Morphological diversity among local and introduced maize (*Zea mays* L.) varieties in Haiti for yield improvement

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Abstract

Maize, the cereal with the largest production in the world, is a staple crop in many developing countries including Haiti. Even if Haiti is the second maize consumer in the Caribbean region, its national yield is low and alleged of aflatoxin contamination. In order to increase grain yield, three introduced and two local maize varieties (these used as comparative control) were evaluated. Three field trials were carried out at Torbeck and Morne Briller (Port-Salut) during different growing seasons. Experiments were designed in a three-replicate randomized block. Each experimental plot was 3.50 m per 10.5 m with a gross area of 36.75 m^2 and 40 000 plants/ha densities. The trials were carried out according to local agricultural practices. The results revealed that the introduced varieties had a smaller yield than the local ones. Moreover, all varieties showed some aflatoxin content below the European Union limit (5µg/Kg). Statistical analyses showed a strong and direct correlation between yield and ear weight and an inverse correlation between yield and male and female flowering day after sowing. The broad phenotypic diversity suggested a rich reserve of alleles to exploit in a breeding program focused to improve food security in Haiti.

Introduction

Maize (*Zea mays* L.), the most important cereal produced worldwide is cultivated under temperate, tropical and subtropical climates. Among cereal crops, maize has the highest average yield per hectare and remains third after wheat and rice as for the total area and production in the world (Gami et al., 2017). In many developing countries, maize is a staple crop transformed in several derived products providing about 15-56% of the total daily calories intake (Nuss and Tanumihardjo, 2010).

In Caribbean region, Haiti with 50 g/person/day is the second maize consumer after Cuba (66 g/ person / day) (Ranum et al., 2014). Haiti imports almost 70% of the consumed maize as the national yield (0.225 MT; FAO, 2016) is less than the domestic consumption (Jolly et al., 2011; Molnar et al., 2015). Since the Haitian climate is suitable for an all year long maize cultivation (Aloys, 2014), the maize low yield, around 1t/ha (Molnar et al., 2015), is due to inappropriate genetic resources and poor agronomic practices.

The South Department is one of the most important maize growing areas of Haiti, and two local varieties, Maquina and Chiken corn, are the most spread among farmers, even if data on their genetic diversity and phenotypic traits are scarce (Aloys, 2014).

Data on the nature and magnitude of variation in the available material and the relationship between yield and other agronomic characters is a prerequisite to plan an effective breeding program to improve productivity (Mohammadi et al., 2003; Nemati et al., 2009; Rafiq et al., 2010).

Among others, the most yield determining traits are: ear weight, ear length, number of grains per row, number of rows per ear, plant height, days to 50% male and female flowering after sowing, number of leaves per plant, tassel length and stem diameter (Pavan et al., 2011; Zarei et al., 2012; Kumar et al., 2015).

The low Haitian yield is worsened by a high aflatoxin (AF) contamination (Aristil et al., 2017). To reduce the on-field AF contamination, good agricultural practices and superior genotypes are needed (Hell et al., 2000;

2013; Maina et al., 2016). The present study aims (i) to evaluate three introduced and two local maize varieties with special emphasis on yield and AF content, (ii) to investigate varieties genetic variability, (iii) to determine the relationships of yield with eleven phenotypic traits. This is the first characterization of Haitian local and introduced maize varieties focused on the selection of agronomic traits useful for a local breeding program.

Materials and Methods

Varieties

Five maize varieties were evaluated (Table 1). Three synthetic populations, never grown in Haiti, and selected for their tropical and subtropical origin (South Africa, Argentina) (Cantaluppi et al., 2017), were kindly provided by University of Milan (UNIMI). Two open pollinated local varieties were used as comparative controls.

Table 1 - Origin of tested maize varieties

Maize varieties	Origin	Characteristics
Maquina	Haiti	Local variety
Chicken corn	Haiti	Local variety
R4185	Argentina	Introduced
R4865	South Africa	Introduced
R4271X4185	Peru	Introduced

Fields trials and plants management

Three field trials (FTs) were conducted in South Haiti during three growth seasons (July 2016 - May 2017) (Table 2). Two fields were placed at the agricultural farm of the University Notre Dame of Haiti (UNDH), (Torbeck 18°10'N, 73°49'W, 13 m altitude) and one at "*Morne Briller*", Port-Salut (18°04'N, 73°55'W, 295 m altitude). The previous cultivated crops in Torbeck and at "*Morne Briller*" were maize and sorghum, respectively. All varieties were evaluated, under the same agricultural practices: three-replicate randomized complete block design, plot 3.50 m per 10.5 m (gross area of 36.75 m^2), fifteen subplots of 3 rows (13 plants) per plot, distance between subplots 1 m, spacing between either plant or row 0.50 m.

Table 2 - Fields trial location, relief, sowing and harvesting date

Field trials	Location	Relief	Sowing date	Harvesting date
E1	Torbeck	Plain	15/7/ 2016	12 /10/2016
E2	"*Morne Briller*"	Hill	14 /12/ 2016	18 /3/2017
E3	Torbeck	Plain	2 /2/2017	12/5/ 2017

Sowing was done by hand drilling at a seeding rate of 25 kg/ha. Two seeds per stand were sown, which were thinned to one plant per stand at V3 growth stage (Ritchieet et al., 1986) (2 weeks after sowing) corresponding to 40 000 plants/ha (Aydinsakir et al., 2013). The fertilization was performed at two growth stages: V4 (4 weeks after sowing), 44 Kg of urea (46-0-0, N-P-K) plus 30 kg of (20-20-10); V10 (10 weeks after sowing), 170 kg of 20-20-10 N-P-K. The total amount of Nitrogen (N), Phosphorus (P_2O_5) and Potassium (K_2O) was 60, 40 and 20 kg /ha, respectively. When rainfall was larger than 7 mm a week, no irrigation was performed, while after one week with no rainfall, plants were watered by hand. In order to keep the water-holding capacity in the optimum range, watering was performed one week before and after flowering. Two hoeing and handling weeding were performed two weeks after sowing and during the second fertilization, respectively, while harvest was carried out when the grain was at around 15% of humidity.

Aflatoxin quantification

The total aflatoxins (B1, B2, G1 and G2) content was assessed with Reveal® Q+ aflatoxin test (Neogen®) (detection range from 2 to 1500 µg/kg) according to the supplier's instruction within one week after harvest.

Data collection

Twelve phenotypic traits (PhTs) were recorded on eighteen plants per subplot namely: number of rows/ear (NRE), number of grains/row (NGR), ear weight (EW) and ear length (EL), grain yield (GY), leaf angle (LA), tassel length (TL), number of leaves per plant (NLP), plant height (PH), stem diameter (SD), female flowering days (FFD), and male flowering days (MFD), the latter two being the number of days from sowing to the time of ~50% flowering of silks and tassels, respectively, within a given subplot. Humidity of the grain was adjusted at 12% and yield (t/ha) was calculated according to Sesay et al. (2016). In addition to the phenotypic traits, rainfalls, maximal and minimal air temperatures were recorded using a pluviometer and a max/min thermometer, respectively. The temperature was recorded twice a day (8.30 AM; 05.30 PM).

Statistical analysis

The SPSS statistical package for Windows, v. 23.0 (SPSS Inc.) allowed assessment of statistical analyses. Shapiro–Wilk and Levene tests gave evidence of the normal distribution and homogeneity of variances, while the analysis of variance (ANOVA) concerned all data except climate parameters (Brown and Rothery, 1993). Combined analysis of variance using genotypes (G), sites (S) and SxG as fixed parameters allowed evaluation of the impact of each of them on PhTs;namely, ANOVA

Table 3 - Mean air temperatures and rainfall occurred during experimentation. FTs = Field Trials* 25 years average data from World Bank climate in 2017

Year	Field trials	Growing season	Location	Month	Average air* temperature (ºC)		Average* precipitation (mm)	
					FTs	25 years	FTs	25 years
2016	E_1	Sprint	Torbeck	July	27.5	26.4	85	126.1
				August	27.5	26.7	240.8	140.75
				September	32.0	26.3	23	174.16
2017	E_2	Autumn	*Morne Briller*	December	26.4	23.6	21	55.91
				January	27.4	22.9	0	41.32
				February	25.0	23.1	352	52.17
2017	E_3	Winter	Torbeck	February	28.4	23.1	54	52.17
				March	28.1	23.9	253	72.77
				April	29.4	24.6	33	152.66
	Cumulate			-	**251.7**	**220.6**	**1061.8**	**868.01**
	Average			-	**28.0**	**24.5**	**117.98**	**96.45**

tests were performed with sites (S) and genotypes (G) as fixed, and replication as random. One-way ANOVA and Ryan-Einot-Gabriel-Welsch-F test (REGW-F) allowed detection of significant differences among characters. Pearson's rank correlation coefficients (ρ) were computed for traits according to Kwon and Torrie, (1964).

Results and discussion

Sites

For each month mean air temperature and precipitations were recorded (Table 3). Remarkably different meteorological trends characterized FTs. The mean air temperatures ranged from 25 to 32 °C. The highest and the lowest air temperatures were recorded during field trial E_1 (September, 2016) and E_2 (February, 2017). Monthly cumulative precipitations recorded during FTs ranged from 0 to 352 mm with a mean precipitation of 117.98 mm. The minimum and maximum precipitations recorded were detected in January and March, 2017 during field trials E_2 and E_3 respectively. Large variations concerned also climate parameters and maize growth. During field trial E_1, the temperature was higher and the precipitations at flowering and early milk stages more intense than during field E_2 at similar stages. Milder and better distributed precipitations occurred during field trial E_1 and E_3 at dough stage and harvest compared with E_2.

FT on hills received less rain than those in plains. Moreover the minimum precipitation period was the trimester December - February while the trimester July - September precipitations peaked.

The recorded meteorological precipitations and temperatures exhibited deviation from the 25 years average. In particular, the distribution of rainfalls was different during growth seasons with respect to the average monthly value, even if weather patterns were suitable for maize growth without abiotic stress during experimentation. To the best of our knowledge, this was the first study conducted in Haiti taking in account meteorological parameters:, the previous investigations being indeed mainly focused on agriculture practices (fertilization) with no relation with wheatear parameters (Isaac et al., 2004).

PhTs data analyzed with ANOVA using S, G and SxG as fixed parameters, revealed that all but one PhTs (i.e. plant height) significantly depended on S, G or SxG

Table 4 - Mean square values from combined analysis of variance using sites (S), genotypes (G) and SxG as fixed. *: p < 0.05; **: p < 0.01; ***: p < 0.001; NS: not significant at p < 0.05 (test REGW-F)

Variables	S	G	S x G
Plant height (cm)	80772.0***	6045.5^{NS}	4667.2^{NS}
Steam diameter(mm)	91.4***	37.114***	10.901***
Male flowering days	7650.2***	640.4***	204.9***
Female flowering days	8995.7***	424.3***	178.4***
Number of leaves per plant	13.2**	94.1***	16.1***
Tassel length (cm)	12332.6***	499.1***	118.5**
Leaf angle	5495.6***	1130.7***	913.8***
Ear length (cm)	1512.0***	140.8***	23.6***
Number of rows per ear	7.7*	57.6***	8.0**
Number of grains per row	6531.4***	1087.1***	119.9***
Ear weight (g)	22500.0***	32141.2***	5227.6***
Grain yield (t/ha)	79.3***	104.7***	17.3***

Table 5 - Analysis of variance of aflatoxin content and phenotypic traits. For each trait, values with same or absence of letters indicate no significant difference (test REGW-F)

Variables	R4185	R4271X4185	R4865	Chicken corn	Maquina
Grain yield (t/ha)	3.6a	4.7b	4.8b	5.0b	7.4c
Ear weight (g)	60.1a	78.1b	80.2b	84.1b	126.2c
Ear length (cm)	13.8a	14.9a	15.6a	14.8a	18.1b
Number of rows per ear	9.8ab	10.7c	9.2a	10.4bc	11.9d
Number of grains per row	11.9b	12.6b	12.5b	10.3a	12.1b
Female flowering days	62.0b	62.0b	57.0a	63.0b	65.0b
Male flowering days	58.9bc	55.6ab	53.1a	59.7bc	61.8c
Steam diameter (cm)	4.7a	5.1a	4.8a	5.8b	6.7c
Tassel length (cm)	32.6a	35.1ab	37.4ab	37.0ab	40.9b
Number of leaves per plant	9.4a	9.1a	9.8a	10.9b	12.3c
Leaf angle (°)	53.0bc	54.0c	53.7c	43.9a	47.1ab
Aflatoxin (µg/Kg)	2.7	2.3	2.3	2.0	2.1

(Table 4). The field trials localities were characterized by great dissimilarities, such as soil types, altitude (hill/plain), previous culture and season: as expected, all but one (plant height) phenotypic traits were affected by sites and sites x genotypes.

Variance analysis

Results of one way ANOVA indicated statistically significant differences between most PhTs (Table 5).

The two local varieties were more productive than the introduced ones. Maquina alone, with 7.0 t/ha, showed a significantly larger yield than others (ranging from 3.6 to 5.0 t/ha). Also for ear weight and ear length, Maquina, with 126 g and 18.1 cm, appeared statistically different from the other varieties that ranged from 84.1g to 60.1 g and from 13.8 cm to 15.5 cm.

The highest and lowest numbers of rows per ear were 11.92 (Maquina) and 9.7 (R4185): the differences between Maquina and all the others varieties were significant. Concerning the number of grains per row, Chiken corn exhibited a significant lower value (10.3) than the other varieties.

Male flowering day (MFD) and female flowering day (FFD) were 53 and 57, respectively, for R4865 that flowered significantly earlier compared with other varieties. Remarkably, Maquina MFD and FFD were 61.8 and 65, respectively. Steam diameter (SD) recorded values ranged from 4.5 to 6.7 mm. Maquina showed the significantly highest SD followed by Chicken corn. Tassel length (TL) ranged from 32 to 41 cm. Once again, Maquina showed the highest value (40.9 cm), while the significant lowest one belonged to R4185. Number of leaves per plant (NLP) ranged from 9 to 12. Maquina with 12.33 and R4271x4185 with 9.1 exhibited the highest and the lowest values. Also for this character, Maquina was significantly different from all the other varieties. Leave angle (LA) was about 54° for R4271x4185 and R4865, i.e., statistically different from 47° and 44° found for Maquina and Chicken corn. Results of one way ANOVA indicated statistically significant differences among PhTs and suggested a broad genetic variability among varieties. This result was in agreement with a study by Wietholter et al. (2008) aimed to characterize the genetic variability in corn local varieties from Southern Brazil, using eleven phenotypic traits and molecular markers (SSR; AFLP).

Moreover, Baretta et al. (2016), in a study on nine Brazilian traditional and four commercial maize varieties aimed to characterize yield potential and genetic variability, found that, among fifteen investigated characters of agronomic importance, the characters with higher importance were: leaf angle, grain yield, height, number of rows in the ear, which overlap with the ours.

Under the experimental conditions of field trials, the two local varieties, Maquina and Chicken, that are very popular and spread throughout the country, yielded respectively 8 and 6 times more than the Haitian average possibly due to better agronomical practices (mainly fertilization and watering). This finding stresses that, beside the breeding program, also the improvement of the plant management allows the enhancement of the yield potential of given varieties.

Aflatoxin

AF content was analyzed by one way ANOVA (Table 5). All tested maize varieties revealed AF content ranging from 2.0 to 2.7 µg/Kg, below the European Union limit (5 µg/Kg) without significantly differences among varieties. Since no susceptible genotypes varieties were used as comparative control we cannot assume that the low contamination is due to tested varieties resistance more than good agronomical practices. It is remarkable that the agronomical practices that increase GY can decrease AF (Bowen et al., 2014).

Table 6 - Correlations among phenotypic traits of tested maize varieties. *: p<0.05; **: p<0.01; ***: p<0.001 and NS: not significant p>0.05. GY: grains yield; EL: ear length; EW: ear weight; NRE: number of rows per ear; NGR: number of grains per row; LA: leaf angle; TL: tassel length; NLP: number of leaves per plant; MFD: male flowering days after sowing; FFD: female flowering days after sowing; PH: plant height and SD: stem diameter

	GY	EW	NRE	NGR	EL	LA	TL	NLP	MFD	FFD	SD
GY											
EW	0.991***										
NRE	0.260***	0.270***									
NGR	-0.007^{NS}	0.005^{NS}	0.137*								
EL	0.164**	0.191**	0.169**	0.608***							
LA	-0.178**	-0.154*	-0.042^{NS}	0.171**	0.244***						
TL	0.055^{NS}	0.075^{NS}	0.052^{NS}	0.651***	0.755***	0.328***					
NLP	0.283***	0.301***	0.201**	0.295***	0.348***	-0.198***	0.238***				
MFD	-0.214***	-0.198**	0.159**	0.665***	0.599***	0.276***	0.632***	0.229***			
FFD	-0.245***	-0.229***	0.137*	0.669***	0.604***	0.238***	0.606***	0.182**	0.942***		
SD	0.107^{NS}	0.124*	0.299***	0.404***	0.279***	-0.202**	0.170**	0.485***	0.382***	0.425***	
PH	0.192**	0.202**	0.021^{NS}	0.019^{NS}	0.178**	0.002^{NS}	0.200**	0.140*	0.051^{NS}	0.025^{NS}	0.003^{NS}

Correlations

Most of the traits exhibited mutual correlation (Table 6). Since this study purpose was the morphological characterization to be used in yield improvement, we focused on the correlations dealing with GY and PhTs. A correlation appeared between yield and all phenotypic characters, except NGR, TL, SD. Strong and positive correlations (p>0.01) appeared between GY and EW (ρ= 0.991), NLP (ρ= 0.283), PH (ρ= 0.192,) and NRE (ρ=0.260), while good correlation (p>0.1) concerned EL (ρ=0.164) and PH (ρ=0.192), in agreement with literature reports (Bavec and Bavec, 2002; Srećkov et al., 2011). GY appeared strongly and negatively associated (p>0.01) with MFD (ρ= -0.214) and FFD (ρ= -0.245); also LA exhibited a negative correlation (ρ= -0.178; p<0.1).

This is a promising result because in countries with subsistence farming, precocity is an often demanded character beyond GY. Positive correlation between MFD and FFD is in agreement with previous studies on thirty six maize hybrids along with their parental lines to assess the genetic correlation among 16 quantitative traits (Malik et al., 2005).

Conclusions

To the best of our knowledge, this is the first study focused on the comparison between local and introduced maize varieties aimed to improve yield and reduce AF contamination in Haiti.

Our findings revealed specifically that better agricultural practices can by themselves increase yield (from 6 to 8 times) and decrease AF contamination below EU limits.

The significant differences of phonotypical characters indicate a broader variability among varieties. Maquina, a local variety, is the best performing.

Correlations among phenotypic traits suggest some characters that may be used for indirect grain yield improvement, namely, MFD, FFD and EW. Both FFD and MFD are negatively correlated with yield while EW shows opposite correlation signs.

Interestingly, the above characters show differences among varieties, Maquina and R4865 exhibiting the best EW and the shortest MFD and FFD, respectively.

All these findings suggest that local and introduced varieties are promising sources of variability for genetic improvement for a breeding program aimed to increase yield. Further studies on varieties obtained by crossing the present genetic material are in progress in Haiti.

Author contribution statement

Junior Aristil performed field trials and data collection, Alberto Spada and Roberto Pilu drafted the manuscript and oversaw the research. All authors performed statistical analysis and discussed the results and commented on the manuscript.

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Compliance with ethical standards

None

Conflict of interest

The authors declare that they have no conflict of interest.

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