ~1km from the experimental sites. Each station recorded the daily maximum and minimum air temperature (ºC) and rainfall (mm) (Figures 1a, b).

The establishment of the essays in each location coincided with the beginning of the rainy season, except for the fall-winter cycle, when sufficient auxiliary irrigation (seven times) was applied so the plants would not suffer from water stress. The experimental unit was two rows, 5m long by 0.8m wide each. Planting was done *tapa pie*, with the feet,

TABLE I
GERMPLASM GROWN IN SEVEN ENVIRONMENTS IN THE STATES OF VERACRUZ AND GUERRERO, MEXICO (2012-2013)

Nº	Genealogy	Lines*	Seeds*	Level
1	SYNTHETIC-1C	20	20	Experimental
2	SYNTHETIC-2C	10	15	Experimental
3	SYNTHETIC-3C	9	20	Experimental
4	SYNTHETIC-4C	12	15	Experimental
5	SYNTHETIC-5C	12	15	Experimental
6	SYNTHETIC-6C	9	20	Experimental
7	SYNTHETIC-7C	8	25	Experimental
8	SYNTHETIC-8C	6	15	Experimental
9	SYNTHETIC-9C	6	20	Experimental
10	SYNTHETIC-10C	8	15	Experimental
11	SYNTHETIC-11C	8	20	Experimental
12	SYNTHETIC-TS6	12	15	Experimental
13	SYNTHETIC-LPSC3	12	15	Experimental
14	VS-536	9	15	Commercial
15	V-537C	10	15	Commercial
16	SYNTHETIC-3SEQ	12	15	Experimental

* Number of lines and seeds that integrate each synthetic variety.

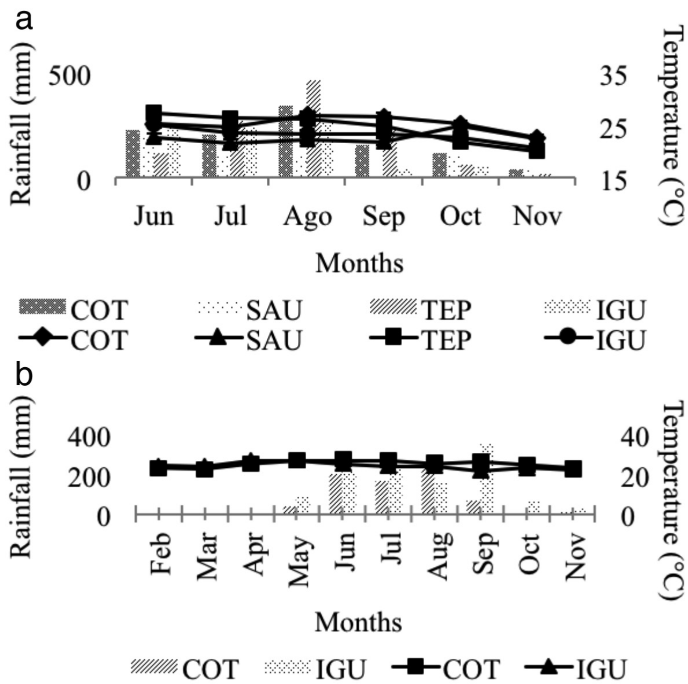

Figure 1. Mean rainfall and temperatures during the years 2012 (a) and 2013 (b) throughout seven environments in the states of Veracruz and Guerrero, Mexico; fall-winter cycle = in the months from December to May; spring-summer cycle= in the months from June to November; COT = Cotaxtla; SAU = El Sauce; TEP = Tepetates; IGU = Iguala.

TABLE II
CHARACTERISTICS OF SEVEN MEXICAN ENVIRONMENTS FOR THE EVALUATION OF 16 SYNTHETIC VARIETIES OF QPM) WITH NORMAL ENDOSPERM (2012-2013)

Identification	Location	State	Geographical location	Altitude (m)	Planting condition	Planting/ harvest dates	Fertilization (Kg·ha^{-1} N-P-K)
E1	Cotaxtla 2012B	Veracruz	18º 56' N, 96º 11' W	18	Rainfed	20/06/12-06/11/12	161-46-00
E2	El Sauce 2012B	Veracruz	18º 42' N, 96º 04' W	19	Rainfed	26/07/12-27/11/12	161-46-00
E3	Tepetates 2012B	Veracruz	19º 11' N, 96º 20' W	18	Rainfed	18/07/12-24/11/12	140-70-00
E4	Iguala 2012B	Guerrero	17º 52' N, 99º 09' W	750	Rainfed	25/07/12-25/11/12	90-60-00
E5	Cotaxtla 2013A	Veracruz	18º 56' N, 96º 11' W	18	Irrigation	21/02/13-12/06/13	161-46-00
E6	Cotaxtla 2013B	Veracruz	18º 56' N, 96º 11' W	18	Rainfed	26/07/13-23/11/13	161-46-00
E7	Iguala 2013B	Guerrero	17º 52' N, 99º 09' W	750	Rainfed	31/07/13-21/11/13	90-60-00

which is the traditional way to plant in the region. Three seeds were planted per hole, 0.4m from each other, to be later thinned to two plants (62500 plants/ha).

Yield and physical characteristics of the kernel

Grain yield (t·ha^{-1}) was estimated on the field, represented by the weight of the cob per lot, adjusted to 14% humidity and multiplied by the ratio of the size of the lot compared against an hectare, thus transforming it to t·ha^{-1}. The weight per 100 kernels (PCG) (Billeb and Bressani, 2001) was recorded. The flotation index (FI), which estimates the relative density of kernels and is an indirect measure of kernel hardness was determined: 100 grains were immersed in sodium nitrate solutions (41g in 100ml of water) with a density of 1.25 at 23ºC; the grains were stirred and after 1min the number of floating grains was counted. Hardness classification of kernels was based on the scale proposed by Salinas *et al.* (1992), such that FI values of 0-12% are very hard kernels, of 13-37% hard, 38-62% intermediate, 63-87% soft and >87% very soft kernels. Finally, visual grading (VG) of the type of endosperm as an indirect hardness of the kernel: values between 1-2 are very hard (VH), 3-4 hard (H), 5-6 intermediate (I), 7-8 soft (S), and 9-10 very soft (VS) endosperm (Vázquez *et al.*, 2012).

Nitrogen, lysine, and tryptophan quantification

Samples of endosperm (without tip cap, pericarp, and germ) as well as from the whole kernel were taken from each genotype. The samples were ground in a Tecator Cyclotec 1093 grinder, sieve size 0.5mm. The obtained flour was subjected to a de-greasing process using petroleum ether in a Soxhlet intermittent extractor for 6h. Afterwards, they were dried in the open air to eliminate excess petroleum ether (Galicia *et al.*, 2009, 2012).

Nitrogen quantification was carried out with a Technicon II Autoanalyzer (#334-74, 1997) following the methodology of Galicia *et al.* (2009). The amount of protein was estimated from the nitrogen value by multiplying the nitrogen percentage (N)×6.25 (conversion factor for maize) (Vivek *et al.*, 2008). Lysine quantification was done following the colorimetric method described by Villegas *et al.* (1984) modified by Galicia *et al.* (2011), which is based on the reaction of 2-chloro, 3,5-dinitropyrine; reading was done in a microplate reader (μQuant MQX200, BioTek®) to determine optical density (DO) at 390nm. Lastly, tryptophan quantification was done according to the method by Nurit *et al.* (2009) modified by Galicia *et al.* (2012), which is based on the reaction of glyoxilyc acid; reading was done in a plate spectrophotometer at 560nm. Both amino acids are expressed as a percentage of protein.

Statistical analysis

Laboratory analyses were performed in duplicate. All the evaluated variables were analyzed under a completely random design, except for the grain yield variable, which was done in a completely random block design with three replicates. Combined variance analyses and mean comparison tests (Tukey) were done with the SAS/STAT®software, version 9.0 (SAS, 1990).

Results

With regard to the combined analysis, there were significant differences (P<0.01) among varieties (V), environments (E), and the variety × environment (V×E) interaction on yield, physical characteristics, and protein quality (Table III). These differences are due to the genetic variability among varieties due to particular genetic characteristics, to the effect of the environment, and to crop management. The variation coefficients fluctuated from 2.33 to 20.84. These values suggest reliability in the obtained results. Research results have demonstrated the competitiveness for grain yield of QPM with the best normal maize cultivars in numerous tropical environments (Vergara *et al.*, 2000). In some works hybrids yielded more grain than open-pollinated cultivars, but mean grain yield did not differ for single cross, three-way, and double-cross hybrids (Cordova and Pandey, 1999; Pixley and Bjarnason, 2002).

Genotype × environment interaction on yield and physical characteristics of the kernel

The differences among varieties (P<0.01) show different levels of productivity that were affected by the V×E interaction. Across all environments, varieties 9C, 10C, and 11C, with mean yields of 3.94, 3.95, and 3.96t·ha^{-1}, respectively, were statistically similar to the better control variety VS-536, which showed the highest mean yield (4.08t·ha^{-1}) (Table IV). On their part, locations Cotaxtla 2012B (E1) and Iguala 2012B (E4) (where 'B' stands for 'spring-summer growing season') showed the highest yields (Table V). The best variety for environment E1 was 9C, and for E4, it was 10C, with mean yields of 6.18 and 6.27t·ha^{-1}, respectively (data not shown). In E1 the flowering took place in August 2012, when the minimum average temperature was 20.6ºC while the maximum average temperature was 31.3ºC, and the rainfall was 347.3mm (Figure 1a). On the other hand, E4 flowering occurred in September 2012, when the minimum average temperature

TABLE III
MEAN SQUARES AND STATISTICAL SIGNIFICANCE OF 16 SYNTHETIC VARIETIES OF QPM WITH NORMAL ENDOSPERM GROWN IN SEVEN ENVIRONMENTS IN THE STATES OF VERACRUZ AND GUERRERO, MEXICO (2012-2013)

Source of variation	DF	GY	Visual grading	WHG	FI (%)	Protein (%)		Tryptophan ¶¶ (g/100g protein)		Lysine ¶¶ (g/100g protein)	
						Kernel	Endosperm	Kernel	Endosperm	Kernel	Endosperm
Variety (V)	15	2.19**	9.18**	25.07**	419.64**	3.89**	1.98**	0.09**	0.05**	1.38**	0.59**
Environment (E)	6	58.13**	8.19**	16.43**	3628.57**	19.79**	14.06**	0.22**	0.31**	10.84**	4.99**
V×E interaction	90	0.77**	3.32**	19.73**	363.58**	1.54**	1.37**	0.04**	0.03**	0.90**	0.41**
Error	224	0.48	0.48	0.99	12.67	0.06	0.22	0.01	0.01	0.14	0.04
CV (%)		20.09	20.84	4.23	9.87	2.33	6.04	3.42	6.54	12.03	7.77
R^2		0.80	0.81	0.98	0.98	0.97	0.91	0.91	0.94	0.98	0.97

*, ** Different from cero at a 0.05 and 0.01 probability, respectively; GY: grain yield; WHG: one hundred kernel weight; FI: flotation index; DF: degrees of freedom; CV(%): coefficient of variation; ¶¶ informed in dry base, oil free samples.

TABLE IV
AVERAGE YIELD, PHYSICAL AND CHEMICAL CHARACTERISTICS OF THE KERNELS FROM 16 SYNTHETIC VARIETIES OF QPM WITH NORMAL ENDOSPERM GROWN IN SEVEN ENVIRONMENTS IN THE STATES OF VERACRUZ AND GUERRERO, MEXICO (2012-2013)

Variety	GY (t·ha^{-1})	Protein¶ (%)		Lysine¶ (g/100g protein)		Tryptophan¶ (g/100g protein)		VG	Hardness type of endosperm	WHG	FI (%)	Hardness of whole kernel
		Kernel	End	Kernel	End	Kernel	End					
1C	3.48 abc*	10.38 de	7.36 d	3.71 ab	3.25 a	0.74 d	0.63 bc	4.67 a	I	24 b	39.00 bcde	I
2C	3.43 abc	10.39 de	7.79 bcd	3.35 abc	2.47 cdef	0.69 fg	0.55 efg	3.10 de	H	23 c	38.50 bcde	I
3C	3.83 ab	9.94 f	7.39 d	3.83 a	2.53 bcdef	0.92 a	0.65 b	3.90 bc	H	25 b	36.33 cdef	H
4C	3.62 abc	10.15 ef	7.79 bcd	3.71 ab	2.79 b	0.74 d	0.63 bc	2.95 def	H	25 b	30.50 fghi	H
5C	3.44 abc	9.83 f	7.83 bcd	3.37 abc	2.62 bcd	0.81 c	0.63 bc	2.90 ef	H	22 d	32.00 efgh	H
6C	3.52 abc	10.29 e	8.55 a	3.05 cd	2.34 def	0.72 de	0.55 defg	2.90 ef	H	21 d	37.50 bcdef	I
7C	3.60 abc	10.64 cd	7.42 d	2.99 cd	2.54 bcdef	0.67 fg	0.5 5defg	2.81 ef	H	25 b	23.17 i	H
8C	3.77 ab	10.42 de	7.35 d	3.06 cd	2.56 bcde	0.70 ef	0.52 g	2.71 ef	H	23 c	34.50 cdefg	H
9C	3.96 a	10.64 cd	7.60 cd	2.55 d	2.63 bcd	0.80 c	0.60 cde	4.33 ab	H	22 d	39.50 bcd	I
10C	3.95 a	11.23 ab	7.74 bcd	3.14 bcd	2.41 def	0.68 fg	0.57 defg	3.10 de	H	24 b	28.00 ghi	H
11C	3.94 a	10.95 bc	7.60 cd	2.62 d	2.50 bcdef	0.66 g	0.58 def	2.81 ef	H	28 a	33.67 defg	H
TS6	3.35 abc	11.46 a	8.34 ab	2.93 cd	2.24 f	0.66 g	0.54 fg	2.33 f	VH	25 b	25.17 hi	H
LPSC3	3.01 c	11.32 a	8.26 ab	3.23 abc	2.73 bc	0.68 fg	0.60 bcd	4.00 ab	H	25 b	34.67 cdefg	H
VS-536	4.08 a	11.51 a	8.20 abc	3.03 cd	2.30 ef	0.61 h	0.54 fg	3.19 cde	H	21 d	44.33 b	I
V-537C	3.11 bc	10.44 de	7.74 bcd	3.10 cd	2.61 bcd	0.84 b	0.76 a	3.90 bc	H	21 d	58.83 a	I
3SC	3.18 bc	10.96 bc	7.46 d	2.81 cd	2.29 ef	0.73 de	0.63 bc	3.67 bcd	H	21 d	41.17 bc	I
HSD	0.44	0.32	0.61	0.59	0.32	0.03	0.05	0.76		2.01	7.35	
R^2	0.8	0.97	0.91	0.91	0.94	0.98	0.97	0.81		0.98	0.98	

* Means with the same letter in a column are not statistically different (Tukey, 0.05). GY: grain yield; VG: visual grading, where: 1-2 Very hard (VH), 3-4 Hard (H), 5-6 Intermediate (I), 7-8 Soft (S), 9-10 Very soft (VS); WHG: one hundred kernel weight; FI: flotation index, where: 0-12% Very hard (VH), 13-37% Hard (H), 38-62% Intermediate (I), 63-87% Soft (S), ≥87% Very soft (VS); End: endosperm; HSD: honestly significant difference; ¶ informed in dry base, oil free samples.

TABLE V
INFLUENCE OF THE ENVIRONMENT ON GRAIN YIELD, PHYSICAL AND CHEMICAL CHARACTERISTICS OF THE KERNELS IN 16 VARIETIES OF SYNTHETIC QPM WITH NORMAL ENDOSPERM GROWN IN SEVEN ENVIRONMENTS IN THE STATES OF VERACRUZ AND GUERRERO, MEXICO. (2012-2013)

Environment	GY (t·ha^{-1})	Visual grading¶¶	Hardness type of endosperm	WHG§	FI§§ (%)	Hardness of whole kernel	Protein¶ (%)		Tryptophan¶ (g/100 g protein)		Lysine¶ (g/100 g protein)	
							Kernel	Endosperm	Kernel	Endosperm	Kernel	Endosperm
E1	4.8 a*	3.4 ab	D	-	-	-	9.75 f	7.22 d	0.71 c	0.57 d	3.00 b	2.78 b
E2	3.2 cd	3.0 c	D	-	-	-	11.95 a	8.60 a	0.62 e	0.53 e	2.93 b	2.20 c
E3	3.3 c	3.3 bc	D	-	-	-	10.93 c	8.12 bc	0.67 d	0.56 d	3.00 b	2.05 d
E4	5.1 a	2.6 d	D	-	-	-	9.95 e	7.15 d	0.67 d	0.63 c	2.67 c	2.78 b
E5	3.9 b	3.8 a	D	23.1 b	30 b	D	10.31 d	8.43 ab	0.79 b	0.46 f	4.17 a	2.94 a
E6	2.8 d	3.6 ab	D	23.0 b	30 b	D	10.40 d	6.96 d	0.81 a	0.67 b	-	-
E7	1.9 e	3.6 ab	D	24.3 a	48 a	I	11.33 b	7.96 c	0.83 a	0.76 a	-	-
HSD	0.44	0.76		2.01	7.35		0.32	0.61	0.59	0.32	0.03	0.05

* Means with the same letter in a column are not statistically different (Tukey, 0.05). GY: grain yield; ¶¶ visual grading, where: 1-2 is very hard (VH), 3-4 hard (H), 5-6 intermediate (I), 7-8 soft (S), 9-10 very soft (VS); WHG: one hundred kernel weight; FI: flotation index, where: 0-12% is very hard (VH), 13-37% hard (H), 38-62% intermediate (I), 63-87% soft (S), ≥87% very soft (VS); ¶ informed in dry base, oil free samples. For environment identification see Table II. -: no record.

was 17.8ºC, the maximum average temperature was 31.5ºC, and rainfall was 48.8mm (Figure 1a).

The location Iguala 2013B (E7) had the lowest grain yield. In this location, the best performance was that of variety 10C with a mean yield of 3.17t·ha^{-1} (data not shown). The results found in this location can be attributed to the fact that during the crop cycle, the climatic conditions were atypical, particularly so during the month of September, when it rainfall was 357mm, followed by a period of drought in October, with rainfall of 38.2mm and relatively hotter than the E6 location (Cotaxtla 2013B), thus affecting grain filling (Figure 1b). According to Stapper and Fischer (1990), during grain filling, for each degree centigrade of temperature rise, development increases more than growth, which reduces grain yield up to 4%.

In Cotaxtla 2013A (E5) (where 'A' stands for 'fall-winter growing season'), under irrigation, the mean yield of all varieties was greater than those of Cotaxtla 2013B (E6), rainfed, at 3.9 and 2.8t·ha^{-1}, respectively. Environment E6 differed from E5 because the establishment of the crop was late, since according to the technical recommendations by INIFAP, late plantings are more susceptible to pests and diseases. In the latter environment, the best performance was that of variety 4C with 3.45t·ha^{-1} (data not shown).

With regard to the physical characteristics of the kernel, the lowest weight per 100 kernels (WHG) (smallest kernel size) and the highest flotation index (IF) (intermediate hardness) was obtained for varieties VS-536, V-537C and 3SEQ throughout all seven study environments. Also, when visually classifying for the type of endosperm, these showed a hard kernel (Table V). Kernel hardness is related with the time that it needs for nixtamalization; the harder the kernel, the more cooking time is needed to obtain a nixtamal (*tortilla* dough) with the adequate characteristics for quality dough (Serna-Saldivar and Rooney, 2003; Rooney and Serna-Saldivar, 2003).

All the maize varieties evaluated had small kernels (≤33g/100 kernels); however, those planted in E7 had a greater WHG (24.3g/100 kernels), and were statistically different ($P \leq 0.05$) from those in E5 and E6 (Table V). The reduced weight and size of the kernels is attributed to the lower temperature in E5 during the first three months of the year (Figure 1b). Environment E6 is characterized for suffering the climatological phenomenon known as *canicula* or 'midsummer drought', which lasted for 40 days from August to September, mainly affecting the individual weight of the kernels (Figure 1b). Maize hardness has been shown to have an influence on the production efficiency or quality of the final product (Fox and Manley, 2009).

Protein, lysine, and tryptophan content

The mean content of total raw protein in QPM varieties in all seven test environments varied from 9.8 to 11.5% in kernels and from 7.4 to 8.6 in the endosperm (Table IV). These values are similar to those reported for whole kernels (7-10.1%) by Fufa *et al.* (2003) in five Ethiopian quality protein and normal maize cultivars; although they are higher than those reported for whole kernels (9.2-9.4%) by Pixley and Bjarnason (2002) in a group of tropical QPM with a wide genetic base. The QPM varieties in environment E2 developed greater protein content in the kernels and endosperm. In this location, the best registered values were those of the variety TS6 with 13.8% in kernels and 10.3 in the endosperm (data not shown).

The endosperm of QPM is different from that of normal maize endosperm in that it synthesizes a greater amount of the lysine and tryptophan fractions (albumines, globulines, and glutelines), and less prolamines, which is the major fraction in maize with normal endosperm and deficient in these amino acids. Thus, the protein concentration between these two types of maize might be the same, but what distinguishes QPM is that their endosperm has a greater amount of these amino acids. In this case, the mean protein reduction from the removal of the germ was 2.9% (Table IV).

Throughout all environments, genotypes 1C, 2C, 3C, 4C, 5C, and 10C had a greater concentration of lysine in the kernel than did the QPM control, while 6C, 7C, and 8C were statistically equal to the control V-537C. As far as the endosperm, only variety 1C was better than the control, and eight genotypes were statistically equal to the control in lysine concentration (Table IV). In environment E5, whole kernels and endosperm of all the varieties showed the highest levels of lysine; particularly, the control variety V-537C showed a high mean lysine content in the kernel and endosperm, 4.90 and 3.35g/100g protein, respectively. These increases are because in this cycle, especially, irrigation was applied and therefore water availability was greater, which in turn reflected positively in this variable (Figure 1b).

Regarding the mean tryptophan content in whole kernels and endosperm, it varied from 0.61 to 0.92 and from 0.52 to 0.76g/100g protein, respectively; particularly so in variety 3C, where the level of tryptophan in whole kernels was statistically greater than the control variety V-537C, with 0.92g/100g protein. On the other hand, the tryptophan content of the endosperm was greater in the control (Table IV). In this regard, the maize varieties grown in environment E2 showed low concentrations of this amino acid, while those in environment E7 had the highest levels. It is inferred that great climatic variations positively affected tryptophan content.

Discussion

With regard to grain yield, throughout all the test environments, all the QPM varieties performed statistically equal ($P<0.05$) to the best control with normal grain, VS-536, but better than the control QPM variety V-537C. It can be said that these maize varieties show agronomic advantages over varieties with normal endosperm; therefore, they are good options to improve the nutritional level of consumers. In all seven environments, the QPM varieties showed a better performance, except for environments E2 and E3, where the normal endosperm variety VS-536 had the best performance. Previous studies have reported yields of hybrid QPM from the CIMMYT that are competitive against the best local normal endosperm cultivars in several tropical locations (Bjarnason and Vasal, 1992; Pixley and Bjarnason, 1993).

According to the physical properties of the kernels, all the evaluated varieties had small kernels (≤33g/100 kernels). In this regard, it is well-known that kernel weight is an indicator of kernel size and density; bigger kernels have a greater ratio of endosperm than do smaller kernels. Therefore, they have a greater yield in flour, which is an important characteristic for the dough and *tortilla* industry. Serna-Saldivar and Rooney (2003) indicate that this characteristic favors kernel hydration during the nixtamalization process, which affects the dough and gives off *tortillas* with better texture. The effect of the crop cycle significantly affected this characteristic ($P<0.05$), being environment E7 where the biggest kernels developed. Environments E5 and E6, planted with irrigation and rainfed, had no significant differences in kernel size; thus, the expression of the varieties was not affected by the water conditions.

According to the FI, as an indirect measurement of kernel hardness, 56% of the varieties were proven to have a hard grain, while the rest of them developed intermediate kernels. No relationship was found between FI and kernel texture through visual classification regarding the type of endosperm. The terms hard and soft are used to designate the ratio of floury and crystalline areas present within the endosperm of the kernel, a characteristic that influences kernel hardness. Salinas and Vazquez (2006) mention that the nixtamalized dough industry (IHN) and the *tortilla* dough industry prefer to process maize with intermediate sized kernels.

The highest protein content, both in the kernel and in the endosperm, was observed in the varieties with normal endosperm. Zarkadas *et al.* (2000) mention that the difference in protein content in favor of normal kernel maize is attributed to a greater presence of prolamines (zeins), especially alpha-zeins. Gutiérrez *et al.* (2008) reported that prolamines make up around 50-70% of the total proteins contained in the endosperm, deficient in lysine and tryptophan. Hasjim *et al.* (2009) mention that QPM are considered to have high quality proteins since they have sufficient lysine and tryptophan.

With regard to the amount of lysine and tryptophan present in the kernel and in the endosperm, there were statistical differences ($P<0.05$) among the synthetic maize varieties through all seven test environments (Table V). The highest lysine contents (g/100g protein) were those of varieties 1C, 3C, 4C, 5C, 6C, 8C, and V-537C, which in turn are related to the highest values of tryptophan and grain yield. According to De Groote *et al*. (2013), the current quality of maize improved for these characteristics is due to a decrease in the level of total protein. Zeins are the most affected, since they are the prevalent section, relatively increasing the presence of other proteins that are not deficient in lysine or tryptophan, such as albumines and globulines. Given that these cultivars show agronomic advantages as well as in kernel quality, they are a good option to improve the nutritional level of the people living in rural zones, who greatly depend on this cereal.

Conclusions

The effects of variety, environment and their interaction affected size, hardness, and kernel quality. The QPM varieties were competitive with regard to the controls VS-536 with normal kernel and QPM V-537C. No relationship was observed between IF and kernel texture through visual classification in the type of endosperm. All varieties developed small kernels and are thus accepTable for household processing as well as for the dough and *tortilla* industry. Out of the 16 evaluated varieties, only nine of them presented hard kernels, which could be useful for the nixtamalized dough industry. Environment E6 registered maize with the greatest concentrations of tryptophan; under irrigation, the synthetic varieties produced a higher percentage of lysine in the kernels and in the endosperm. The results of the current study indicated the existence of a high level of variability and the possibility of improving QPM cultivars for the traits evaluated. QPM is a nutritional enhanced crop awaiting widespread dissemination and the opportunity its potential for global health improvement.

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