INTEGRATED APPROACHES TO AGRICULTURE

IN DEVELOPING COUNTRY: HAITI

By

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PUBLICATIONS

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Aristil, J., Venturini, G., & Spada, A. (2017). Occurrence of Toxigenic Fungi and Aflatoxin Potential of Aspergillus spp. Strains Associated with Subsistence Farmed Crops in Haiti. *Journal of Food Protection*, *80*(4), 626-631.

Manuscripts in preparation

Aristil, J., Venturini, G., & Spada, A. Study of Toxigenic fungi and Aflatoxin Contamination of Haitian Food Chain.

Aristil, J., & Spada, A. Characterization of five Improved and Varieties of Maize (*Zea mays*) Tested in Haiti For yield and Aflatoxin Content.

DEDICATION

This dissertation is dedicated to my brothers and sisters and my son, Max C. J. Aristil for their confidence and love. It is also for all Haitian students, most common persons who could found inside the profound expression of patriotism, love and other strategies for singing Haiti, our charming Island forever.

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DECLARATION of Author

This PhD dissertation is a result of my original work. Texts of fragments from other studies are notified with reference of authors and work.

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ABBREVIATIONS

AF: Aflatoxin

AFB1: Aflatoxin B1

AFB2: Aflatoxin B2

AFG1: Aflatoxin G1

AFs: Aflatoxins

AGP: Agricultural practice

AGPs: Agricultural practices

ANOVA: Analysis of variance

ASC: *Aspergillus* section *Circumdati*

ASF: *Aspergillus* section *Flavi*

ASN: *Aspergillus* section *Nigri*

Aug: August

A_w: Activity water

Cav: Carvaillon

CC: Cultural cycle

CdB: *Croix des bossal*

CFU/g: Colony forming units per gram

CP: Culture provenience

DA: Discriminate analysis

DiSAA: Department of Agricultural and Environmental Sciences - Production, Landscape, Agroenergy

E_1: First field trial

EL: Ear length

ELISA: Enzyme-linked immunosorbent assay

EU: European Union

EW: Ear weight

F_2: Second generation

F_3: Third generation

FA: Ferulic Acid

Fert: Fertilization

FF: Fields of farmers

FFD: Female flowering days after sowing

FUS: *Fusarium* spp

G: Genotypes

GY: Grains yield

HM: Harvesting month

HPLC: High Performance Liquid Chromatography

IA: Immunoaffinity

IF: Isolation frequency

Irr: Irrigation

IV: Independent variable

Jul: July

Jun: June

LA: Leaf angle

LOD: Limit of detection

LOQ: Limit of quantification

MA: Maize in association

Mar: March

MeF: *Marché en fer*

Mers: Mersan

MF: Maize flour

MFD: Male flowering days after sowing

MH: Month of harvest

Miser: Miserne

MM: Maize in monoculture

Mor: Morency

Mox: Mycotoxins

mPDA: Modified potato dextrose agar

MS: Meal samples

MSS: Maize seeds supplier

MUFA: Monounsaturated fatty acids

NGR: Number of grains per row

NLP: Number of leaves per plant

NRE: Number of rows per ear

NS/H: Number of seeds per hole

NS: Not significant

NW/C: Number of weddings per cycle

OS: Oleaginous seeds

PB: Peanut butter

PC: Previous culture

PC1: First principal component

PC2: Second principal component

PH: Plant height

PhTs: Phenotypic traits

S: Sites

SA**:** Salicylic Acid

SD: Stem diameter

SFA: Saturated fatty acids ratio

SxG: Sites per genotypes

TL: Tassel length

USFDA: US Food and Drug Administration

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GENERAL SUMMARY

Food safety and food security are two of the core issues in developing countries such as Haiti located in tropical area where climate could be favorable to toxigenic fungal (TF) growth and mycotoxins (Mox) accumulation such as aflatoxins (AFs). AFs are secondary metabolites of fungal species belonging to *Aspergillus* section *Flavi*. Good agricultural practices (GAPs) and selected genotypes could reduce AF content in food and increase crop yield (GY). In order to investigate the safety of Haitian food chain, 78 seeds (SS) and processed products (PPs) were sampled in South Haiti and tested for TF and AF content. Results obtained revealed that SS samples were more TF contaminated compared to PPs. *Aspergillus* genus was predominant in seeds (84%) whilst *Penicillium* spp. in PPs (70%). *Aspergillus* section *Flavi* and *Nigri* compete with *Fusarium* spp. under Haitian climate (ρ=-0.997). Eighty percent of Haitian ASF strains were aflatoxigenic and peanut and maize were colonized with heterogeneous chemotypes pattern and moringa with homogenous (P = 0.45). Two AF types contaminate Haitian food chain with AFB1>AFB2. Fifty percent SS and PPs were positive to AF with concentrations ranging from 1.20 to 1033.20 µg/kg and 14.90 to 166 µg/kg, respectively. Maize derived products such as maize flour and meal samples were more TF and AF contaminated compared to cassava flour, moringa leaves powder, and peanut butter. All contaminated maize derived products had AF concentration greater than the 5 µg/kg, accepted in European Union (EU). Peanut samples were less AF contaminated compared to maize grains. Fifty-five percent of maize grain samples had AF greater than the 20 µg/kg, maximum concentration fixed by US Food and Drug Administration (USFDA). Oleaginous AF contamination seems due to bad drying whilst almost 71% of that of maize is a result of improper AGPs. Harvest month, weeding frequency and storage duration are the principal practices responsible.

In the frame of food security, a breeding program was performed in Haiti using three introduced maize varieties (INMVs) selected in Italy and two local landraces, Maquina and Chicken corn as testers. The aim of the breeding was to investigate on INMVs GY and AF content at harvest. INMVs as well as landraces yielded ranging from 5 to 7 t/ha,

which represent more than 5 to 7 times of National maize yield (0.96 t/ha). Local maize varieties yielded more and were less AF contaminated compared to INMVs. Moreover, landraces were genetically more different than INMVs. Indeed landraces formed two separate clusters whilst INMVs, are grouped in one. The two principal traits that are responsible of 90% of variation were GY and ear weight (EW). AF content ranging from 2.03 to 2.73 µg/kg, (less than EU limit: 5 µg/kg). EW was positively and negatively related with GY and AF respectively. Therefore, EW is the target character in order to reduce AF and increase yield. Meanwhile, five improved maize varieties (IMVs) were developed during the first field trial by crossing INMVs with Chicken corn. During the following field trials, IMVs were also tested for GY and AF content. Results showed that 80% of IMVs yielded up to 6 t/ha, with an increasing of 39% after three crossing cycle. Conversely 40% of tested IMVs were AF contaminated up to the 20 µg/kg (higher than USFDA limit). Thus, IMVs yielded more and were more AF contaminated compared with parents. On the base of this study, ear weight and tassel length are the two most important characters link to GY increasing and AF reduction.

Beyond experimental researches an investigation was carried out by exploiting the literature available on Moringa in order to better know its nutritional values. Results showed that *Moringa oleifera* could be cultivated in Haiti all year long and used as nutraceutical food supplement in mitigating malnutrition.

GENERAL INTRODUCTION

Integrated approach is a combination of different aspects for solving identified problems. For developing countries located in tropical areas such as Haiti, problems of food contamination is one of the most preoccupant because its offer natural climate condition for toxigenic fungal (TF) growth and mycotoxins accumulation, which could consequently increase the exposure of local population to mycotoxicosis cases, cancer and limit overall the commercialization of agricultural substrates on the international markets. Food security and safety problems are predominant in Haiti. Several strategies could be envisaged such as identifying the steps of contamination and using available resources for increasing production and reducing contamination exposure. As in developing countries researches and technologies are very limited, several Southern Universities develop international relationship with Northern ones. Since 2006, it is established, an agreement for international cooperation between UNIMI (University of Milan Faculty of Agriculture and Food Science, Italy) and UNDH (University Notre Dame of Haiti, Faculty of Agriculture, Torbeck, Haiti) mutually promote and encourage scientific and academic cooperation in agronomy for the purpose of improving the efficiency of education programs, and providing a deeper knowledge for the exploitation of agricultural resources to the benefit of rural Haitian community. The present PhD dissertation writing in the framework of this relationship was aimed to evaluate integrated approaches for reducing malnutrition and aflatoxin (AF) exposures in Haiti. Safety of Haitian food chain was studied. A maize breeding program with aims to increase yield and reduce AF using local resources was developed in South, Haiti. Possibility to use Moringa leaves and seeds in Haiti as row materials was documented.

FIRST PART:

Fungal and Aflatoxin Contamination of Haitian Food Chain

Summary. Activity water (a_w), potential toxigenic fungal (PTF) distribution, ability of 49 *Aspergillus* section *Flavi* strains for producing aflatoxin (AF) *in vitro*, and AF quantification were performed for 78 seed samples (SS) and processed products (PPs) from 2014-15 cropping seasons collected in South Haiti during 2015 year in order to evaluate the safety of Haitian food chain and the exposure of local population to AF contamination. Maize grains were sampled from farmers and agricultural practices (AGPs) data were registered. Meal and peanut seeds were purchased from local markets. Moringa seeds were from Torbeck and Cavaillon. *Unitė de transformation* (UdT) provided maize and cassava flour, moringa leaves powder and peanut butter. A_w measurement results revealed that SS were less dried than PPs with respectively an average of 0.62 and 0.50. SS were more contaminated by PTF compared with PPs. *Aspergillus* genus was predominant in seeds (84%) whilst *Penicillium* spp. in PPs (70%). *Aspergillus* section *Flavi* and *Nigri* compete with *Fusarium* spp. under Haitian climate (ρ=-0.997). Eighty percent (80%) Haitian ASF strains were aflatoxigenic and peanut / maize were colonized with heterogeneous chemotypes pattern and moringa with homogenous (P = 0.45). Two AF forms contaminated Haitian substrates with AFB1 > AFB2. Fifty percent (50%) SS and PPs were positive to AF with concentrations ranging from 1.20 to 1033.20µg/kg and 14.90 to 166µg/kg, respectively. Maize PPs were more AF contaminated compared with peanut and cassava. Means AF concentrations of meal from *Marché en fer* and *Croix des Bossals* markets were respectively 4.97 and 105.40µg/kg. Means AF concentrations of moringa from Torbeck represent 60 times of those from Cavaillon. Lack drying process is responsible of AF contamination of moringa and peanut seeds. Oleaginous Haitian seeds were less AF contaminated than maize. Almost 71% of fungal and AF contamination of Haitian maize grains are due to bad AGPs. Harvest month, weeding frequency and storage duration are the principal responsible factors. Therefore, Haitian food chain is highly contaminated with fungi and AF. So, integrated approach including good AGPs and post harvest management could reduce AF exposure.

Keywords. Seeds, foodstuffs products, toxigenic fungi, aflatoxin, agricultural practices, Haiti

Chapter 1.1. Introduction

Mycotoxins (Mox) are toxic low molecular weight compounds produced by fungi which infect agricultural products, causing severe health exposure risks by accumulation in different human and animal organs /tissues (Williams et al.,2004; Sirot et al.,2013; Arroyo-Manzanares et al., 2017). The consequent food contamination is a continuing threat to food and public health safety (Wu, 2006; Marin et al., 2013). Mox contaminate approximately 25% of the cereals in the world and are mainly common in rural communities of developing countries (Smith et al., 1994; Bhat et al. 1997; Mannon and Johnson 1985). Agricultural commodities could be contaminated with Mox during production, harvesting and processing (Wagacha and Muthomi, 2008; Chulze, 2010; Misihairabgwi et al., 2017).

Genotypes, drought, insect activity, delayed harvest, slow and inadequate drying, improper storage, humid and warm temperature, typical in tropical and subtropical regions but extended in other areas by climate change, can contribute to their occurrence (García-Cela et al., 2015; Kabak et al.,2006; Lanyasunya et al., 2005 ;Medina et al., 2017). Also, factors such as host stature, nutrients availability, water activity, pH, and presence / absence of specific gases are suitable conditions for potential toxigenic fungi (PTF) growth and Mox accumulations (Martins and Martins, 2002; Lanyasunya et al., 2005; Magan et al., 2003).

Out of more than 400 Mox isolated, Aflatoxins (AFs), Fumonisins and Ochratoxins are the most toxic, and they are subjected to regulations worldwide (Otsuki et al., 2001; Atroshi et al., 2002; Richard et al., 2003; Petersen, 2015; Cheli et al., 2014). These secondary metabolites are produced by saprophytic fungi, belonging to *Aspergillus* genus, *Fusarium*, and *Penicillium* spp (Placinta et al., 1999; Sweeney et al., 1998; Pitt and Hocking, 2009; Kabak et al., 2007; de Souza et al., 2017).

AFs are produced by *Aspergillus flavus*, and *A. parasiticus,* which are worldwide distributed and relatively predominant in tropical and subtropical areas. Other species such as *A. nomius, A. pseudotamarii,* and *A. bombycis* produce also AFs (Pildain et al., 2008; Varga et al., 2011; Diedhiou et al., 2011; Kurtzman et al., 1987), but their occurrence is rarer. These aflatoxigenic species have been recovered globally from a wide range of foods and derived products as maize, wheat, cassava, peanut and other nuts, and other oil seeds (Amaike and Keller, 2011; Georgiadou et al., 2012; González-Salgado et al., 2008; Faria et al., 2017; Klich and Pitt, 1988; Adjovi et al., 2014; Bordin et al., 2014). Four AF

forms are mostly found in nature. Called aflatoxin B1, B2, G1 and G2, referring to their respective fluorescence under ultraviolet light (B=Blue, G= Green) and their mobility during thin layer chromatography (TLC) (Thathana et al., 2017; da Rocha et al., 2014). Classified on the first group of natural substances active as human carcinogens, the most widespread AFs are AFB1 and AFG1 (De Ruyck et al., 2015; IARC, 1993).

A large majority of the world population is chronically exposed to AF (Gnonlonfin et al., 2013) because most of staple foods and food basic products in tropical /subtropical areas resulted contaminate with those secondary metabolites.

Maize is staple crop in 22 countries where it provides a large percentage of energy to the local population. Sixteen of those countries are located in Africa (Dowswell et al., 1996). Contrarily to Asian and European people that take a large amount of their protein needed from wheat, maize is largely preferred in South and Central America (Ranum et al., 2014). In Venezuela, Peru, Bolivia and Ecuador, maize provides 2–14% of dietary energy. Similarly, in Central American countries such as Honduras and Nicaragua, it provides respectively to 27%, and 25% of daily dietary energy (Tscharntke et al., 2012; Ray et al., 2013). After rice, which covers almost 16-22% of daily Haitian energy, maize is the second most consumed cereal (Ray et al., 2013). In Haiti, maize is consumed before/at maturity (*mayi boukanen* and *mayi bouyi*) and in various derived products such as Acamil and Acassan, and flour against anemia and malnutrition (Ivers et al., 2014; Shak et al., 2011; Cayemittes et al., 2007; Halsey et al., 1990; Jelliffe, 1960; Klipstein et al., 1968). Most of times, maize is prepared with black bean (*Phaseolus vulgaris)* and since 1979, each Haitian habitant consumes 2-3 kg/year and takes about 7.6% of his needed protein from it (Van Schoonhoven and Voysest, 1989). Other commodities suitable to AF and PTF contamination are peanut, moringa and cassava (*Manihot esculentum.L*): they are also important crops of Haitian diet (Johnson et al., 2003; Henry and Hershey, 2002; Aso et al., 2015).

For a long time, Haitian drinking water (Schwartzbord et al., 2013), maize (Castor et al., 1987) and oleaginous seeds (Schwartzbord et al., 2015) were highly reported to be contaminated by heavy metals and AF. AF exposure was confirmed by monitoring urine samples from Haitian adults and children with an AF biomarker (Gerding et al., 2015). Even so, till now, no studies have been conducted on the occurrence of PTF in Haitian seeds and processed products cultivated in South Haiti for

providing insights on fungal ecology in this department/district. The current survey was assigned to (**i**) determine the distribution of fungal mycobiota associated with Haitian seeds (maize, moringa, peanut, bean) and processed products (PPs) as peanut butter, meal and maize flour, moringa leaves powder, and cassava flour; (**ii**) evaluate the AF contamination of Haitian food samples, and (**iii**) screen ASF strains isolate for production of AFB1 and AFG1 *in vitro*.

Chapter 1.2. Materials and methods

1.2.1. Study region and sampling procedure

1.2.1.1. Study region

The main objectives of the research were: i) Investigate and identify potential toxigenic fungi (PTF), with particular attention to *Aspergillus* genus. ii) Study ability of ASF strains to produce aflatoxin B1 and G1 *in vitro.* iii) Quantify aflatoxins (AFs) in Haitian seed/grain samples (SS) and processed products (PPs). The investigations were carried out on agricultural products cropped during 2 years (2014 and 2015).

Study region. Figure 1 shows Haiti Republic location in the world. Figure 2 shows all *arrondissements* of Haiti Republic with particular attention to Les Cayes. Figure 3, communes of Les Cayes *arrondissement* across them, Torbeck. Figure 4 shows some sections *communales* of Torbeck, where is located the agriculture faculty of University Notre Dame of Haiti (UNDH) and the unit of transformation (UdT).

Figure 1. Haiti Republic location with red coloration.

Figure 2. Arrondissements of Haiti, Les Cayes is indicated with the red row and arrow.

Figure 3. Communes of Les Cayes *arrondissement,* Torbeck is indicated with the red arrow.

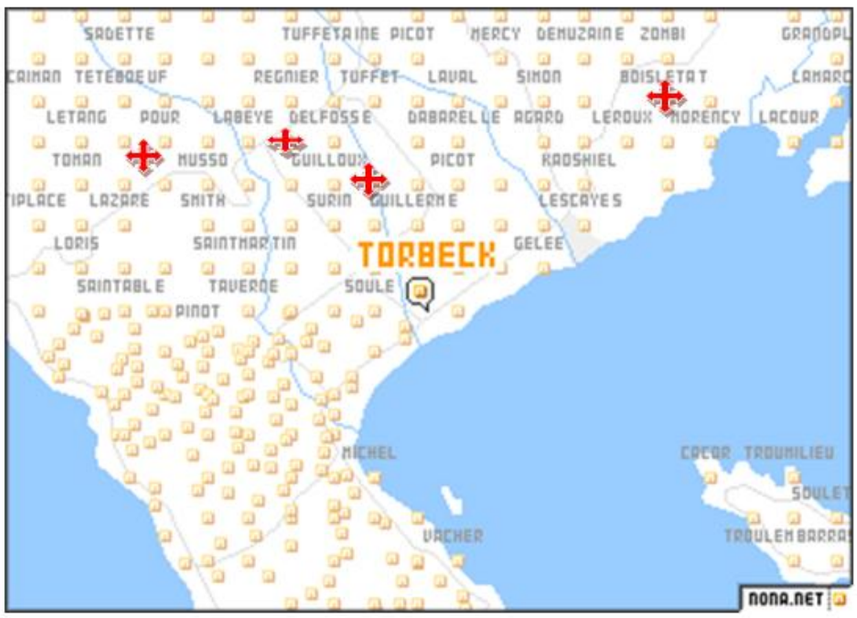

Figure 4. Sections communal of Torbeck commune and other localities where crops were sampled are indicated by the red crosses.

1.2.1.2. Samples collection. Two sampling were conducted in February and August 2015 in Les Cayes *arrondissement* of Southern department, Haiti (Figures 2 and 3).

a) February 2015 – (first sampling)

Twenty-eight samples included 9 maize (*Zea mays*), 17 seeds i.e. peanut (*Arachis hypogaea*), moringa (*Moringa oleifera)* and bean (*Phaseolus vulgaris*) cropped in 2014 year and 2 processed products (PPs) i.e. cassava (*Manihot esculenta)* flour and moringa (*Moringa oleifera)* leaves powder were collected in Haiti in February 2015. Grains and seeds were sampled from farmers whilst PPs were provided by the UdT (Table 1).

During samples collection a very short questionnaire was elaborated and questions relative to cropping and post-harvesting management were asked. In Table 2 are inserted data recorded per sample during the investigation.

Figure 5. Unit of transformation and some of local crops transformed.

Table 1. Staple crops sampled in Haiti in February 2015

Code	Species	Origin	Type
1	*Zea mays*	Redon	Grain
2	*Z. mays*	Bourry	G.
3	*Z. mays*	Moreau	G.
4	*Z. mays*	Chantal	G.
5	*Z. mays*	Guillerme	G.
6	*Z. mays*	Durcis	G.
7	*Z. mays*	Levy	G.
8	*Z. mays*	Durcis	G.
9	*Z. mays*	Minet	G.
10	*Moringa oleifera*	Redon	Seed
11	*M. oleifera*	Durcis	S.
12	*M. oleifera*	UdT	Leaves powder
13	*M. oleifera*	Belle Source	S.
14	*Arachis hypogaea*	Guillaux	Seed
15	*A. hypogaea*	Bourjouly	S.
16	*A. hypogaea*	Torbeck	S.
17	*A. hypogaea*	Moreau	S.
18	*A. hypogaea*	Durcis	S.
19	*A. hypogaea*	Durcis	S.
20	*Manihot esculenta*	UdT	Flour
21	*Phaseolus vulgaris*	Levy	Seed
22	*P. vulgaris*	Redon	S.
23	*P. vulgaris*	Redon	S.
24	*P. vulgaris*	Mineur	S.
25	*P. vulgaris*	Ducis	S.
26	*P. vulgaris*	Beraud	S.
27	*P. vulgaris*	Torbeck	S.
28	*P. vulgaris*	Maillard	S.

Table 2. Production and post-harvesting data recorded during the first investigation

Sample	Production			Post-harvest
Code	**Harvesting month**	**Pest control**	**Cultural cycle (days)**	**Storage duration (days)**
1	Jan.	Yes	120	60
2	Dec.	Yes	120	90
3	Jan.	No	150	60
4	Feb.	No	90	30
5	Sept.	No	90	210
6	Sept.	No	90	210
7	Nov.	No	120	120
8	Jan.	Yes	90	60
9	Feb.	Yes	90	30
10	Oct.	No	270	30
11	May	No	270	30
12	Aug.	No	270	30
13	May	No	270	30
14	Jan.	No	120	60
15	Jan.	No	150	60
16	Nov.	No	120	90
17	Dec.	No	120	60
18	March	No	120	30
19	Jan.	No	120	30
20	Feb.	Na*	240	15
21	Jan.	Na	90	30
22	Feb.	Na	120	15
23	Jan.	Na	90	30
24	Jan.	Na	120	15
25	Feb.	Na	150	15
26	Jan.	Na	120	30
27	Jan.	Na	90	30
28	Feb.	Na	90	15

***Na:** not available

b) August 2015 – (second sampling)

Fifty Haitian agricultural products of major consumption were sampled in Les Cayes department, Haiti in August 2015. Sampled products included 20 maize (*Zea mays)* grains (Table 3), 22 oleaginous seeds i.e. (*Moringa oleifera*) and peanut (*Arachis hypogaea)* (Table 5), and eight PPs (Table 4). Similarly to 2014 sampling, two of PPs, mainly maize flour (MF) and peanut butter (PB) were provided by the UdT (Table 4). Meal samples were purchased from retails sellers of the two markets of Les Cayes: *Marché en fer* (MeF) and *Croix des bossal* (CdB) (Table 4). Moringa seed samples were collected at Cavaillon and Torbeck communes (Table 5). Maize grains were sampled directly from farmer's depot (Table 3).

During the second sampling a new questionnaire with more data relative to AGPs were prepared and filled out with farmers. Data relative to maize grains AGPs and storage duration as well as seeds provenience are inserted in Table 3. In Table 5 are reported data relative to only provenience and market where moringa and peanut seeds were purchased. Table 4 and Figure 7 summarize the data relative to Haitian PPs sampled in August 2015.

Collected grain and seed samples have been sun dried for 5 days close to the agricultural farm of UNDH and were put in sterile polyethylene bags, stored at 4°C for limiting insect activity until transportation in Italy (DiSAA, Milano) for activity water (a_w) test, mycological analyses and AF quantification as seen on Figure 6.

Figure 6. Samples preparation before transporting to Italy.

Table 3. History of each maize grain sampled in August 2015

	Agricultural practices									
Code	**CP**	**PC**	**ST**	**NS/H (u)**	**CC (days)**	**Irr.**	**Fert.**	**NW/C (u)**	**HM**	**MSS**	**STd (days)**
29	Guil.	Bean	yes	3	150	no	no	1	Jul.	TWP	30
30	Guil.	MM	yes	2	180	no	no	1	Aug.	TWP	15
31	Bourry	MA	yes	2	180	no	Yes	2	Aug.	TWP	8
32	Bour.	MA	yes	2	150	no	Yes	1	Jul.	TWP	30
33	Redon	Bean	yes	3	90	no	no	1	Jul.	TWP	30
34	Red.	MM	no	4	120	no	no	2	Aug.	FF	22
35	Miser.	MA	no	3	90	no	Yes	2	Mar.	FF	150
36	Cance	MM	no	2	90	no	Yes	1	Jun.	FF	60
37	Mers.	Bean	no	2	120	Yes	Yes	3	May	FF	120
38	Mers.	Bean	no	2	90	Yes	Yes	3	May	FF	120
39	Mers.	Bean	no	2	90	Yes	no	3	May	SEP	75
40	Durcis	Bean	yes	2	150	Yes	no	2	Jun.	SEP	60
41	Durcis	MM	yes	2	150	Yes	Yes	2	Jul.	SEP	60
42	Beraud	Bean	yes	3	120	no	Yes	2	Jun.	FF	90
43	Ber.	MA	no	3	150	yes	no	2	Jul.	FF	45
44	Ber.	Bean	no	2	150	Yes	no	2	Jul.	FF	45
45	Ber.	Pt	no	2	150	no	no	1	Jul.	FF	60
46	Mor.	Sorg	no	3	90	no	no	2	Jul.	FF	30
47	Cav	MA	no	4	90	no	no	1	Jul.	FF	90
48	Cav	Bean	yes	2	90	no	Yes	1	Jun.	FF	120

Legend. CP: culture provenience; PC: previous culture; ST: seeds treatment; MSS: maize seeds supplier; MA: maize in association; MM: maize in monoculture; NS/H: number of seeds per hole; NW/C: number of weddings per cycle; CC: cultural cycle; MH: month of harvest; Irr: irrigation; Fert: fertilization; STd: storage duration; Cav: Carvaillon; Mor: Morency; Mers: Mersan; Miser: Miserne; TWP: Taiwan project; SEP: Secal project; FF: fields of farmers; Pt: peanut; Sorg: sorghum; Jun: June; Jul: July; Aug: August; Mar: March and U: unit.

Table 4. Inventory of Haitian processed products sampled in August 2015

Code	Host	Purchased
49	Meal	*Croix des bossals*
50	M.	*Croix des bossal*
51	M.	*Marché en fer*
52	M.	*Marché en fer*
53	M.	*Croix des bossals*
54	M	*Marché en fer*
55	Peanut	*UdT*/Torbeck
56	Maize	*UdT*/Torbeck

Figure 7. Flour and meal products obtained from processed maize grains.

Table 5. Data recorded for Haitian moringa and peanut seeds in August 2015

Code	Species	Growing	Purchased
57	*Arachis hypogaea*	Les Anglais	Les Cayes
58	*A. hypogaea*	Mersan	Mersan
59	*A. hypogaea*	Chantal	Cavaillon
60	*A. hypogaea*	Cance	Cance
61	*A. hypogaea*	Miragoane	Cavaillon
62	*A. hypogaea*	Mersan	Mersan
63	*A. hypogaea*	Cavaillon	Cavaillon
64	*A. hypogaea*	Durcis	Cavaillon
65	*Moringa oleifera*	Cavaillon	Carvaillon
66	*M. oleifera*	Cav.	Cav.
67	*M. oleifera*	Cav.	Cav.
68	*M. oleifera*	Cav.	Cav.
69	*M. oleifera*	Cav.	Cav.
70	*M. oleifera*	Cav.	Cav.
71	*M. oleifera*	Cav.	Cav.
72	*M. oleifera*	Torbeck	UdT
73	*M. oleifera*	Torb.	UdT
74	*M. oleifera*	Torb.	UdT
75	*M. oleifera*	Torb.	UdT
76	*M. oleifera*	Torb.	UdT
77	*M. oleifera*	Torb.	UdT
78	*M. oleifera*	Torb.	UdT

1.2.2. Staple crops analyses

All the 78 samples collected in Haiti during February and August 2015 were processed following these procedures:

1.2.2.1. Water activity measurement

Firstly, twelve grams were taken from each sample with an automatic balance. Then materials were pulverized using automatic machine and three grams per subsample were put in special plastic tube for water activity measurement. The procedure relative water content measurement specific to powder, flour and seed matrices as oleaginous/ cereals ones, elaborated by AQUALAB CX-2 (Decagon

Devices Inc., Pullman, Wa, USA) was followed (Figure 8). Briefly, the machine was started 2 hours prior the test and specific solution of control was used and tested three times for evaluating the machine calibration. Then, corresponding solution according to matrix samples like cereals or oleaginous was used. Once sure of the machine precision, each sample was subdivided in three representatives subsamples and analyzed three times for a_w at 25^0C. The averages obtained determine the exact value of a_w of sample.

Figure 8. Sample preparation and AQUALAB CX-2 (Decagon Devices Inc., Pullman, Wa, USA) machine used for water activity tests.

1.2.2.2. Mycological analyses and fungal isolation

The analysis of the viable mycobiota associated with samples was estimated using a) direct plating technique for seed and grain samples after surface sterilization and b) serial dilution techniques for PPs.

a) Fungal isolation from seeds and grains

First of all, a total of hundred and fifty grains or seeds per sample were randomly chosen. Each sample was divided into three subsamples of fifty grains or seeds. The three subsamples of 50 grains /seeds were indicated and noted with letters ranging from A to C. Each sample was surface washed three times with sterilized solution for three minutes. Solution used for surface sterilization was prepared with:

- NaOCl (7%) 80 ml,
- Ethylic alcohol 100 ml,
- Distilled water 820 ml.

After sterilization, grains /seeds were rinsed three times with deionized water for 6 minutes. Seeds/grains were, then, put on adsorbent paper under floor laminar hood cabinet during 3- 4 minutes for facilitating water evaporation. After drying, grains/ seeds were placed on Petri dishes 90 mm Ø containing mPDA (modified potato dextrose agar) medium. The mPDA having the following composition:

- PDA (Potato Dextrose Agar, Difco®, Becton and Dickinson Company, Sparks, MD, USA) 20 g/L,
- Tetracycline 50 mg/L,
- Chloramphenicol 100 mg/L,
- Dichloran 2 mg/L.

The medium was sterilized using autoclave at 2 atmospheres and at 121°C for 20 minutes, while antibiotics (chloramphenicol and tetracycline) and the fungicide Dichloran were added to the substrate after sterilization and mainly immediately prior plating.The distribution of the seeds or grains into plates was carried out under the floor laminar hood cabinet. The fifty kernels per subsample were distributed in five plates containing 10 kernels each. Each grain /seed has a number ranged from 1 to 50. The inoculated Petri dishes plates were transferred into a thermostat (incubator) at 25 °C for 5 days (Figure 9).

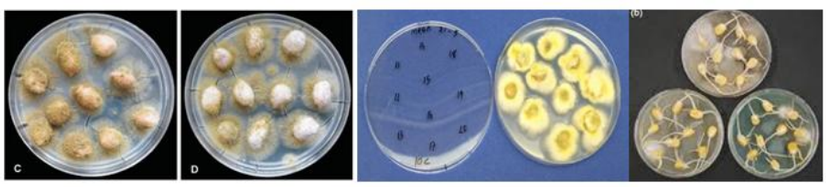

Figure 9. Fungal isolated from seeds and grains.

b) Fungal isolation from processed products

Fungal isolation from PPs samples was performed following serial dilution technique. This technique allowed considering internal fungal contamination, since the sample was milled. Each sample was divided in three subsamples equivalent to ten grams which were marked with letters ranging from A to C. Each subsample (5 g) was put in 50 ml Falcon conical centrifuge tube containing 45 ml sterile solution of peptone (Bacto® Difco) (v/v water - peptone 0. 1%) in an amount equivalent to 9 times the weight of the sub-sample. The Falcon vials were orbital shacked at 200 rpm for 30 minutes. The obtained suspension was utilized for preparing out a series of successive decimal dilutions. Each decimal dilution has been obtaining by putting 1 ml of previous solution into 9 ml of solution of sterile peptone, until dilution factor of 1:100000(10^{-5}). For each obtained dilution, 0.1 ml suspension was inoculated into three Petri dishes plates 90 mm Ø filled with mPDA. The plates were then treated as described in the previous paragraph. In this sense, each flour or powder sample was tested for fungal mycobiota eighteen (18) times (3 replicates (A, B and C) x 6 dilutions (from 1:10 to 1:10^{-5}).

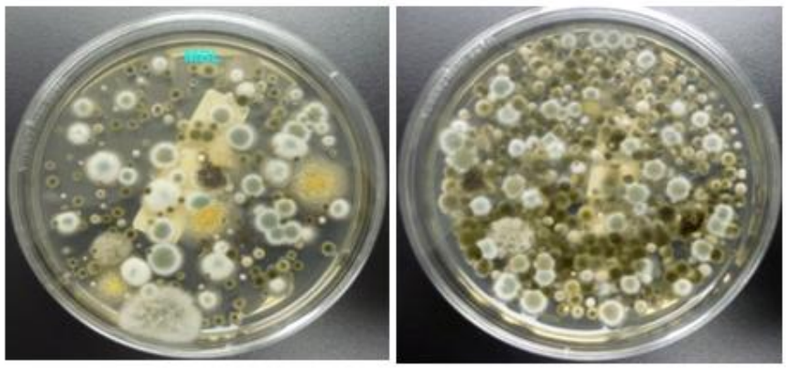

Figure 10. Fungal isolated from processed products.

1.2.2.3. Fungal identification

a) **Fungal identification at genus level**

Fungal identification was carried out after 5 days post inoculation (dpi) in thermostat at 25°C. The number and types of colonies developed on each Petri dish was recorded. Developed fungi were identified and screened according to macromorphological and cultural criteria assigned to genera *Aspergillus*, *Fusarium* and *Penicillium*, as indicated by Pitt and Hocking (2009), Samson et al (2010), and Watanabe (2010), considering the color of the air mycelium, the plate backside, and the possible production of exudates or pigments. The observations were repeated 5-6 days after inoculation, because some mycelia associated with some taxa, such as PEN, often took more days for taking an aspect that may allow morphological recognition. The most representative strains of each taxon were isolated in pure culture in Petri plates of 60 mm Ø containing PDA (Difco®, 39 g/L). The inoculated plates were incubated in a thermostat at 25 °C for 7 days.

b) Fungal identification at section level

In order to confirm the identification of isolated fungal genera and to allow subsequent identification at section or complex level, strains associated with toxigenic genera were obtained as monosporial culture, originated from single spore and obtained by the method of exhaustion on a poor potato-dextrose agar (using the streaking method).

A conidia suspension of each isolated colony was prepared in test tubes containing Tween® 20 polysorbate at 0.01% in sterile water by scraping conidia with a sterile needle on the colony in active growth. The final concentration of the conidia present in the suspension was determined by KOVA™ Glasstic™ Slide 10 with Grids. After adjusting conidia concentration to 10^3 conidia /ml by diluting with sterile water, 0.1 ml of this suspension were inoculated on Petri dishes plates, 90 mm Ø

containing 15 g/L (1.5%) agar. The plates were incubated at 25°C in thermostat. Twenty-four hours after inoculation, at the moment of early germination phase, the single colony was detected under 4x objective magnification with a Leitz Orthoplan optic microscope (Ernst Leitz GmbH, Wetzlar, HEL, Germany), taken from the plates by a sterile scalpel and transferred into PDA plate. After 5 days of incubation in thermostat at 25 °C, the monosporial cultures of *Aspergillus* section *Flavi* (ASF) and section *Nigri* (ASN) and *Penicillium* spp (PEN) were transferred to 4°C for storage. Similarly, colonies referred to *Fusarium* spp (FUS) were inoculated on PDA supplemented with 5 g/L of KCl (Fisher et al., 1983) in order to be used for identification of the micro-conidial chains typical of the *F. fujikuroi* (FFSC) species complex (Leslie and Summerell, 2006) frequently associated with maize and derivates (Figure 11).

The colonies of *Fusarium* spp., that after 7 dpi at 24 °C had not developed micro-conidial chains were inoculated onto SNA (Spezieller Nährstoffarmer Agar, composed by 1 g/L KH_2PO_4, 1 g/L KNO_3, 0.5 g/L $MgSO_4 \cdot 7H_2O$, 0.5 g/L KCl, 0.2 g/L glucose, 2 g/L sucrose and 20 g/L agar; (Nirenberg, 1976)) in order to detect the microscopic characteristics of the isolates. SNA plates were incubated at 22-24°C in alternating light/dark conditions every 12 hours. After the incubation, observations were made by using the appropriate optical microscope at different magnifications on the shape and size of macroconidia, microconidia, phialides and clamidospores. Data collected during macroscopic and microscopic observations were used to identify the various strains following dichotomy keys (Balmas et al., 2000; Leslie and Summerell, 2006). From a taxonomic point of view, the taxa of *Fusarium* were classified according to the review proposed (Aoki et al., 2014). The identification at species level within each toxigenic taxon was not relevant to the aim of the present study.

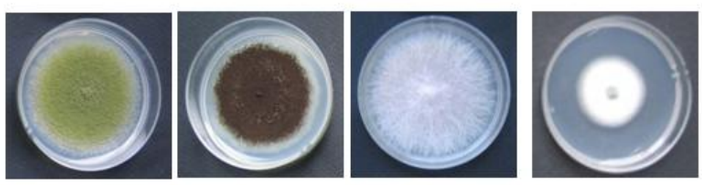

Figure 11. *Aspergillus* section *Flavi*, *Aspergillus* section *Nigri*, *Fusarium* and *Penicillium* spp. identified from Haitian samples.

1.2.2.3. Aflatoxigenic potential of *Aspergillus* section *Flavi* strains

The ability of forty-nine arbitrarily selected *Aspergillus* section *Flavi* monosporial strains to synthesize AF *in vitro* was assessed following the procedure described by El Mahgubi et al. (2013). For this purpose, agar-mycelium disks of 0.6 cm of diameter were withdrawn from active cultures on PDA and inoculated on yeast extract sucrose agar (YESA), having the following composition: 20 g/L yeast extract (Difco®), 150 g/L sucrose, 15 g/L agar (Oxoid), 0.5 g/L $MgSO_4 \cdot 7H_2O$, 10 mg/L $ZnSO_4 \cdot 7H_2O$, 5 mg/L $CuSO_4 \cdot 5\ H_2O$, final pH 6.5 (Samson et al., 2010). Evaluation of AF production was carried out after 14 days of incubation in thermostat at 30°C. Ten agar plugs of 0.6 cm Ø were taken from Petri dishes where ASF single strain was grown and placed into 4ml-Wheaton® vials (Wheaton Industries Inc., Millville, NJ, USA) containing 2 ml of an extraction solution consisting of a mixture of Ethyl acetate/Methanol (1:1, v/v). After 24 hours, the plugs were removed and the solvent left to evaporate under an extractor hood. The crude extract was suspended again in 0.4 mL of a mixture of chloroform/methanol (1: 1, v/v) and chromatographed by thin layer chromatography (TLC) on Aluminum TLC plates of silica gel coated with fluorescent indicator F254 (TLC Silica gel 60 F_{254}, Cod. 105554 - Merck Millipore S.p.A., Italy). The elution mixture (mobile phase) of TLC plates was constituted by a solution containing toluene/ethyl acetate/formic acid 6:6:1 (v: v: v). Volumes of 1 µl of extract solution obtained from individual strain and reference AFB1 and AFG1 (Sigma-Aldrich,) were placed on each plate. To quantify results, the production of AF by single ASF strain was detected by comparison of Rf (Rate of flow) of the crude extract with the reference standards B1 and G1 under backlight (366 nm wavelength) by using UV lamp (CAMAG Chemie-Erzeugnisse & Adsorptionstechnik AG., Muttenz, Switzerland) as seen on Figure 12.

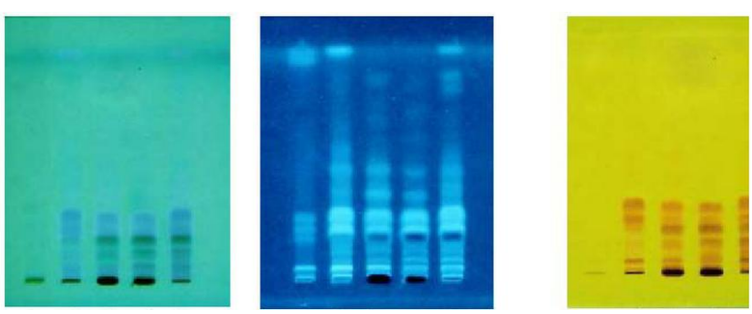

Figure 12. Aflatoxin production by *Aspergillus* section *Flavi* strains under 366 nm light.

1.2.2.4. Aflatoxin quantification

Aflatoxin quantification of Haitian crops was performed following enzyme-linked immunosorbent assay (ELISA) and High Performance Liquid Chromatography (HPLC) techniques. For February 2015 samples (first sampling), AF was quantified by ELISA. ELISA is a test that uses antibodies and colour change to identify or quantify substance as AF.

August 2015 samples (second sampling) were submitted to AF quantification using HPLC. HPLC is an analytical chemistry technique used to separate, identify, and quantify each component in a mixture. The procedures of AF quantification in cereal grains and oleaginous seeds as well as PPs were very different and were relative to material matrix.

a) Aflatoxin quantification with ELISA test

To determine the content of AF in February 2015 samples, the extraction and quantification protocol were provided by the company producing the immuno-enzymatic kit (RIDASCREEN® Aflatoxin Total from R-Biopharm AG, Darmstadt, Germany). Briefly, the extraction consisted of the initial mechanical milling of a representative sample, prepared after careful mixing of samples matrix. For extracting AF, 70% of methanol was added to the ground material (5 g) and to oleaginous seeds. Also sodium chloride (2.5 g/L) was added, in order to absorb water. Formed mixture was shaken manually and energetically for 10 minutes. In the case of oil seeds, i.e. peanut and moringa, the extract was centrifuged for 15 minutes at 3000 g at room temperature and then the supernatant fat layer was removed. Using Whatman N.1 paper, specially bent for funnel, the extracts were filtered and placed in polypropylene tubes and stored at -20 °C until ELISA dosing. The detection limit of the kit was 1.75 µg/kg. ELISA was performed following the procedure provided with the kit by the manufacturer. Diluted extracts (1: 2, v/v) and aflatoxin B and G standard solutions (0 zero standard, 0.05, 0.10, 0.45, 1.35 and 4.05 µg/kg AFB1 + AFG1 in methanol/water) were subjected to ELISA tests. The reading of the individual reaction complex was performed in absorbance at 450 nm. The absorbance results obtained by reading the Sunrise® Absorbance Reader (Tecan Group Ltd, Männedorf, CH) microplate microscopy readings were analyzed using the Logit-log function converted into total aflatoxin concentrations (µg/kg) using the RIDA® SOFT Win software (R-Biopharm) software.

b) Aflatoxin quantification with HPLC

Extraction and purification protocol provided by the company producing the immuno affinity columns (IAC) EASI-EXTRACT® Aflatoxin (R-Biopharm) were used for AF quantification in August 2015 samples.

About 100 g of seed samples was milled for 1-2 minutes in a laboratory mill (Foss Cyclotec Sample Mill 1093; Foss Italia S.r.l.) set at the finest setting and a representative sub-sample of 50 g was used for subsequent analyzes. HPLC-grade analytical reagents, such as sodium chloride (NaCl), potassium bromide (KBr), phosphate buffer saline (PBS) and nitric acid (HNO_3) solution, and solvents, such as water, methanol and water, were purchased from Merck (Merck KGaA, Darmstadt, Germany). AF extraction consisted of high speed mixing for 2 minutes of ground material added of 5 g of sodium chloride with methanol 80% (v/v) for maize (milled, flour and grains), and methanol 100% for oleaginous products (peanuts, moringa and peanut butter). Water was added into peanut and moringa preparations and a further high speed mixing was performed for 1 minute and absolute methanol was added and the mixture was blended again for 2 minutes. After centrifugation at 4500 *g* for 10 min, the supernatant was filtered through a fluted Whatman no. 4 filter paper (GE Healthcare Ltd). Filtrates obtained from maize and derivates blends were diluted 1:8 (v/v) in saline phosphate buffer (PBS, Oxoid) while filtrates of oleaginous seeds were adjusted to pH 7.4 by using 2 M NaOH. All the filtrates were passed through the IA (immunoaffinity) columns as indicated by manufacturer. The final collection of AFs "captured" from column IA was transferred to amber glass tubes, and 1 ml of the vial was immediately diluted with water in a ratio of 1:1 (v/v).

The AFs contained in purified filtrates were quantified by injecting 100 µl of sample into the HPLC system a flow rate of 1 ml/ min Agilent 1260 Infinity HPLC system (Agilent Technologies Inc. Santa Clara, CA, USA). This system was equipped with a quaternary pump (G1311B), vacuum degasser (G1379B), self-sampler (G1329B), fluorescence detector Agilent 1260 Infinity/1200 series (G1321B), analytical column of Inertsil ODS-3V (5 µm, 4.6 x 150 mm; GL Sciences Inc. Tokyo, Japan) protected with a guard column (5 µm, 10 x 3.2 mm, Hicrom Ltd., Berkshire, UK).

Both columns were kept at a temperature of 40°C. The mobile phase consisted of isocratic elution with daily prepared mixture of water: methanol (60:40, v/v) adjusted with 119 mg/L KBr and 35% HNO_3 (4

M). The flow was maintained at 1 ml/min. AFs were detected by fluorescence: AFB1 - AFB2 with λ excitation at 362 nm and λ emission at 425 nm; AFG1 - AFG2 λ excitation at 365 nm and λ emission and 455 nm. In order to increase the natural ultraviolet AF fluorescence and make it more easily identifiable before reading the fluorimeter, the eluent samples were derivatized post-column with Br_2, generated by KBr present in the mobile phase, using the electro-chemical cell Kobra Cell (R- Biopharm) calibrated at 100 µA. The AFs were quantified by comparing the peak areas obtained from the samples eluted with those of the standard AFLASTANDARD ready-to-use solution (R-Biopharm). The standard solution was constituted with AFs at a concentration of 1000 µg/kg in 50% methanol and each of the 4 aflatoxins (AFB1 - AFB2 - AFG1 - AFG2) was present in 25% solution. A calibration curve was constructed daily with 5 standard concentrations: 0 (standard zero), 2, 10, 20, 40 and 100 µg/kg (Figure 13). The quantities of the 4 aflatoxins were expressed in µg/kg of the initial injected sample (0.033 g). Detection limits 1.2 µg/kg.

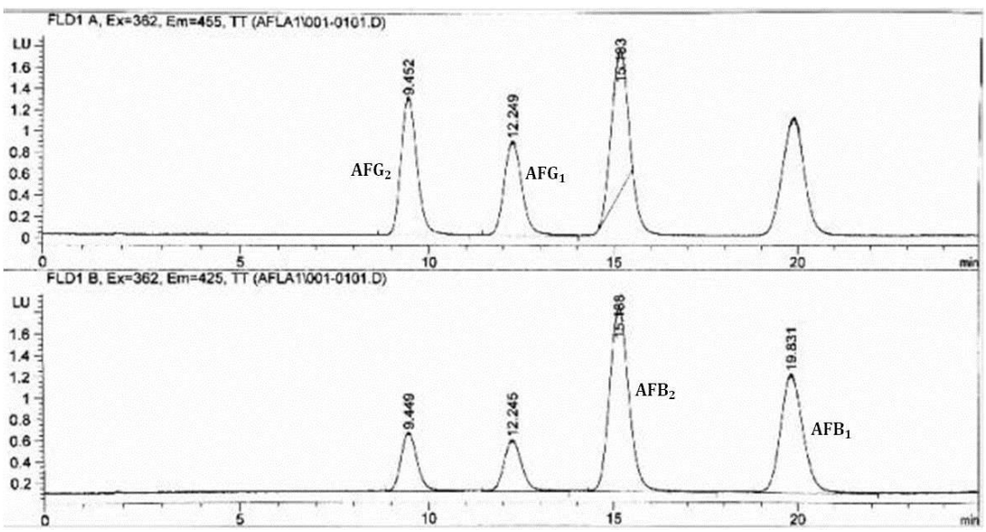

Figure 13. HPLC aflatoxin chromatograms.

1.2.3. Statistical analyses

The SPSS statistical package for Windows, v. 23.0 (SPSS Inc.), was used for all statistical analyses (Corp, 2013). First of all, data expressed in percentage such as isolation frequency (IF) of PTF in the grains and seeds, with the equation y = arc sin (√p/100) were transformed to normalize distribution of residuals and remove heterogeneity of error variances prior to analysis of variance (ANOVA). Normal distribution and homogeneity of variances were verified using the Shapiro–Wilk test and the Levene's test, respectively (Brown and Rothery, 1993). One-way analysis of variance was carried out and the Ryan-Einot-Gabriel-Welsch-*F* test (REGW-*F*) was applied to detect significant differences

among means observed for each collected datum except a_w, occurrence of PTF in PPs (CUF/g) and aflatoxigenic ASF strains. Contingency tables were produced with AFs chemotype frequencies, which were then compared using the Chi-square test or a two-tailed Fisher's exact test, when appropriate. Pearson's rank correlation coefficients (ρ) were calculated to estimate associations among means AFs (aflatoxin B1, B2 and total), a_w and IF of PTF with particular attention to ASF in seed and grain samples with exception to bean ones. Correlation matrix was also calculated for meal samples. Medians aflatoxin (µg/kg), and IF ASF were assigned to Student t test ($p<0.05$, Levene's test) for establishing comparison when necessary. Fungal mycobiota and AFs found in Haitian maize grains data were analyzed using the multivariate analysis i.e. the principal component analysis. Agricultural practices recorded during maize sampling 2015 year were used as independent variables (IVs) in order to detect the contribution of each one to the total fungal and AF contamination found in maize grains. The relationships between AGPs were displayed by means of Gabriel's Biplot (Rawling, 1988). A biplot illustrates relationships among the IVs, the relative similarities of the individual datum point, and the relative values of the observations for each IV.

Chapter 1.3. Results

Samples from 2014 and 2015 cropped season collections were measured for a_w. Fungal mycobiota of samples were also evaluated with particular attention to PTF such as ASF, ASN, *Aspergillus* section *Circumdati* (ASC), FUS, PEN. Forty-nine ASF strains isolated from maize, moringa and peanut – from the first sampling were also tested for their ability to produce AFB1 and AFG1 *in vitro*. AFs were quantified using ELISA in February-samples, while in 2015 samples HPLC was utilized.

1.3.1. Activity water (a_w)

1.3.1.1. Samples from February 2015

In February 2015, 28 samples were analyzed. Twenty-six were seeds/grains (SS) and two PPs, moringa leaves powder (sample 12) and cassava flour (sample 20). SS were subdivided in grain (GS) and oleaginous samples (OS). GS were constituted of maize and bean and, OS regrouped moringa and peanut seeds.

A_w results obtained from first sampling are presented in (Table 6, 7). Low variation was observed in substrates. SS were less dried compared with PP. SS samples showed a_w ranging from 0.59 to 0.77 and almost 37% showed $a_w \geq 0.70$. For PPs, the maximum a_w recorded was 0.55 obtained in moringa flour (Code 12).

Dissimilarity was also observed among SS. Maize samples were well dried compared with bean samples. Maize grains showed a_w ranging from 0.65 (Code 1 and 2) to 0.77 (Code 5). Bean samples had a_w ranging from 0.60(Code 23) to 0.75 (Code 21 and 26). More than 38% of bean samples have $a_w > 0.70$ while almost 78% of maize revealed $a_w < 0.70$.

OS revealed a_w ranging from 0.59 (Code 14) to 0.74 (Code 13). More than 44%, showed $a_w \geq 0.70$. Moringa samples were less dried compared to peanut. Sixty seven percent moringa samples had $a_w \geq 0.70$, comparing to less than 34% of peanut samples that overlapped this value (Table 6). All 2014 PPs showed $a_w < 0.70$. Cassava flour was better dried compared with moringa powder (Table 7).

Table 6. Activity water and potential toxigenic fungi isolated from seeds of the first sampling

Host	Code	Origin	a_w	Isolation Frequency (IF)				
				ASF (%)	ASN (%)	ASC (%)	FUS (%)	PEN (%)
Maize	1	Redon	0.65	48.00ab	28.00bc	nd#	33.30ab	10.70a
	2	Bourry	0.65	9.30a	6.70 a	nd	69.30bc	24.70ab
	3	Moro	0.69	9.30a	6.80 ab	nd	70.00 c	24.00ab
	4	Chantal	0.68	34.00ab	23.3abc	nd	50.70ab	45.33ab
	5	Guillerme	0.77	97.30c	58.00e	nd	50.80abc	52.67b
	6	Durcis	0.67	80.70c	51.30de	nd	32.70ab	42.00ab
	7	Levy	0.74	31.33ab	20.70abc	nd	31.30a	53.33b
	8	Durcis	0.68	47.70abc	39.30cd	nd	26.00a	32.00ab
	9	Minet	0.68	40.00ab	33.30cd	nd	26.70a	37.33ab
Moringa	10	Redon	0.68	80.70c	12.70	10.70	nd	2.30
	11	Durcis	0.71	54.70b	12.00	10.60	nd	17.30
	13	Belle Source	0.74	1.30a	nd	nd	14.70	9.30
Peanut	14	Guilloux	0.59	4.00ab	60.00e	nd	nd	nd
	15	Bourjoly	0.72	46.67d	11.33bc	nd	nd	26.00b
	16	Post-Droit	0.68	28.00c	38.00d	nd	nd	nd
	17	Beraud	0.66	nd	nd	nd	nd	nd
	18	Moreau	0.70	16.67bc	22.00c	nd	1.30	2.67a
	19	Durcis	0.67	6.67ab	10.67ab	nd	nd	2.67a
Bean	21	Levy	0.75	nd	nd	nd	nd	nd
	22	Redon	0.62	nd	nd	nd	nd	nd
	23	Redon	0.60	nd	nd	nd	nd	nd
	24	Minet	0.64	nd	nd	nd	nd	nd
	25	Durcis	0.69	nd	nd	nd	nd	nd
	26	Beraud	0.75	nd	nd	nd	nd	nd
	27	Torbeck	0.68	nd	nd	nd	nd	nd
	28	Maillard	0.70	nd	nd	nd	nd	nd

Legend. nd: not detected; a_w: Activity water; ASF: *Aspergillus* section *Flavi*; ASN: *Aspergillus* section *Nigri*; ASC: *Aspergillus* section *Circumdati*; FUS: *Fusarium* spp; PEN: *Penicillium* spp. Values with same letters indicate there is no significant difference for p<0.05 (test REGW-*F*).

Table 7. Activity water and potential toxigenic fungi isolated in processed products from first sampling

Host	Code	Origin	a_w	Fungal isolation				
				ASF (CFU/g)	ASN (CFU/g)	ASC (CFU/g)	FUS (CFU/g)	PEN (CFU/g)
Moringa	12	Redon	0.55	6.00	2.80	2.10	nd	2.80
Cassava	20	Redon	0.52	nd#	nd	nd	nd	nd

Legend. nd: not detected; a_w: Activity water; ASF: *Aspergillus* section *Flavi*; ASN: *Aspergillus* section *Nigri*; ASC: *Aspergillus* section *Circumdati*; FUS: *Fusarium* spp; PEN: *Penicillium* spp.

1.3.1.2. Samples from August 2015

In August 2015, 50 samples were analyzed, 42 SS and 8 PPs. SS analyzed were 20 maize grains and 22 OS. OS were eight peanut and 14 moringa seeds. Eight PPs analyzed included six meal samples (MS), one maize flour (MF) and one peanut butter (PB). Results obtained for a_w of 2015 samples are showed in (Table 8), (Table 9), (Table 10 and 11) for OS for respectively maize grains, PPs, and OS.

Similarly to February2015 samples, SS were less dried compared with PPs. PPs revealed a_w ranging from 0.38 (code 56) to 0.57 (code 52). Dissimilarity was also observed among August 2015 year PPs. MS were less dried compared with MF and PB. Means a_w obtained from MS was 0.53 compared with 0.38 and 0.40 detected in MF and PB respectively (Table 9). Also, important dissimilarity was detected among analyzed SS. Maize grains were more humid compared with OS. Maize samples showed a_w varied from 0.57 (Code 39) to 0.73 (code 33) and 30% revealed $a_w \geq 0.70$ (Table 8). OS showed a_w ranging from 0.42 to 0.67(Table 10 and 11). Like in February 2015 year samples, important variation was also detected among OS. Moringa seeds were less dried compared with peanut. The lowest and highest a_w detected in peanut seeds were respectively 0.42(Code 63) and 0.57(Code 59) with an average of 0.52(Table 10). Moringa seeds showed 0.47(Code 75) and 0.65 (Code 65) as minimum and maximum values obtained for a_w. Mean a_w observed in moringa samples was 0.57(Table 11). Moringa sampled at Cavaillon were less dried compared with those collected at Torbeck. Means a_w detected in Cavaillon and Torbeck moringa were respectively 0.58 and 0.55 (Table 11).

Considering all the 78 samples collected during the two sampling, SS were less dried compared with PPs. Almost 21% (14/68) of tested SS had $a_w \geq 0.70$. Fifty percent of those samples were maize grains.

Table 8. Activity water, potential toxigenic fungi and aflatoxin found in maize grains from second 2015 year sampling

Code	Origin	a_w	Isolation Frequency (IF)				Aflatoxin		
			ASF (%)	ASN (%)	FUS (%)	PEN (%)	AFB1 (µg/kg)	AFB2 (µg/kg)	AFtot (µg/kg)
29	**Guillerme**	0.72	38abcde	65.33defg	24.67abcdef	18.67a	144.8	11.90	156.70
30	**Guillerme**	0.71	66def	71.33defg	2.67ab	16.67a	643.70	36.40	680.10
31	**Bourry**	0.72	58.67defg	26.67abcde	30bcdef	11.33a	448.40	37.30	485.70
32	**Bourry**	0.71	48.67abcdef	58cdefg	41.33def	52b	nd#	nd	nd
33	**Redon**	0.73	88efg	54.67abcdefg	4abcd	16a	681.00	57.40	738.40
34	**Redon**	0.68	52.67bcdefg	30abcdef	2.67ab	23.33a	134.80	13.40	148.20
35	**Miserne**	0.66	76.67defg	49.50abcdefg	29.50abcdef	6.50a	nd	nd	nd
36	**Cance**	0.64	3.33ab	23abcd	43def	9a	nd	nd	nd
37	**Mersan**	0.65	2.67a	8.67a	0.67a	6a	29.50	nd	29.50
38	**Mersan**	0.63	29.33abcde	9.33ab	61.33f	22a	nd	nd	nd
39	**Mersan**	0.57	56.67cdefg	52abcdefg	17.33abcde	7.33a	nd	nd	nd
40	**Durcis**	0.68	90.67fg	22.67abcd	3.33abc	28ab	250.10	23.10	273.20
41	**Durcis**	0.60	15.33abcd	62cdefg	18.67abcdef	3.33a	nd	nd	nd
42	**Beraud**	0.58	46abcdef	59.33cdefg	13.33abcde	14a	19.20	1.60	20.90
43	**Beraud**	0.64	86.67defg	22abc	nd	19.33a	nd	nd	nd
44	**Beraud**	0.62	96g	93fg	nd	5.33a	nd	nd	nd
45	**Beraud**	0.60	35.33abcde	56.67bcdefg	36.67cdef	2.67a	62.10	5.30	67.30
46	**Morency**	0.64	4.67abc	86cfg	0.67a	1.33a	958.70	74.60	1033.20
47	**Cavaillon**	0.76	19.33abcd	64.67cdefg	46ef	52.67b	nd	nd	nd
48	**Cavaillon**	0.67	58.67defg	93.33g	nd	16a	75.97	8.47	84.40

Legend. nd: not detected; a_w: Activity water; ASF: *Aspergillus* section *Flavi*; ASN: *Aspergillus* section *Nigri*; ASC: *Aspergillus* section *Circumdati*; FUS: *Fusarium* spp; PEN: *Penicillium* spp. AFB1: aflatoxin B1; AFB2: aflatoxin B2, AFtot: aflatoxin total. Values with same letters indicate there is no significant difference for $p<0.05$ (test REGW-*F*).

Table 9. Activity water, potential toxigenic fungi and aflatoxin found in Haitian processed products from second 2015 year sampling

Hosts	From	Code	A_w	Potential toxigenic fungi				Aflatoxin content		
				ASF (CFU/g)	ASN (CFU/g)	FUS (CFU/g)	PEN (CFU/g)	AFB1 (µg/kg)	AFB2 (µg/kg)	AFtot (µg/kg)
MBMG	CdB	49	0.48	29.50 $x10^2$	27.50 $x10^2$	31.80 x 10^3	0.10 x 10^3	148.50	17.5	166.00
MBMG	CdB	50	0.51	0.50 x10	nd	nd	0.50 x 10^5	nd	nd	nd
MBMG	MeF	51	0.52	0.50 x10	nd	nd	0.10 x 10^7	nd	nd	nd
MBMG	MeF	52	0.57	nd	0. 20 x 10^2	nd	10.90 x 10^2	nd	nd	nd
MMMG	CdB	53	0.54	6.05 x 10^2	0.35 $x10^2$	16.35 $x10^3$	0.80 x 10^2	132.10	18.10	150.20
MMMG	MeF	54	0.54	0.50 x10	nd	nd	10.05 x 10^2	14.90	nd	14.90
PB	UdT	55	0.40	nd	nd	nd	nd	nd	nd	nd
MF	UdT	56	0.38	0.15 $x10^2$	nd	nd	nd	59.90	5.70	65.60

Legend. nd: not detected; MBGM: milled big maize grain; MMMG: milled middle maize grain; PB: peanut butter and MF: maize flour; UdT: Unit of Transformation; MeF: *Marché en fer* and CdB: *Croix des bossal*. AFB1: aflatoxin B1; AFB2: aflatoxin B2, AFtot: aflatoxin total.

Table 10. Activity water, potential toxigenic fungi and aflatoxin found in August 2015 peanut seeds

Provenience	Code	a_w	Isolation frequency				Aflatoxins content		
			ASF (%)	ASN (%)	FUS (%)	PEN (%)	AFB1 (µg/kg)	AFB2 (µg/kg)	AFtot (µg/kg)
Les Anglais	**57**	0.52	14.00ab	8.35a	nd	10.35	nd	nd	nd
Mersan	**58**	0.46	8.00ab	2.00a	2.70	5.30	nd	nd	nd
Chantal	**59**	0.67	28.00bc	34.00bc	nd	11.30	68.60	25.40	94.00
Cance	**60**	0.50	7.30ab	24.00bc	6.00	0.70	nd	nd	nd
Miragoane	**61**	0.55	41.30c	34.00c	nd	5.30	152.60	34.00	186.60
Mersan	**62**	0.50	nd	nd	nd	nd	nd	nd	nd
Cavaillon	**63**	0.42	4.70a	23.30b	nd	2.00	nd	nd	nd
Durcis	**64**	0.55	16.70ab	8.70a	nd	6.70	316.90	58.20	375.10

Legend. nd: not detected; a_w: Activity water; ASF: *Aspergillus* section *Flavi*; ASN: *Aspergillus* section *Nigri*; ASC: *Aspergillus* section *Circumdati*; FUS: *Fusarium* spp; PEN: *Penicillium* spp. AFB1: aflatoxin B1; AFB2: aflatoxin B2, AFtot: aflatoxin total. Values with same letters indicate there is no significant difference for p<0.05 (test REGW-*F*).

Table 11. Activity water, potential toxigenic fungi and aflatoxin found in August 2015 moringa seeds

			Isolation frequency				Aflatoxin content		
Origin	**Code**	**a_w**	**ASF (%)**	**ASN (%)**	**FUS (%)**	**PEN (%)**	**AFB1 (µg/kg)**	**AFB2 (µg/kg)**	**AFtot (µg/kg)**
Cavaillon	**65**	0.65	6.70c	5.70d	nd#	4.70i	nd	nd	nd
Cav	**66**	0.59	6.70c	1.30a	4.70d	1.30b	nd	nd	nd
Cav	**67**	0.57	24.00j	8.70f	0.60b	6.70j	2.80	nd	nd
Cav	**68**	0.57	2.70a	57.30l	nd	1.30b	nd	nd	nd
Cav	**69**	0.56	8.70d	56.00k	8.00f	1.30b	nd	nd	nd
Cav	**70**	0.63	16.70i	34.00i	0.70c	2.70d	1.20	nd	1.20
Cav	**71**	0.49	12.00f	5.30d	nd	0.70a	7.80f	3.90	11.70
Torbeck	**72**	0.60	14.70g	8.00e	26.00h	17.30k	nd	nd	nd
Torb.	**73**	0.48	10.70e	45.30j	nd	4.00f	581.00	57.70	638.70
Torb.	**74**	0.54	15.30h	20.70h	0.70c	2.00c	1.60	nd	1.60b
Torb.	**75**	0.47	3.30b	57.30l	nd	2.00c	66.50	4.20	70.80
Torb.	**76**	0.64	6.70c	2.70b	8.70g	3.30e	31.20	3.40	34.70
Torb.	**77**	0.52	3.30b	4.00c	6.70e	0.70a	nd	nd	nd
Torb.	**78**	0.62	48.00k	15.30g	nd	4.00f	449.40	49.50	498.90

Legend. nd: not detected; a_w: Activity water; ASF: *Aspergillus* section *Flavi*; ASN: *Aspergillus* section *Nigri*; ASC: *Aspergillus* section *Circumdati*; FUS: *Fusarium* spp; PEN: *Penicillium* spp. AFB1: aflatoxin B1; AFB2: aflatoxin B2, AFtot: aflatoxin total. Values with same letters indicate there is no significant difference for p<0.05 (test REGW-*F*).

1.3.2. Fungal mycobiota of samples

In addition to a_w measurement, SS and PPs were also tested for fungal occurrence as described in M&M. Seedborne and PTF were investigated. The most important PTF searched were *Aspergillus* genus, *Fusarium* and *Penicillium* spp. *Aspergillus* genus was mostly investigated for section *Flavi*, section *Nigri* and section *Circumdati*. Fungi isolated from PPs was expressed by colony forming units (CFU/g) and those from SS was expressed by isolation frequency (IF). Results obtained for PPs and SS are showed in (Tables 6 and 7) and (Tables 8, 9, 10 and 11) for respectively the first and second sampling.

1.3.2.1. First sampling- February 2015 year

Seed samples were more contaminated compared with PPs. More than 68% (17/26) of SS were contaminated with PTF compared to 50% (1/2)

PPS. Moringa leaves powder was contaminated with five PTF. *Aspergillus* genus with a total of 10.9 CFU/g was more common compared with *Penicillium* spp, 2.80 CFU/g. *Fusarium* spp was not detected in moringa powder. Cassava flour was fungal free (Table 7). Significant variation was also observed among SS. Bean were free of PTF. *Mucoraceae*, *Sclerotiniaceae*, *Cladosporium* spp., *Eurotium* spp. and *Trichoderma* spp were the main fungal genera found in bean samples (not showed data). Contaminated SS were colonized also by five PTF. More than 94% (16/17) of infested SS were colonized with ASF and ASN. Also PEN colonized almost 89% of samples. ASC and FUS were the less common fungi isolated in February 2015 SS.

Maize grains were more contaminated compared with OS. Five PTF colonized OS compared to four observed in maize grains. All tested maize samples were contaminated simultaneously with the four most important PTF: ASF, ASN, FUS and PEN. PTF contamination showed dependence on location of maize samples ($p<0.05$). Sample from Moro (Code 3) was less ASF and ASN contaminated compared with Guillerme and Durcis (Code 4 and 5). Redon (Code1) was less contaminated with PEN compared with Levy (Code 7). Maize sampled at Bourry (Code 2) was less susceptible to PTF contamination compared with those sampled at Durcis (Code 6 and 8).

OS were differing in regard to PTF. Five PTF contaminated moringa compared to four detected in peanut. *Aspergillus* genus was more occurred in OS samples compared to PEN. FUS was quite absent in all oleaginous substrates. All moringa samples showed similar PTF contamination ($p>0.05$) except for ASF. Moringa from Redon was significantly more contaminated compared with Belle source and Durcis (Table 6). Moringa samples were also infected with seedborne fungi. Twenty-eight per cent of moringa seeds moulds belong to non-toxigenic species, i.e. *Botryosphaeriaceae*, *Mucoraceae*, *Cladosporium* spp., *Nigrospora* spp. Peanut samples were also colonized by seedborne fungi. *Alternaria* spp., *Cladosporium* spp., and *Mucoraceae* occurred in 39% of samples (data not showed).

1.3.2.2. Second sampling- August 2015 year

Contrarily to February 2015 samples, August food materials showed contamination with four PTF. PPs were less contaminated than SS. Maize derived products were more contaminated compared with PB. Only 0.15 $x10^2$ CFU/g of ASF were detected in PB. MF was fungal free.

PEN occurred in all tested meal and ASF were detected in more than 83%. PEN IF was ranging from 0.80 x 10^2 to 0.10 x 10^7 CFU/g detected in sample 53 and 51. Meal samples from MeF were less contaminated compared with CdB. ASF infection was ranging from 5 to 29.50 x 10^2 CFU/g in meal samples. FUS and ASN were the less occurred taxa (Table 9).

Significant variation was also detected among August 2015 year SS. maize grains were more infected compared with OS. Conversely to five PTF isolated in February 2015 samples, ASC was not detected among OS August 2015 year. *Aspergillus* genus was more frequently isolated compared to PEN and FUS. PTF IF in August 2015 samples were ranging from nd levels to 57.30 (Table 10). Peanut samples were less infested compared with moringa. Less than 90% of peanut samples were contaminated by PTF compared with all moringa. *Aspergillus* genus was more predominant in moringa compared with PEN and FUS. The highest and lowest IF ASF was registered in moringa from Torbeck (sample 78 and 77). Fifty per cent of tested samples showed PEN IF ≤ 20 and were quite low contaminated with FUS (Table10).

Similarly to moringa samples, peanut August 2015 samples were more colonized by *Aspergillus* genus compared with PEN and FUS. FUS colonized 25% of tested samples whilst PEN was detected at very low levels (IF<15). ASF and ASN IF showed dependence on origin. Sample from Miragoane was more contaminated compared with Cance and Miserne. ASN occurred more frequently than ASF. The maximum and minimum ASN (IF=34 and nd) were noted in samples 59/61 versus sample 62 (from Chantal/Miragoane vs Mersan) respectively. Similarly, ASF infections were ranged from nd levels to up than 40%. The highest and lowest (IF=41.30 and nd) were still detected in (samples 61, Miragoane) and (sample 62, Mersan). Sample 62 from Mersan was free of PTF (Table10).

Aspergillus genus, *Penicillium* spp and *Fusarium* spp colonized also August 2015 maize grains. ASF and ASN variation was almost similar for the two taxa. ASF occurred in all samples at variation levels ranging from less than 4% (sample 36 and37) to greater than 85% (sample 33, 40, 43 and 44). The highest and the lowest IF were recorded mainly in (sample 44, Beraud and sample 37, Mersan). All samples were also infected with ASN. The highest and the lowest (IF = 93.33 and IF=8.67) ASN were detected at Cavaillon (Code 48) and Mersan (Code 37).

Amplitude of PEN contamination of maize grains was (IF=51.34). The highest and the lowest (IF=52.67 and IF=1.33) were detected at Cavaillon (Code 47) and Morency (Code 46), respectively. Regarding FUS, the maximal and minimal IF recorded in grains were detected in two samples coming from Mersan (IF=0.67, sample 37 and IF=61.33, sample 38).

Fungal mycobiota of maize grain showed dependence of origin of samples ($p<0.05$). Maize from Cavaillon and Morency were mostly colonized by *Aspergillus* genus compared with Mersan and Miserne. Durcis (code 40) and Redon (Code 33) samples were more ASF contaminated compared with Cance (Code 33). Samples from Beraud were more colonized with ASF and ASN compared with PEN and FUS. FUS and PEN were more predominant in samples from Cavaillon (Code 47) compared to Morency (Code 46). *Penicillium* spp. occurrence was quite greater at Redon (Code 34) compared with Beraud. Furthermore 67% (2/3) of samples, collected at Beraud were free from FUS in comparison with Bourry and Cavaillon whose all samples showed highest FUS infection (Table 8).

Regarding all 78 samples collected, SS were more contaminated with PTF compared with PPs. Aspergillus with 48.667 (IF) and 16.533 (IF) for ASF and ASN respectively, was the most occurred genus. PEN with 50.687 (IF) and FUS with 18.567 (IF) completed fungal mycobiota of February and August 2015 SS. Meal samples were more contaminated compared with others PPs. PEN was also the most occurred genus isolated in PPs followed by *Aspergillus*.

1.3.3. Aflatoxigenic capacity of *Aspergillus* section *Flavi* strains *in vitro*

Among all ASF strains isolated from February 2015 samples, forty- nine were arbitrarily selected for studying their ability to produce AFB1 and AFG1 *in vitro* as described in M&M. Those strains were collected from maize grains, moringa and peanut seeds. Results obtained from the tests are presented in Table 12. A clear majority of toxigenic (83 %) Haitian ASF strains was observed in samples compared to atoxigenic ones (17 %) ($\chi^2 = 33.11$; $P < 0.001$). OS strains were more suitable to produce AFs compared with those from maize. Eighty-seven percent (13/15) OS strains produce AFs compared with 76% (26/34) detected in maize grains. Important dissimilarity was also detected among OS strains tested. Ninety percent of tested peanut strains were AFs producer compared to 80% detected in moringa. Moringa seeds were contaminated by a population of ASF strains with a homogenous

chemotype pattern (χ^2 = 1.60; P = 0.45). Peanut was colonized by heterogeneous strains able to produce significant AFB1 > AFB1&G1 (P = 0.007). In all tested strains, AFB1 producers were more common compared with AFG1 and AFB1&G1 producer. Atoxigenic strains represent more than two times of AFB1&G1. No significant association was found between AF profile and host of the Haitian ASF strains. Similarly to peanut samples, maize also was mainly contaminated by chemotypes I and II ASF strains and by atoxigenic ASF strains, to a lesser extent.

Table 12. Aflatoxin chemotypes pattern of *Aspergillus* section *Flavi* strains from maize, moringa and peanut seeds February 2015 year

		Aflatoxin chemotypes				
Host	**Number of tested isolates**	**I AFB1**	**II AFB1+AFG1**	**III AFG1**	**IV None**	**P-values**
Maize	34	24	2	nd	8	**< 0.001**
Moringa	5	3	1	nd	1	**0.449**
Peanut	10	8	1	nd	1	**0.007**

AFB1: aflatoxin B1; AFG1: aflatoxin G1

1.3.4. Aflatoxin content

All the 78 SS and PPs were also tested for AF content. AF content of February 2015 samples was determined by using ELISA test, while a more accurate determination using HPLC was used for August 2015 samples.

1.3.4.1. First sampling- February 2015 year

Significant difference was observed among tested SS and PPs February 2015 year. PPs were AFs free. SS showed significant variation also among maize grains and OS. Bean samples were AFs free. All maize, moringa and peanut recorded AFs contaminations greater than 70% of tested samples.

Maize samples had the limits of detection of the method (LOD, signal-to-noise ratio 3:1) and quantification (LOQ, signal-to-noise ratio 10:1) respectively to 1.70 and 5.10 µg/kg. LODs for moringa and peanut samples were 2.1 and 1.8 µg/kg, respectively. LOQs for moringa and peanut samples were 6.50 and 5.60µg/kg, respectively.

AF contamination in maize samples ranged from nd levels to 1500 µg/kg with a mean concentration of 95.40 µg/kg. The ranges of AFs

contamination in moringa and peanut seeds were from nd levels to 700 and 500 µg/kg, respectively. Means values of AF contamination in moringa and peanut seeds were 70.80 and 45.30 µg/kg, respectively. Considering all samples, the greatest AF concentrations were detected in maize grains followed by moringa and peanut seeds with respectively 1500, 700 and 500 µg/kg (not showed data).

1.3.4.2. Second sampling- August 2015 year

Measuring AFs content of PPs and SS collected in Haiti during August 2015 (Table 8- 11), a similarity across samples can be noted. Fifty per cent of PPs (4/8) and SS (21/42) samples were contaminated with AFs. AFB1 occurred more frequently and in a greater proportion than AFB2. Considering PPs, PB was AF free. Maize PPs were AFs contaminated at variable levels. MF showed AFtot = 65.60 µg/kg levels, which represent more than 13 times of the international regulation limit (5 µg/kg). Moreover, 50% meal samples were AF- contaminated. More than 66% AF-contaminated meals had AFs greater than 20 µg/kg, US Food Drug Administration limits. Meal from CdB appeared more AF- contaminated compared with MeF. Almost 67% of samples from CdB were AF-contaminated compared with less than 34% in MeF. The greatest and lowest values (AF= 150.20 and 14.90 µg/kg) were noted in samples 53 from CdB and 54 from MeF.

AFs mean detected from meals sampled at CdB represented almost 21 times of AFs registered in MeF and were highly differed (Tables 9 and 14).

Significant differences were also detected among SS. Maize samples were more AFs contaminated compared with OS. Less than 46% OS were AFs contaminated compared with 55% maize. OS were AF-contaminated at levels ranging from nd to 636.70 µg/kg (Table 10 and 11). Moringa samples were more AF-contaminated compared with peanut. More than 62% peanut samples were AFs free. Contaminated samples showed AF ranging from 94 to 375.10 µg/kg, which were all greater than 20 µg/kg (Table 10).

Fifty percent of tested moringa samples were AF contaminated. The greatest and the lowest (AFtot= 638.70 and 1.20 µg/kg) concentrations were detected (samples 73 from Torbeck and 7 from Cavaillon).

All tested moringa seeds from Cavaillon had AFs< 20 µg/kg. AF of contaminations showed dependence on seeds provenience. Moringa sampled at Cavaillon were less AF-contaminated compared with Torbeck. Eighty percent of contaminated moringa sampled at Torbeck

showed AF concentration >20 µg/kg. Mainly, forty percent (2/5) had AF>100 µg/kg, five times of US Food Drugs Administration limits. Total of means AF contamination of moringa seeds from Cavaillon and Torbeck were respectively 2.49 and 177.81 µg/kg which were highly different ($p<0.001$) (Table 11 and 13).

Regarding maize, AF contamination of grains was ranging from nd to 1033.20 µg/kg with an average of 189.50 µg/kg per sample. Mean AFB1 detected was equivalent at 172.40 µg/kg, which represented almost 13 times AFB2 registered in samples. Twenty four percent of tested maize samples had AFs>100 µg/kg. Two percent had AFs>1000 µg/kg, which represented from five to 50 times the US Food Drugs Administration limits. All maize grains sampled at Guillerme (Code 29 and 30), Redon (Code 33 and 34) and Morency (Code 46) were contaminated and all AF quantified in those places were AFs>100 µg/kg. Samples from Miserne and Cance were AFs free. Samples collected at Mersan and Beraud were less AFs contaminated than those samples at Durcis (Table 8).

Table 13. Comparison between activity water and aflatoxins found in moringa seeds from Cavaillon and Torbeck

	Seeds provenience		
Variables	**Cavaillon**	**Torbeck**	**P-values**
a_w	0.58	0.55	0.06NS
AFB1(µg/kg)	1.93	161.39	0.001**
AFB2(µg/kg)	0.58	16.40	0.001**
AFtot(µg/kg)	2.49	177.81	0.001**

;**;: p<0.05; 0.01; 0.001 and NS: not significant at p<0.05(test REGW-*F*).**

Table 14. Comparison between activity water and aflatoxins found in meal from *Marché en fer* and *Croix des bossals*

	Purchased from		
Variables	**CdS**	**MeF**	**P-values**
a_w	0.51	0.54	0.005**
AFB1(µg/kg)	93.53	4.97	0.002**
AFB2(µg/kg)	11.87	nd	0.001**
AFtot(µg/kg)	105.40	4.97	0.002**

Legend. CdS: *Croix des bossal*; **MeF**: *Marché en fer.*

;**;: p<0.05; 0.01; 0.001 and NS: not significant at p<0.05(test REGW-*F*).**

1.3.5. Bivariate analysis

The values obtained from PTF isolated, a_w measured and AF quantified from each substrate were submitted to bivariate analysis. AF and a_w found from meal samples were also tested for correlation. PTF submitted to bivariate analysis included ASF (%), PEN (%), FUS (%), and ASN (%). Bean samples, PB, MF and cassava flour, moringa leaves powder were not submitted to bivariate analysis, since those samples were free of PTF and AF contaminations.

1.3.5.1. Bivariate analyses of February 2015 year

A_w and PTF found in February 2015 year maize and OS were analyzed for their relationships. PTF and a_w were more related in OS compared with maize grain. Data were more correlated in oleaginous seeds compared with maize grains. Only three coupled data showed correlation in maize grains compared with four and 11 detected respectively in peanut and moringa seeds. Moringa data were more strongly correlated compared with others substrates. PTF were less related among them compared with a_w. ASF and ASN were positively and strongly ($p<0.001$) related in all samples except in peanut. FUS was inversely and strongly ($p<0.001$) associated with ASF (ρ=- 0.947) and ASN (ρ= -0.962) in moringa seed samples. Also in maize FUS and ASN were negatively related (ρ= -0.552, $p<0.05$). Regarding PEN, it was showed positively correlated with a_w in maize and peanut samples (Table 15).

Table 15. Correlation matrix related activity water and potential toxigenic fungi in February 2015 year samples

Hosts	Variables	a_w	ASF (%)	ASN (%)	FUS (%)
Maize					
	ASF (%)	NS			
	ASN (%)	NS	0.974***		
	FUS (%)	NS	NS	-0.552*	
	PEN (%)	0.761**	NS	NS	NS
Peanut					
	ASF (%)	0.706*			
	ASN (%)	-0.651*	NS		
	FUS (%)	NS	NS	NS	
	PEN (%)	0.601*	0.818*	NS	NS
Moringa					
	ASF (%)	-0.981***			
	ASN (%)	-0.889*	0.962***		
	FUS (%)	0.886*	-0.947***	-0.999***	
	PEN (%)	NS	NS	NS	NS

Legend. a_w: Activity water; ASF: *Aspergillus* section *Flavi*; ASN: *Aspergillus* section *Nigri*; FUS: *Fusarium* spp; PEN: *Penicillium* spp. *;;***: p<0.05; 0.01; 0.001 and NS: not significant at p<0.05.**

1.3.5.2. Bivariate analyses of August 2015 samples

Similarly to February 2015 samples, maize grains and OS data were submitted to bivariate analysis. More positive and strong ($p<0.001$) correlation was observed among parameters compared with inverse and weak association. AFs were more associated among them compared with PTF. All AFs were associated at $p<0.001$ levels compared to four relation observed at similar levels in PTF. Also great variation levels were also detected among tested substrates. Parameters were less associated in maize grains compared with OS. No association was detected between AFs contamination and PTF. Only PEN was associated with a_w in addition to relationship detected among AFs.

Moringa and peanut parameters were associated at similar proportion (10/21). More strong correlations were found in moringa compared with peanut samples. ASF was associated with all AFs types in moringa

samples. AFB1 showed any relation with ASF in peanut samples. AFs types detected in moringa showed any relation with aw. AFB2 was weakly related with aw (ρ=0.493, $p<0.05$) in peanut samples. Also positive correlation was detected between ASF and ASN (ρ=0.655, $p<0.01$) in the same substrate.

Similarly to maize grains and OS, a_w and AF found in meal samples were also tested for their relationship. All AF types were related among them at $p<0.001$ levels. No correlation was observed between a_w and AF (Table 16). Compared with February data, PTF were lower correlated in August 2015-samples. The most correlated among all remains ASF (%). ASF were related with ASN (ρ=0.577, $p<0.001$) in peanut samples.

Table 16. Correlation matrix associated activity water, potential toxigenic fungi and aflatoxins in seeds August 2015

Hosts	Variables	a_w	ASF (%)	ASN (%)	FUS (%)	PEN (%)	AFB1 (µg/kg)	AFB2 (µg/kg)
Maize								
	ASF (%)	NS						
	ASN (%)	NS	NS					
	FUS (%)	NS	NS	NS				
	PEN (%)	0.609**	NS	NS	NS			
	AFB1(µg/kg)	NS	NS	NS	NS	NS		
	AFB2(µg/kg)	NS	NS	NS	NS	NS	0.968***	
	AFtot(µg/kg)	NS	NS	NS	NS	NS	1***	0.988***
Peanut								
	ASF (%)	0.577**						
	ASN (%)	NS	0.655**					
	FUS (%)	NS	NS	NS				
	PEN (%)	0.537**	0.603**	NS	NS			
	AFB1(µg/kg)	NS	NS	NS	NS	NS		
	AFB2(µg/kg)	0.493*	0.499*	NS	NS	NS	0.978***	
	AFtot(µg/kg)	NS	0.409*	NS	NS	NS	0.999***	0.984***
Moringa								
	ASF (%)	0.317*						
	ASN (%)	-0.392**	NS					
	FUS (%)	NS	NS	NS				
	PEN (%)	0.306*	NS	NS	0.755***			
	AFB1(µg/kg)	NS	0.482***	NS	NS	NS		
	AFB2(µg/kg)	NS	0.527***	NS	NS	NS	0.997***	
	AFtot(µg/kg)	NS	0.486***	NS	NS	NS	1***	0.997***
Meal								
	AFB1(µg/kg)	NS						
	AFB2(µg/kg)	NS					0.992***	
	AFtot(µg/kg)	NS					1***	0.994**

Legend. a_w: Activity water; ASF: *Aspergillus* section *Flavi*; ASN: *Aspergillus* section *Nigri*; FUS: *Fusarium* spp; PEN: *Penicillium* spp. AFB1: aflatoxin B1; AFB2: aflatoxin B2, AFtot: aflatoxin total. *;;***: p<0.05; 0.01; 0.001 and NS: not significant at p<0.05.**

1.3.6. Agricultural practices

As described in M&M and mainly in sampling procedure, the impact of AGPs on PTF and AF contamination of samples was evaluated. The questionnaire used in the investigation of AGPs includes all activities realized from soil preparation to harvest.

Peanut and maize grain from February 2015 year samples were evaluated for association detected with AGPs. August 2015 samples were collected with more AGPs data and results are presented Tables 18, 19 and 20. AGPs tested were cultural cycle, harvesting month, seeds treatment, and pest control, weeding frequency, irrigation, fertilization and previous culture. Although seeds suppliers and storage duration were not part of AGPs, they were also evaluated.

1.3.6.1. Samples from February 2015 year

Across February 2015 samples, only peanut seeds and maize grains were evaluated with IF ASF. Those samples were not evaluated for AF content under those practices. As seen in Table 17, AGPs modified considerably ASF occurrence in substrates. Comparing AGPs and substrates, maize grains mycobiota were more suitable to be influenced than peanut seeds. All evaluated AGPs influenced considerably ASF contamination. Cultural cycle influenced maize at $p<0.05$ levels and all the rest at $p<0.001$. The cultural cycle influence also ASF isolated from peanut. Maize grains harvested in September showed more ASF compared with December ($p<0.001$). Similarly, IF ASF was higher in 3 months CC compared with 4 and 5 months. Maize grains stored for 7 months showed more ASF IF compared with 1 and 2 months.

Table 17. Cultural cycle, harvest month, storage duration effect on IF *Aspergillus* section *Flavi i*solated in maize and peanut February 2015

Maize		Peanut	
Month	**AFS (%)**	**Month**	**AFS (%)**
STd			
1	37.00a	1	3.30
2	34.67a	2	25.33
3	9.33a	3	28.00
4	31.67a	10	16.67
7	89.33b		
	P<0.001		P> 0.05
CC			
1	59.87b	4	11.07a
2	29.56a	5	46.67b
3	9.33a		
	P<0.05		P**<0.001
HM			
January	34.67a	January	14.33
February	37.00a	March	16.67
September	89.33b	November	28.00
November	31.33a		
December	9.33a		
	P<0.001		P>0.05

CC: cultural cycle; STd: storage duration; HM: harvest month.

****: t-Student test. Within agricultural practice, values with different letters are different at p<0.05 (test REGW-*F*).**

1.3.6.2. Samples August 2015 year

AF, ASF and a_w found in August 2015 year maize samples were also evaluated with AGPs. Results are showed in Table 18-20 and on Figure 14. Forty and seventy percent of AGPs influence respectively IF ASF (%) and AF concentration in samples. Storage duration of maize grain modified simultaneously IF ASF (%) and AF. AF content varied from 22.47 to 463.19 µg/kg by a decreasing of storage duration.

Irrigation, fertilization and number of seeds per hole affected also AF contamination. Means AF detected in irrigated and fertilized maize were respectively 43.24 and 96.98 µg/kg whilst AF concentrations were 262.68 and 258.62 µg/kg when plants are neither watering nor fertilized.

Maize grains harvested in August were more AF-contaminated compared with March / May with respectively 438 and not detected /7.38 µg/kg. Grains from 2 seeds/hole were more AF-contaminated compared with 2 and 4 seeds. No significant difference was observed between 2 and 4 seeds/hole.

Considering all AGPs, only 30% showed AFtot ≤ 20 µg/kg, the US Foods Drug Administration limits. Seeds provided by Secal project, maize grains harvested in March/May and three weeding realizing during maize cycle showed respectively 6.97, nd/7.38 and, 9.83 µg/kg (Tables 17, 18 and 19). Moreover, IF ASF and/ AF contamination of Haitian maize grains depended on harvesting month and storage duration and previous culture (Tables 18 and 19).

a) Principal components analysis

In order to evaluate the contribution of all AGPs and their degree of dependence to the total PTF and AF contamination found in Haitian maize grains, a Principal component analysis test was performed using each AGP as predictor/vector. On Figure 14 all information regarding the contribution of each predictor are inserted. The two first principal components computed 70.81% of divergence detected among samples with a Cronbach's α equivalent to 0.752. Principal component one (PC1) accounting 37.25% while principal component two (PC2) computed 33.56%. The lengths of these AGPs reflect their contribution to the variation in the total AFs and PTF, whereas the angle between vectors reflects the degree of association between AGPs.

Correlation matrix associated all vectors was also used for computing the relation degree among them exactly. Forty percent of AGPs were positively and significantly associated with all IVs. Correlations detected among IVs ranged from -0.026 to 0.968. Regarding loading matrix, all AGPs are positively associated with PC1 and PC2. The most important vectors responsible to divergence among tested maize grains are weddings frequency, harvesting month and irrigation, which are positively associated with PC1 and respective loading vectors equivalent to 0.857, 0.813 and 0.537. Previous culture and fertilization with respectively 0.454 and 0.371 as loading values are also important predictors associated with PC1. In addition, seeds suppliers, storage duration, and cultural cycle with respectively 0.786, 0.769 and 0.464 as loading values are the most important AGPs associated with PC2. Weddings frequency and harvesting month are strongly and positively correlated (ρ=0.968). Also cultural cycle and previous culture are

positively correlated (ρ=0.307) and contribute also significantly to the divergence detected in tested maize grains. Seeds supplier and storage duration are strongly and positively related also (ρ=0.307). They constitute the third categories of vectors responsible of divergence. Fertilization and irrigation participated more to the variability detected among maize grains samples compared with seeds per hole and seeds treatments (Figure 14).

Table 18. Irrigation, fertilization and seed treatment effect on *Aspergillus* section *Flavi* and aflatoxins found in August 2015 maize grains

AGRICULTURAL PRACTICES			
Irrigation			
Variables	**Yes**	**No**	**P-values**
ASF (%)	53.90	45.85	0.317NS
AFB1(µg/kg)	39.94	243.75	**0.00*****
AFB2(µg/kg)	3.30	18.95	0.001**
AFtot(µg/kg)	43.24	262.68	**0.006*****
Fertilization			
ASF (%)	58.67	40.48	0.017*
AFB1(µg/kg)	89.33	240.39	0.025*
AFB2(µg/kg)	7.66	18.24	0.055*
AFtot(µg/kg)	96.98	258.62	0.026*
Seed treatment			
ASF (%)	56.67	42.12	0.057NS
AFB1(µg/kg)	251.46	107.74	0.044*
AFB2(µg/kg)	19.58	8.48	0.041*
AFtot(µg/kg)	271.04	116.20	0.044*

ASF: *Aspergillus* section *Flavi*; AFB1: aflatoxin B1; AFB2: aflatoxin B2, AFtot: aflatoxin total. Within agricultural practices, values with different letters are different at p<0.05 (test REGW-*F*).

Table 19. Cultural cycle, seeds suppliers, seeds per hole, weeding frequency effect on *Aspergillus* section *Flavi* and aflatoxins found in August 2015 maize grains

Agricultural practices	Variables			
Cultural cycle (days)	**ASF (%)**	**AFB1 (µg/kg)**	**AFB2 (µg/kg)**	**AFtot (µg/kg)**
90 (n*=8)	42.08	214.46	17.85	232.00
120 (n=3)	33.78	61.17	5.00	66.20
150 (n=7)	58.67	65.29	5.76	71.02
180 (n=2)	62.33	546.05	36.85	582.90
Seeds suppliers				
SCP (n=3)	50.67	6.40	0.53	6.97
TWP (n=5)	59.87	383.58	28.60	412.18
FF (n=12)	43.50	125.93	10.41	136.32
Seeds per hole				
2 (n=12)	46.78	117.62a	8.63a	126.23a
3 (n=6)	56.67	356.90b	28.78b	385.66b
4 (n=2)	36.00	67.40a	6.70a	74.10a
Weeding frequency				
1 (n=8)	44.67ab	200.95	14.94	215.86
2 (n=9)	58.59b	201.24	16.67	217.91
3 (n=3)	29.56a	9.83	nd#	9.83

n: number of samples; ASF: *Aspergillus* section *Flavi*; FF: farmer's fields, TWP: Taiwan project; SCP: Secal project; AFB1: aflatoxin B1; AFB2: aflatoxin B2, AFtot: aflatoxin total. Within agricultural practices, values with different letters are different at $p<0.05$ (test REGW-*F*).

Table 20. Previous culture, harvest month, storage duration effect on activity water, *Aspergillus* section *Flavi* and aflatoxins found in August 2015 maize grains

Agricultural practices	Variables				
Previous culture	Aw	ASF (%)	(µg/kg)		
			AFB1	AFB2	AFtot
Bean (n*=9)		56.22b	133.40a	11.39a	144.79a
Peanut (n=1)		35.33ab	31.05a	2.65a	33.65a
Sorghum (n=1)		4.67a	479.35b	37.30b	516.60b
Maize (n=4)		34.33ab	259.50ab	16.60ab	276.10ab
Maize ass⁺(n=5)		58.00b	112.10a	9.33a	121.43a
Harvesting month					
March (n*=1)	**0.66abc**	**76.67**	nd#	nd	nd
May(n=3)	**0.61a**	**29.56**	7.38a	nd	7.38a
June(n=4)	**0.64ab**	**49.67**	86.33ab	8.30ab	94.62ab
July(n=9)	**0.67bc**	**48.00**	205.18b	16.58bc	221.73b
August(n=3)	**0.70c**	**59.11**	408.97c	29.03c	438.00c
Storage duration (days)					
8≤α ≤30 (n*=7)	**0.70c**	**50.95**	430.19b	33.00b	463.19b
45≤α ≤75 (n=7)	**0.62a**	**54.86**	44.60a	4.06a	48.64a
90≤α ≤150 (n=6)	**0.66b**	**38.78**	20.78a	1.68a	22.47a

n: number of samples and Maize ass⁺: maize associated with other crops; a_w: Activity water; ASF: *Aspergillus* section *Flavi*; AFB1: aflatoxin B1; AFB2: aflatoxin B2, AFtot: aflatoxin total. Within agricultural practice, values with different letters are different at p<0.05 (test REGW-*F*).

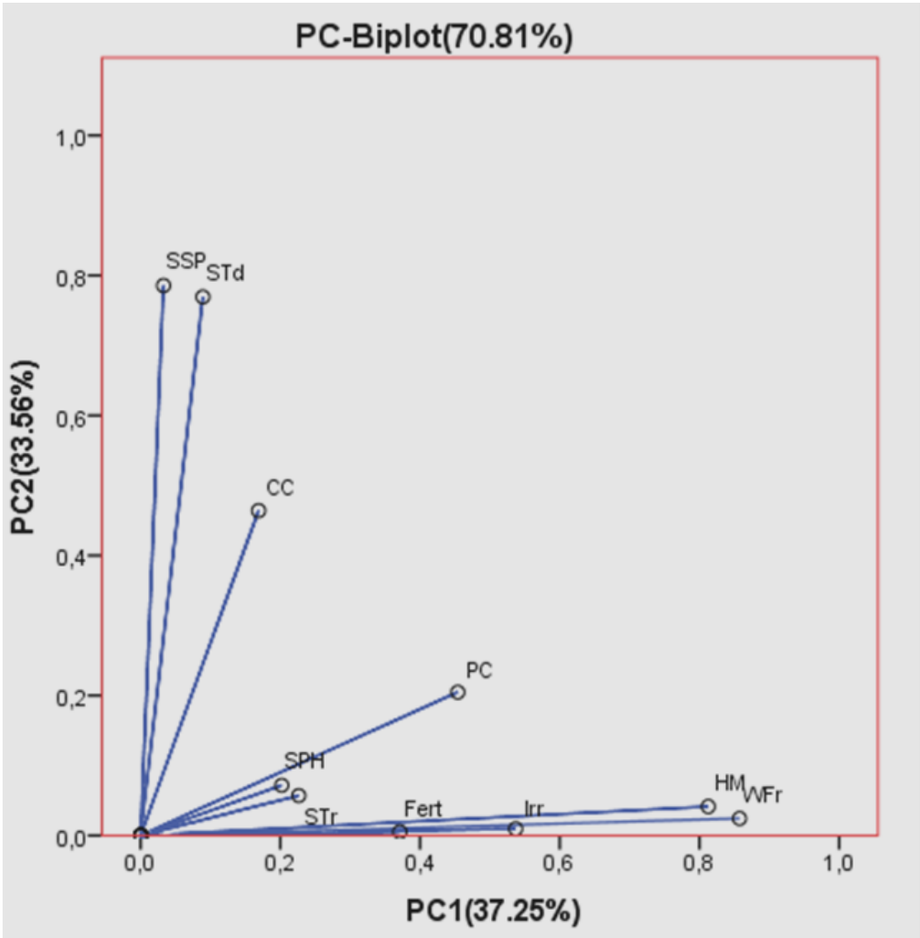

Figure 14. Biplot of first (PC1) and second (PC2) principal components expressing the proportion of variation derived from the ten agricultural practices (AGPs) and contribution of each one in total fungal and aflatoxin contamination in Haitian maize grain in 2015. SPP: seeds suppliers, STd: storage duration, CC: cultural cycle, PC: previous culture, SPH: seeds per hole, STr: seeds treatment, Fert: fertilization, Irr: irrigation, HM: harvesting month and WFr: weddings frequency.

Chapter 1.4. Discussion and conclusions

This study is the first one conducted on water activity, fungal mycobiota and AF contamination in substrates in South Haiti. A_w is the ratio of the vapor pressure of water in equilibrium with a food to the saturation vapor pressure of water at the same temperature. Moreover, it could be defined also, as the lower limit of available water for microbial growth (Fontana, 1998). Thus, a_w is one the most important indicator for studying microorganism and toxins production. Relation of microorganism and a_w of food material was studied and modeled (Harris, 1981; Davey, 1989; Broughall et al., 1983). Limit for fungi growth and toxins productions were estimated at 0.61 and 0.78 respectively (Beuchat, 1981). Rockland and Beuchat (1987) fixed fungal growth at 0.70. Studies related to the influence of temperature, water activity and food materials are also available (Marín et al., 1998; Marin et al., 1998; Andrews and Pitt, 1987). Microorganism metabolism is complex and a_w alone is not enough for a deep study but it could give data for appreciating food quality (Beuchat, 1983 and Fontana, 1998). The equipment Aqualab, used to measure a_w of samples in this study was ranging 0.030 and 1.000 a_w (Fontana, 1998). Most of Haitian SS were less dried compared with PPs and showed a_w up to the range of 0.70. Thus, SS samples were more susceptible to be contaminated with PTF and AF. Referring to limit (Fontana, 1998), a large majority of tested SS samples offered suitable conditions for fungal growth and toxins productions. Almost all maize samples evaluated during the two years were in the range to support PTF growth. Peanut and moringa samples were also in the range for moulds growth. Findings suggest a possible exposure of Haitian population to toxigenic fungi and toxins assumption. A_w varied across samples and locality. Findings suggested that dried method is modified across places and substrates in South Haiti. This study is the first realized in Haiti presenting a_w of samples. Thus, it is really difficult to have comparison of data. The two most investigated products in Haiti are peanut and maize. Maize grains were more humid compared with peanut. Similar findings were expected because all maize grains are dried in field whilst peanut seeds at maturity are transported home for drying process. Similar AGP reduce the exposure of peanut seeds to climate parameters like precipitation that could increase humidity and a_w of substrate. Moringa seeds from

Torbeck were well dried compared with Cavaillon. Similar results were also expected because farmers at Torbeck are more experimented in moringa cultivation and processing compared with Cavaillon. At Torbeck is located the UdT, which provided adequate formation for moringa farmers: for this reason, background to post-harvest moringa seed management in Torbeck is better than in Cavaillon.

Almost all PPs were under the limit of PTF growth and toxins productions. Therefore, findings predict very low levels of PTF and toxins contamination in samples. Similar results were also expected because PPs are well dried and more stored compared with grains in Haiti. More attentions were paid to the last category because they are used in baby's foods.

Fungal mycobiota. Almost 84% and 70% of Haitian SS and PPs were contaminated with PTF and confirm that those products are contaminated as well as before and during processing. SS were contaminated with *Aspergillus* genus followed by *Penicillium* spp and *Fusarium* spp. Those findings were expected because *Aspergillus* genus under tropical and subtropical climates finds better conditions to develop in comparison with *Fusarium* spp, which are more common in temperate areas.

Maize, peanut and moringa were contaminated with important PTF. Findings support that those substrates used in Haiti could be sources of fungal and toxins contaminations. The predominance of *Aspergillus* genus in maize grains (48%) is consistent with data obtained in Haiti previously by other authors (Castor et al., 1987). Castor and colleagues working with 268 samples collected in different departments during January, February 1983 and July and August 1984, reported a high contamination of maize grains with toxigenic fungi related to *Aspergillus* genus. This observation is also congruent with data recorded in other countries having similar climate characteristics to Haiti like Nigeria (Atehnkeng et al., 2008; Ezekiel et al., 2011), Pakistan (Saleemi et al., 2012), Libya (Attitalla et al., 2010) and Algeria (Riba et al., 2010). Furthermore, ancient as well as more recent data reported (Dalcero et al., 1998; Aliyu et al., 2016; Mendoza et al., 2017 and Xing et al., 2017) confirmed also those findings. Similar results were obtained in Argentina (Astoreca et al., 2010), Ecuador (Pacin et al., 2003), Costa Rica (Robledo-Robledo, 1991) and in others cereals such as wheat (Riba et al., 2010), rice (Park et al., 2005), and all over the world. Conversely, current findings refute some data published (Doko et al. 1996; Orsi et al., 2000;

Marasas et al., 2001 and Almaguer et al., 2012), where reported *Fusarium* spp as the most frequently identified fungus occurring in maize and rice in France, Spain, Italy, Brazil and Cuba. The difference observed from present findings to cited papers could be due because fungal contamination of samples depended on ecological niche.

Similarly to maize grains, OS were more contaminated with *Aspergillus* genus compared with *Penicillium* and *Fusarium* spp. The present findings were also expected because oleaginous seeds are often colonized by *Aspergillus* genus compared with *Fusarium* spp. A large part (85%) of peanut seed samples was contaminated with *Aspergillus* genus. This study is the first one conducted on peanut fungal mycobiota in Haiti. Thus it was not possible to compare data. Great colonization of peanut seeds and pistachios by *Aspergillus* genus was worldwide mentioned (Mphande et al., 2004; Lamboni et al., 2016; Jogee et al., 2017; Kazemi et al., 2014, Bhattacharya et al., 2002; Njobeh et al., 2009). Results of this investigation are differing from those reported by (Nakai et al., 2008; Gonçalez et al., 2008 and Reis et al., 2012).

Concerning moringa, this research is the first one mentioning contamination with PTF. A great part (65%) of samples was colonized by *Aspergillus* genus. A quite low incidence of *Aspergillus* section *Circumdati* was detected. The low incidence of this section is concordant with that reported by Joosten et al. (2001), who studied mycoflora associated with coffee cherries in Thailand. Also a quite low contamination equivalent to 11 CFU/g of *Aspergillus* genus was detected in moringa leaves powder. Moringa leaves powder is used and promoted as tea for its beneficial properties. Several investigations conducted in Asia and Africa reported contamination of tea products with PTF (Mao et al., 2017 and Toman et al., 2017) belonging to *Penicillium* spp and *Aspergillus* genus.

Generally, investigations conducted on moringa reported its richness in proteins and amino acids (Leone et al., 2016 and Baptista et al., 2017), its use in water purification (Madrona et al., 2010 and Camacho et al., 2017), its biodiesel importance (Arruda et al., 2017) and its application in crop protection as insecticide (Agra-Neto et al., 2014 and de Oliveira et al., 2017), and fungicide (Donli and Dauda, 2006 and El–Mohamedy and Abdalla, 2014) and finally its contribution to animals growth (Khan et al., 2017).

Only seedborne fungi colonized bean samples. Findings are consistent with those of Castillo et al (2004) in Argentine and Tseng et al (1995) in

Canada. Nevertheless, respectively in Thailand and Taiwan, *Aspergillus* genus and *Fusarium* spp were the most frequent PTF detected in samples (Pitt et al., 1994 and Tseng et al., 1995). More reliable data must be collected for a deeper analysis of PTF associated with Haitian beans. Overall, results of bean samples suggest that this staple crop is neither a source of fungal nor AF contamination in Haiti.

Meal and maize flour were contaminated with *Penicillium* spp. followed by *Aspergillus* section *Flavi* 83.67% of samples. Contrary to the findings of the current survey, Katta et al (1997) mentioned *Fusarium* spp as the major fungus colonized meal.

Peanut butter was PTF free. This result refutes with data reported by Reddy et al (2011). Free PTF contamination of Haitian PB and cassava flour are important data because all those substances are used as basic baby's foods.

Cassava (*Manihot esculenta*) is an important source of energy for Caribbean people. In the UdT, this tuber is worked for baby food preparations. It is mixed with peanut butter; moringa leaves powder and MF, for *mama plus* preparation. *Mama plus* is a nutritional supplement prepared for pregnant women and babies and is intended for reducing malnutrition. During this survey, any form of PTF was detected in cassava flour sample. Findings obtained during this survey reject those reported by (Wareing et al., 2001; Adjovi et al., 2014; Gnonlonfin et al., 2008; Essono et al., 2007 and Gnonlonfin et al., 2012).

So, the absence of fungi in Haitian cassava sample could be explained by the correct processing. In fact, after growing and harvesting of cassava, product was submitted to different steps of washing and sterilization. Sample was sun dried in controlled condition. After well dried, sample was milled to flour and put in plastic bag. Fortunately, production phase of the cassava was realized in UNDH farm in Torbeck, which means deep and significant attention was paid. Moreover, the difference observed among this cassava sample and others mentioned in the current survey could be also explained by the provenience and step of food chain. In the current study, cassava flour was directly sampled during processing, whilst most data available in literature and cited in this document were collected on fresh cassava or chips.

However, cassava sample (n=1) was relatively not significant to establish a good panorama of fungal population of cassava in Haiti. Further studies and researches must be done on PTF associated with

cassava freshly harvested and/or stored with relatively more significant samples.

Capacity of *Aspergillus* section *Flavi* strains to produce AFs. A large percentage (83%) of wild Haitian ASF strains in this study were toxigenic, especially those isolated from maize and peanuts, in agreement with previous reports on Latin American ASF strains (Astoreca et al., 2011 and Rocha et al., 2012). Similar results suggest that ASF strains isolated from substances in South Haiti could be an important source of AF contamination because this indicates also that Haitian conditions clearly favor the colonization of toxigenic ASF strains over atoxigenic strains. The most frequent AF chemotype was that producing only AFB1, confirming the dominance of this group in maize and peanuts (Atehnkeng et al., 2008; Sweany et al., 2011 and Riba et al., 2010 and Reddy et al., 2011). Separately, AFB1 producer strains also dominated in oleaginous seeds as in cereals. Similar data were already reported (Vaamonde et al., 2003) and a greater number of AFB1 producer's strains were found in peanuts (69%) compared with wheat (13%). The low incidence of chemotype strains producing both (AFB1 and AFG1) and absence of only AFG1 producer strains are consistence with data reported from Italian maize (Giorni et al., 2007).

Maize and peanut samples were contaminated with heterogeneous strains and moringa with homogenous strains. Similar findings could suggest great variability among ASF strains population of Haitian substrates. Deeper studies and on a bigger number of samples should be conducted on ASF strains isolated from oleaginous seeds moringa and peanut for confirming the possible existing diversity.

Aflatoxins. HPLC methods revealed that 50% of SS and PPs products tested for AFs content were positively contaminated. This incidence of maize grains contamination with AF was less comparing to 88% obtained by (Castor et al., 1983) and upper to the 14% reported by (Jeremy and Brown, 2014). Moreover, almost all samples were contaminated by AFB1 and AFB2 together with predominance of the first one, in agreement with the observation of (Castor et al., 1983; Abbas et al., 2006; Ogara et al., 2017 and Granados-Chinchilla et al., 2017).

Maize samples were AF contaminated more than OS samples. Similar findings are different from those reported from another author (Schwartzbord and Brown, 2015), who found peanut samples more AF contaminated compared with maize. Also, findings demonstrate widespread AF contamination of maize within southern farmers and

Les Cayes markets. Great proportion (55%) of maize samples from farmers in South Haiti had total AFs levels greater than the US Foods Drugs Administration limit of 20 µg/Kg. Seven samples (35%) had AFs content > 100 µg/Kg. Ancient as well as more recent investigations conducted in Haiti reported quite similar data (Castor et al., 1987; Filbert and Brown, 2010 and Schwartzbord and Brown, 2015). Castor and colleagues have been reported that more than 20% and 10% respectively of Haitian maize grain samples tested have been showed > 20 and 100 µg/kg. Similar data were also reported in Kenya (Lewis et al., 2005).

Most of peanut samples were free of AFs. Only three samples (37.5%) were contaminated with >20 µg/kg and this is quite less compared to 97% and 89% measured (Jeremy and Brown, 2014; Filbert and Brown, 2010). Regarding AF concentration of samples, results seem differ from previous data mentioned in Haiti. None of samples showed total AFs greater than 400 µg/kg. Maximum AFs contamination levels detected in peanut was 375 µg/kg comparing to 12.5% of peanut samples that were contaminated with AFs greater than 1000 µg/kg reported by (Schwartzbord and Brown, 2015).

Moringa seed samples were positive to AF contamination. AF contamination of moringa seeds appeared a result of location. Location could be considered as the interaction of factors such as weather, agronomic practices, storage conditions, and fungal population and AF characteristics (Venturini et al., 2015). Therefore, moringa seeds sampled at Torbeck appear as potential sources of AF contamination for rural habitants and overall, farmers and their families.

Maize derived PPs were more contaminated with AF compared with peanut. Upper than 57% of maize derived samples were AF contaminated. Although a deeper investigation is needed to be conducted in Haiti with more reliable samples, the unique information recorded presents a darkness picture of malnutrition in Haiti, since maize flour and meals (maize derived PPs) are basic ingredient of almost all derivate food (from baby's food to nutritional supplements) used against child's and pregnant women malnutrition in Haiti. It suggests the necessity to deal with strategy and technological procedures to lower the population exposure risk. Similar alarming and conflicting results about AF contamination of meal and wheat derived products were reported in Africa, in Asia and in other developing countries (Lewis et al., 2005; Egbontan et al., 2017; Nduti et al., 2017; Mottaghianpour et al., 2017; Lerda, 2017), all claiming for a deeper and

more extensive investigations and assumption of new techniques and strategies for fighting and solving the problem.

Peanut butter was free of AFs contamination. Peanut butter (n=1) was analyzed, it is not possible to draw any general evaluation. May be the reason why findings are not congruent with those reported in literature on Haitian peanut butter samples (Schwartzbord and Brown, 2015; Filbert and Brown, 2012).

Comparing peanut and maize food chains in South, Haiti, it appears that peanut food chain was safer compared with that of maize in Les Cayes, South Haiti. This observation is not consistent with (Schwartzbord and Brown, 2015) who had been reported to be observed that Haitian maize chain was safer than peanut chain. Difference observed could be explained by ecological situation and technology of peanut and maize post-harvesting management that are different among Haitian districts. It is quite important to remember that material was different, being in sampled products from Port-au-Prince in West district, and Cap-Haitian in Northern district (Schwartzbord and Brown, 2015), whilst those presently investigated are from Les Cayes in Southern department. Variations on AFs content as dependent on agroecological zones were reported for groundnut in Zambia (Kachapulula et al., 2017). Results are similar with those obtained by (Nakai et al., 2008 and Zorzete et al., 2013; Barro et al., 2002; Kamika and Takoy, 2011).

Correlation matrix. Bivariate analyses findings revealed that PTF and AFs contamination of Haitian have strong correlations. Great competitions between FUS and two section of the genus *Aspergillus* were observed in maize and oleaginous seeds. Similar results suggest that the decreasing of FUS isolation in Haitian substrate is explained by the proliferation of the genus *Aspergillus* including section *Flavi* and section *Nigri* in Haiti. Almost 90% of FUS reduction was explained by ASF and ASN proliferation (Table 15). Similar findings were supported in a previous paper (Marin et al., 1998). Moreover, ASF and ASN were strongly and positively associated in substrates. Findings confirm their co-occurrence in tropical climate and revealed also the capacity of different sections of a genus to cohabitate for colonizing food materials. It is noteworthy the predominance of *Aspergillus* genus on *Fusarium* spp in all samples as a result of its stable settlement in storage products and under tropical climate (Abbas et al., 2006; Torres et al., 2003; Taghi et al.,

2010; Kazemi et al., 2014; Riba et al., 2010; Alassane-Kpembi et al., 2017 and Ogara et al., 2017).

Fusarium spp was not correlated with AFs contamination in maize grains in Haiti, since FUS is linked to Fumonisin production, another important Mox type (Abbas et al., 2006) which could act as synergetic compounds with AFs (Lim et al., 2015; Domijan et al., 2005; Kovalsky et al., 2016).

Most of AFs (92%) quantified in samples were not correlated with a_w. Only OS had a_w associated with AFs. From 17 %(R^2=0.167) to 43% (R^2=0.427) of AFs detected in OS were due to bad drying. Similar results could be suggested that an important part of AF contamination detected in OS could be reduced by controlling the drying procedure. A_w is not correlated with AFs in maize grains substrates. Finding mean that AF management in sampled maize could not be controlled by dried. May be other factors in field or during post-harvest management are favorable to AF production.

Multivariate analysis and agricultural practices. Student t-test and ANOVA findings showed how AGPs such as irrigation, fertilization, and weeding frequency, previous culture and storage duration contribute to ASF and AFs contamination in maize grains. Increasing of maize growth length and harvest prior raining month (October) make increase AF contamination in maize grains. Those results were previously reported by divers authors in others countries (Bilgrami and Choudhary, 1998; Jones et al., 1981; Wiatrak et al., 2005). Similar finding suggest that Haitian AGP consisting of letting grain dried on stalk must be avoided for reducing fungal and AF contamination. Instead, it should be recommended to collect ears at 15% moisture and storage should be made in silos mostly metallic (Tefera et al., 2011).

A Principal Component Analysis test was performed for completing data obtained from ANOVA and Student t-test relative to AGPs. Findings of PCA-biplot showed that almost 71% of AFs and PTF variation computed in maize grains could be explained by AGPs. This proportion (70.81%) suggests that others factors responsible of fungal and AF contamination in maize samples were not investigated in the current study. Those findings were expected because during sampling, farmers were not able to give exactly the values associated with each AGP. As example, they could say if “fertilizer was applied or not” but not exactly the quantity of Kg fertilizer applied, the number of weeding, period of weeding. In this sense, collected data relative to AGPs seem

more qualitative than quantitative. Moreover, others important AGPs and pest activity were not investigated during the current study whilst similar factors could contribute to fungal and AF contamination in field conditions (Cotty and Jaime-Garcia, 2007; Cotty and Lee, 1990; Guo et al., 2004; Ni et al., 2014). Also, storage site quality was not evaluated and it was reported affected fungal and Mox contamination (Prakash et al., 2013; Hell et al., 2000; Lane et al., 2017). The confidence of the PCA test was confirmed by Cronbach's α equivalent to 0.752. It was an acceptable indicator because many authors fixed the limit as 0.70 (Leech et al., 2014; Peterson, 1994). PC1 and PC2 computed almost similar variation (37.25 and 33.56%). This result suggests that there is not great difference among AGPs associated with the first and second levels in regard to their contribution to the differentiation detected among tested maize grains. Forty percent (4/10) of AGPs were positively and significantly associated among them and a large majority of vectors (80%) are contributed to more than 30% of variation detected among analyzed samples. The results suggest that Haitian maize grain contamination with fungi and AF is a complex system including field and post-harvest favorable conditions. Thus, reduction exposure of Haitian population to fungal and AF contamination might include an integrated approach.

The most important AGPs responsible to divergence among maize are weeding frequency, harvesting month, and irrigation, which are positively associated with PC1. Previous culture and fertilization with respectively 0.454 and 0.371 as loading values are also important IVs associated with PC1. Most of them were positively related. Similar results confirm the first responsibility and the importance of their co-occurrence to the total fungal and AFs detected in maize grains and are consistent with those reported by (Kennedy et al., 2011; Tang et al., 2013 and Than et al., 2017; Fandohan et al., 2003; Blandino et al., 2009; Bilgrami and Choudhary, 1998 and Pringle, 2017; Uchino et al., 2005 and Snapp et al., 2005; Cotty et al., 2008; Probst et al., 2010; Teich and Hamilton, 1985; Liu et al., 2016; Olsen et al., 2008).

Furthermore, seeds suppliers, storage duration, and cultural cycle with respectively 0.786, 0.769 and 0.464 as loading values are the most important APGs associated with PC2. Thus, their second responsibility to the total fungal and AFs contamination is clarified by the results. A large part of seeds planted in Haiti were not certified. Most were from farmer's field (60%) with selection focusing based on phenotypic traits such as length and color of the ear (Table 19). No study was conducted

for seeds selection taking in consideration fungal and AF contamination in seeds prior planting. Also lack of seeds quality in Haiti was documented and reported by Food Agriculture Organization (Aloys, 2014). Therefore, found seed suppliers classified as the second important AGPs added to storage duration and cultural cycle responsible of variation detected among maize grains in regard to total fungal and AFs contamination is not surprising (Prakash et al., 2013; Hell et al., 2000; Lane et al., 2017).

Conclusions. This study is the first one conducted on a_w, fungal mycobiota and AF contamination in substrates in South, Haiti. It provided adequate information for fungal and AF management in Haiti. Seeds as well as processed products are source of PTF and AF contamination in Les Cayes, South Haiti. Almost all main Haitian food chains were fungal and AF contaminated. Peanut food chain in Les Cayes, South Haiti is less PTF and AF contaminated compared with that of maize. Fungal and AF contamination of moringa and peanut seeds are associated with bad drying procedure whilst those of maize grains in Haiti are related with bad agricultural practices. Certified seeds, increasing weeding frequency, and avoiding harvesting prior raining month and reducing exposure of maize grain in field during drying could decrease AF contamination found in Haitian maize. Earlier maize variety with appropriate gene resistant to water and fertilizer stresses could also reduce Haitian population exposure to fungal and AF contamination. Great variation ASF strains was found in different food materials and communes give a clear picture for studying DNA for a first classification of Haitian *Aspergillus* section *Flavi* strains. Other studies on mycotoxins such as Ochratoxins and Fumonisins must be conducted in Haiti due to the occurrence of FUS and ASN found in samples. Overall results revealed the exposure of Haitian population to PTF and AF in Haiti. Inclusive strategy relative to integrated approach is the only way for reducing Haitian exposure to fungal and AF contamination in Haiti.

Chapter 1. 5. References

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SECOND PART:

Characterization of five Maize (*Zea mays*) varieties tested in Haiti

Summary. Maize, one of the three most produced cereals in the world, is a staple crop in many developing countries including Haiti where it provided largely to the total daily calories intake. Even if Haiti is the second maize consumers of Caribbean region, National yield is around 0.96 t/ha and local maize grain is often mentioned highly contaminated with aflatoxin (AF). A breeding program aiming to increase grain yield (GY) and reduce AF contamination at harvest in rainfed condition was performed in South Haiti. Three introduced maize varieties (INMVs) (R4185, R4271X4185 and R4865) already improved at University of Milan were tested, with two landraces, Maquina and Chicken corn, used as testers. Field trials were carried out at agricultural farm of the University Notre Dame of Haiti (UNDH), (Torbeck $18^{0}10'$N, $73^{0}49'$W, 13 m altitude) and at *Morne Briller*, Port-Salut ($18^{0}04'$N, $73^{0}55'$W, 295 m altitude) during 2016-17 cropping season. Experiments were designed in a three-replicate randomized complete block. Each experimental plot was 3.50 m per 10.5 m with a gross area of 36.75 m^2 for a density of 60 000 plants/ha. Agricultural practices similar to those of local farmers were applied. AF content and phenotypic traits recorded were elaborated using dedicate software program (SPSS). Results revealed great genetic variability among maize varieties (MVs) i.e. INMVs were regrouped into one cluster and the local MVs in two separate groups. Maquina was greatly differing from INMVs. Almost 90% of divergence of MVs was explained by GY and ear weight (EW). Maquina with 7.398 t/ha was significantly ($p<0.05$) more yielded than Chicken corn, and R4865 with 5.043, and 4.814 t/ha respectively. AF concentration varied from 2.033 to 2.733 µg/kg i.e. < 5 µg/kg, the maximum level of AFB1accepted in European Union. GY was strongly and directly associated with EW (ρ= 0.991) and inversely related with AF (ρ= -0.347), male and female flowering days after sowing with (ρ= -0.214 and ρ= -0.245 respectively). AF was negatively related with EW (ρ=- 0.344). Thus, landraces and INMVs with high EW potentiality such as R4865 could be used as parents in breeding program with the aims of high GY and less AF contamination in Haiti.

Keywords. *Zea mays*, variability, yield, aflatoxin, agricultural practices

Chapter 2.1.1. Introduction

Maize (*Zea mays*), is one of the three most important cereals produced world-wild, cultivated under temperate, tropical as well as subtropical climates. The enormous popularity of this cereal is due to its great yield potentiality. The top three maize-producing countries are United States (274 million MT), China (208 million MT) and Brazil (71 million MT) especially in 2011year (Sprague and Dudley, 1988; Rong-Lin et al., 1999; Liao et al., 1995; Wu and Guclu, 2013; Ranum et al., 2014).

Maize is a staple crop in many developing countries where it is transformed in several derived products and provided about 15-56% of the total daily calories intake (Pressoir and Berthaud, 2004; Bellon and Berthaud, 2004; Tariq and Iqbal, 2010; Witcombe et al., 2003; FAOSTAT, 2010; Nuss and Tanumihardjo, 2011; McCann and McCann, 2009; Ray et al., 2013; Wilson, 2016).

In Caribbean region, Haiti with 50 g/ person / day is the second consumers after Cuba with 66 g/ person / day (Ranum et al., 2014). Almost 70% of cereals consumed in Haiti is imported because National maize yield (less than 1t/ha) is not enough for providing internal consumption (Timothy et al., 1988 ; Clermont-Dauphin et al., 2003; Jolly et al., 2011 ; Molnar et al., 2015; Romero-Aguilar and Miranda, 2014).

The maize low yield could be due to rainfed farming, inappropriate genotype along with poor agronomic practices.

Haitian tropical climate seems suitable for growing maize all year long in dry plains as well as in humid hills, thus, 50% is planted in sprint (rainy season), 30% in summer (dry season), and 20% in winter/autumn (Aloys, 2014).

South department is the second most important maize producer after Plateau Central. Only two local varieties, Maquina and Chiken corn, are the most commonly planted by farmers in South department, while data on genetic diversity and phenotypic traits are rare for those landraces (Aloys, 2014; Timothy et al., 1988). Therefore, necessity to develop better genotypes and improve landraces focusing on grain yield is unmistakable. Grain yield is a multiple/complex character conditioned by the interaction of various growth and physiological processes throughout the life cycle. Consequently breeding for high yield crops require information on nature and magnitude of variation in the available material, relationship of yield with other agronomic characters and the degree of environmental influence on the expression of these

component characters (Mohammadi et al.,2003; Rafiq et al.,2010 ; Reddy et al.,2013; Sangoi, 2001; Lobell and Field, 2007).

Study correlation between yield and other agronomic traits is a prerequisite to plan an effective breeding program (BP) (Ahmad and Saleem, 2003; Nemati et al., 2009). The characters know to be associated with maize yield 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 (Kumar *et al.*, 2006; Pavan *et al.,* 2011; Mural and Shailaja, 2012; Sadek *et al.,* 2006; Venugopal et al., 2003; Zarei et al., 2012; Kumar et al., 2014).

Beyond Haitian maize low yield, highly aflatoxin (AF) contaminated grains were also reported (Castor et al., 1987; Aristil et al., 2017; Schwartzbord and Brown, 2015). To reduce AF contamination, which could be occurred during all food chain steps, good agricultural practices (GAPs) and genetic improvement (GI) are needed (Hell et al., 2000; Wagacha et al., 2013; Maina et al., 2016).

Sustainable food production and quality required superior genotypes selection adapted to many environments (Kayaga et al., 2016; Rodrigo et al., 2012). Moreover diversity among parents and heterotic effect are the most starting axes on which breeders focused their effort in design BP (Castiñeiras et al, 2007; González et al, 2013). It is possible to investigate on genetic diversity among crops using adequate biometrical/statistical methods (Hierarchical Clustering and Multivariate) such Canonical Discriminating Function that integrate one ways Analysis of Variance, Student t test and Pearson rank correlation. All those analysis provide deeply knowledge about genetic material relationship (Wietholter et al., 2008; Rao, 1952).

Present study is aimed to (**i**) evaluate grain yield potentiality and aflatoxin content of three novel maize varieties and two landraces; (**ii**) determine the relationship among eleven phenotypic traits with grain yield and aflatoxin content found in those varieties; (**iii**) classify all maize varieties referring to their genetic diversity in order to select the best ones for starting a maize breeding program in South Haiti.

Chapter 2.1.2. Materials and methods

2.1.2.1. Fields trials and management

Three field trials (FTs) were conducted in South Haiti during cropping season 2016-2017 (Figure 15). Two were performed at agricultural farm of the University Notre Dame of Haiti (UNDH), (Torbeck $18^{0}10'$N, $73^{0}49'$W, 13 m altitude) and one at "*Morne Briller*", Port-Salut ($18^{0}04'$N, $73^{0}55'$W, 295m altitude). A sample of UNDH maize field soil was taken and analyzed at University of Milan laboratories (Annex A). Maize was the previous crop in Torbeck sites whilst at "*Morne Briller*", was sorghum. Seven maize varieties (MVs) were evaluated during FTs: five were selected for their performance in similar climate areas (i.e. South Africa, Argentina), and already tested and crossed with Haitian MV in a breeding program running at UNIMI "*Azienda agricole Angelo menozzi, Landriano*". The other two were testers: Maquina and Chicken corn (the most spread local varieties) (Tables 21 and 22). Two of the five introduced maize varieties (INMVs) were missing during the first FT (E_1) due to Matthew cyclone. The other five MVs (three INMVS and two landraces) were tested during the two successive FTs (E_2 and E_3). During E_1, a local breeding program was started and five improved maize varieties (IMVs) were developed (see 2.1.2.2 section). The same agricultural practices were applied to all MVs. All the experiments were laid out in a three-replicate randomized complete block design. Each experimental plot was 3.50 m per 10.5 m with a gross area of 36.75 m^2.Each plot consisted of fifteen subplots of 3 rows (13 plants each). The distance between subplots was 1 m. Spacing between plant to plant and row to row were 0.50 m and 0.50 m, respectively. 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 stage growth (2 weeks after sowing) corresponding to 60 000 plants/ha. According to the southern Haiti markets availability and soil analysis data, urea (46-0-0, N-P-K) and (20-20-10, N-P-K) were applied at two growth stage (GS). From V3-V4 (2-3 weeks after sowing), 44 Kg of urea plus 30 kg of (20-20-10) were applied and 170 kg of (20-20-10), at V10 (10weeks after sowing). The total amount of Nitrogen (N), Phosphorus (P_2O_5) and Potassium (K_2O) was 60, 40 and 20 kg /ha respectively. When plants received >5-7mm of rain weekly, no irrigation was performed while after one week with no rainfall, plants were watering by hand. One week before and after flowering, irrigation was performed in order to

keep the water-holding capacity in optimum range. Adequate agricultural practices (AGPs) similar to those of local farmers were applied for pest control. Two hoeing and handling weeding were made. The first, two weeks after sowing and the second, was realized two days prior the second fertilizer application. Harvest was done at humidity of 16-17%.

2.1.2.2. Improved maize varieties

In order to develop IMVs, performances of INMVs grown, during (E_1) were evaluated for the following characters: plant height, steam diameter and number of leave per plant at flowering. Local MVs were selected based upon precocity of male and female flowering. Nine male parents and seven female parented were selected and crossed as following: twenty-four hours before crossing, male florescence were covered with paper bags to collect the pollen. The day after, in the morning (9-10 hrs) the pollen was pooled and used to perform the crosses of the selected female parents. In the case of raining, crossing was delayed. Five IMVs were developed and seeds were stored. They are the starting materials for a maize breeding program (MBP) in Haiti.

2.1.2.3. Aflatoxin quantification

The screening for total aflatoxins (B1, B2, G1 and G2) concentration was performed according with Reveal® Q+ aflatoxin test (Neogen®) supply instruction. Reveal® Q+ for AFs is a single-step lateral flow immuno chromatographic assay based on a competitive immunoassay format for the quantitative testing of AFs in maize and derived product. The range of AFs detection of kit is 2- 1500 µg/kg. Briefly, 200g of maize grain was collected and milled in a laboratory (Foss Cyclotec Sample Mill 1093; Foss Italia S.r.l.). Ten grams of powder was mixed with 50 ml of ethanol 65% (ethanol: water; 65: 35, vol/ vol) in a plastic cylinder (1:5, maize flour: ethanol 65%, weight: vol) and shacked manually for three minutes. With a Fisher pipette, 100 µl of extract were transferred into sample cup and 500 µl of diluents added. One hundred µl of the dilution were transferred into another sample cup. A strip was immerged into the solution and incubated at room temperature six minutes. At the end of incubation, the tip was put into an AccuScan Gold detector for quantifying total AFs (Figure 16). AFs test was performed less than one week after harvest. Each MV was replicated three times.

2. 1.2.4. Data collection

Twelve phenotypic traits (PhTs) were recorded during experimentations. They were: 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). FFD and MFD were evaluated as the number of days, from sowing to 50% mature tassels and silks in the plot. Humidity of the grain was adjusted at 12% and yield (t/ha) was calculated according to (Sesay et al., 2016). Eighteen plants or ears per subplot were used to collect PhTs. In addition to PhTs, during FTs, using a pluviometer and a max/min thermometer, rainfalls, maximal and minimal air temperatures were also recorded. The temperature was record twice a day (9 AM; 05.30 PM). The precipitation was registered after each raining.

2.1.2.5. Statistical analysis

The SPSS statistical package for Windows, v. 23.0 (SPSS Inc.), was used for all statistic analyses (Corp, 2013). Combined analysis of variance using genotypes (G), sites (S) and SxG as fixed effects was performed for evaluating impact of each fixed point on PhTs. Normal distribution and homogeneity of variances were verified using the Shapiro–Wilk test and the Levene's test, prior analysis of variance, for all data collected except climate parameters (Brown and Rothery, 1993).

One-way analysis of variance (ANOVA) was carried out and the Ryan-Einot-Gabriel-Welsch-*F* test (REGW-*F*) was used to detect significant differences among means for each collected datum. ANOVA were performed with sites (S) and genotypes (G) as fixed, and replication as random. Pearson's rank correlation coefficients (ρ) were computed for all recorded traits and AF quantifying at harvest in order to evaluate the relationship among PhTs and genotypes according to (Kwon and Torrie, 1964). Student t-test ($p<0.05$, Levene's test) was performed to compare INMVs and landraces for all PhTs and AF (Landau, 2004). Clustering Analysis using Hierarchical Cluster Method based on Euclidean distance test was performed for identifying the similarity among tested MVs and classified them according to (Crossa and Franco, 2004). A Discriminate Function Analysis test was performed for completing the clustering Analysis. All data recorded except AF content were used as vectors in order to evaluate the contribution of each one in the divergence detected among tested MVs. All loading (<0.30) was removed in the structure matrix and considered as not significant contributed in the variation according to (Bian, 2012).

Figure 15. Maize plants before harvest.

Figure 16. Aflatoxin kits (A) and AccuScan Gold detector (B) used for quantifying aflatoxin content of maize grains.

Table 21. Code, provenience and genetic constitution of maize materials tested

Code	Provenience	Genetic constitution	Origin
R4185	South Africa	Open pollinated	UNIMI/Farm
R4271X4285	Haiti	Population	UNIMI/Farm
R4865	Argentina Salta	Open pollinated	UNIMI/Farm
Chiken corn	Haiti	Open pollinated	UNDH/Farm
Maquina	Mexico/Haiti	Open pollinated	UNDH/Farm
R4543	Peru	Sib pool	UNIMI/Farm
R47777	Argentina	Open pollinated	UNIMI/Farm

Table 22. Location, relief, season, sowing and harvesting period of each field trial conducted in Haiti

Field trials	Location	Relief	Sowing season	Sowing date	Harvesting date
E_1	Torbeck	Plain	Sprint	15 July 2016	12 October 2016
E_2	"*Morne Briller*"	hill	Autumn	14 December 2016	18 March 2017
E_3	Torbeck	Plain	Winter	2 February 2017	12 May 2017

E_1: First field trial; E_2: Second field trial; E_3: Third field trial.

Chapter 2.1.3. Results

2.1.3.1. Sites study

Two out of the three FTs were conducted in plain and the other in hill at 295 m from the sea level. Means air temperature and precipitations recorded in FTs are showed in (Table 23). Remarkably different meteorological trends characterized FTs. Means air temperatures were ranging from 24 to 32 ^{0}C. The highest and the lowest air temperatures were registered during E_1 (September, 2016) and E_3 (April, 2017). Cumulative precipitations monthly recorded during FTs were ranging from 0 to 253 mm. The minimum and maximum precipitations recorded were detected mainly in January and March, 2017 during E_2 and E_3 respectively. FTs conducting in 2016 showed less variation in precipitation and temperature compared with 2017 year. Mean precipitation recorded per FT was approximately 354 mm/month.

Regarding climate parameters and maize growth (MG), great variations were also observed. E_1 was characterized with high temperature and precipitations at flowering and early milk stage compared with min temperature, and limited precipitation in E_2 at similar stage. Low precipitation characterized E_1 and E_3 at dough stage and harvest compared with E_2. Precipitation was well distributed in E_3 and E_1 compared with E_2 in regard to MG.

FT conducted in hill received less rain than those conducted in plain (Table 3). Two main precipitation periods were distinguished during this study. From Dec to Feb sites were less watered compared with others trimesters. August and Mach were characterized by precipitations pick.

All collected PhTs data were submitted to one way analysis of variance using S, G and SxG as fixed (Table 24). All evaluated PhTs were significantly affected by S. Respectively 10 and one of the 12 evaluated traits corresponding to 83.33% and 8.33% of the PhTs were highly ($p<0.001$) and moderately ($p<0.01$) affected by S with number of grains per row was the less affected ($p<0.05$). Furthermore, 75% of PhTs were affected by SxG highly ($p<0.001$), while plant height was not affected by SXG (Table 24).

Table 23. Mean air temperatures and rainfall registered in Haiti during conducting of field trials

Year	Field trials	Sowing season	Location	Month	Average air* temperature (^{0}C)		Average* precipitation (mm)	
					FTs	**25 years**	**FTs**	**25 years**
				July	27.52	26.40	85	126.10
2016	**E_1**	Sprint	Torbeck	August	27.50	26.67	240.8	140.75
				September	31.92	26.32	23	174.16
				December	26.39	23.55	21	55.91
2017	**E_2**	Autumn	*Morne Briller*	January	27.42	22.90	0	41.32
				February	24.95	23.12	352	52.17
				February	28.41	23.12	54	52.17
2017	**E_3**	Winter	Torbeck	March	28.05	23.85	253	72.77
				April	29.40	24.60	33	152.66
-	**Avg./Cum**		**-**	**-**	**27.95**	**24.50**	**1061.8**	**868.01**

Cum: Cumulate; AVg: Average

*** 25 years average data downloaded from World Bank climate in 2017.**

Table 24. Mean square values resulted from analysis of variance using sites(S), genotypes (G) and SxG as fixed

Source	Sites	Genotypes	Sites x genotypes
Aflatoxin (µg/kg)	3.276***	3.910***	6.268***
Plant height (cm)	80772.001***	6045.492^{NS}	4667.019^{NS}
Steam diameter(mm)	91.388***	37.114***	10.901***
Male flowering days	7650.178***	640.319***	204.849***
Female flowering days	8995.615***	424.254***	178.351***
Number of leaves per plant	13.170**	94.046***	16.124***
Tassel length (cm)	12332.581***	499.087***	118.512**
Leaf angle	5495.539***	1130.744***	913.789***
Ear length (cm)	1512.026***	140.783***	23.558***
Number of rows per ear	7.737*	57.578***	7.978**
Number of grains per row	6531.381***	1087.070***	119.909***
Ear weight (g)	22500.022***	32141.213***	5227.615***
Grain yield (t/ha)	79.316***	104.698***	17.284***

***: p<0.05;**: p<0.01; ***: p<0.001 and NS: not significant p>0.05 (test REGW-*F*).**

2.1.3.2. Variance analysis

Results of one way variance analysis of all PhTs collected from MVs are shown in (Tables 24 and 25). More than 91% (even out of 12) evaluated PhTs were highly significant (p<0.001) while PH showed no significant difference (p>0.05). More than 63% (7 out of 11) of the significant traits formed at least three clusters. The most clustered of PhTs was number of rows per ear (NRE) with 4 clusters formed while the less segmented was PH that formed one cluster with data relative to all tested MVs.

GY were ranged from 3.61 to 7.40 t/ha. Maquina with more than 7 t/ha was the most yielded followed by Chicken corn, with 5.043 t/ha. The two local MVs showed significantly different in GY (p<0.05). The variety R4185 with less than 4 t/ha, was the most unproductive. R4271X4185 and R4856 were not significantly different from Chicken corn and are grouped in the intermediate cluster. The most yielded of INMVs was R4856 with more than 4.81 t/ha (Table 25). Regarding EL, the maximum and minimum values 13.75 and 18.05 cm were showed respectively by R4185 and Maquina. The three other tested MVs have EL around 14.78, 14.89 and 15.59 cm which were not significantly different from R4185.

Sixty percent of tested MVs had more than 10 rows per ear. Maquina showed the highest number of rows per ear, 11.92 and R4185, with an average of 9.76 the smallest value.

Maximum and minimum values recorded for EW were noted in Maquina, 126.16g and R4185 with 60.13g, which were significantly different. The other MVs weighted 78.07, 80.24 and 84.09g, which were not significantly different ($p<0.05$). MFD and FFD, was 53 and 57 days for R4865 that flowering earlier compared with other varieties. Maquina with averages of 62 and 65 days respectively was the latest flowering variety.

Values recorded for SD range from 4.50 to 6.70 mm. Maquina showed the bigger SD with about 7mm followed by Chicken corn and R4185, with 5 and 4.8 mm. The variety R4185 with 4.65 mm was the thinnest.

TL values ranged from 32 to 41 cm. The highest value was showed by Maquina (40.85 cm average) whilst the shortest was R4185 with an average of 32.61cm. The other tested MVs showed values ranging from 35 to 37.50 cm which constituted the intermediate segment and were not statistically different from Maquina and R4185.

NLP was ranged from 9 to 13. Maquina with 12.33 and R4271x4185 with 913 respectively showed the highest and the lowest values. Maquina was different from all other tested MVs whilst R4271x4185 was not different from R4185 and R4865. Chicken corn with almost 11 leaves per plant represents the intermediate segment.

Variation of LA detected among tested MVs was more than 10^0. Sixty percent of tested MVs showed more than 50^0. R4271x4185 and R4865 with almost 54^0 showed the high value but not statistically different ($p<0.05$). Maquina and Chicken corn were quite similar ($p<0.05$) with 47 and 44^0 (Table 25).

Table 25. Analysis of variance of aflatoxin content and phenotypic traits recorded from the five maize varieties tested in Haiti during 2016-17 cropping season

Source	R4185	R4271X4185	R4865	Chicken corn	Maquina
Aflatoxin (µg/kg)	2.733c	2.25ab	2.25ab	2.033a	2.133ab
Plant height (cm)	142.482	133.222	136.361	145.889	160.37
Steam diameter(mm)	4.65a	5.035a	4.847a	5.785b	6.665c
Male flowering days	58.852bc	55.593ab	53.111a	59.629bc	61.815c
Female flowering days	61.963b	61.630b	57.315a	63.389b	64.741b
Number of leaves per plant	9.370a	9.130a	9.778a	10.874b	12.333c
Tassel length(cm)	32.611a	35.148ab	37.426ab	37.037ab	40.852b
Leaf angle	52.963bc	54c	53.703c	43.888a	47.092ab
Ear length (cm)	13.759a	14.889a	15.593a	14.778a	18.056b
Number of rows per ear	9.759ab	10.629c	9.185a	10.371bc	11.926d
Number of grains per row	11.94b	12.56b	12.47b	10.33a	12.08b
Ear weight(g)	60.137a	78.071b	80.236b	84.091b	126.156c
Grain yield(t/ha)	3.611a	4.684b	4.814b	5.043b	7.398c

For each trait values with same letters indicate there is no significant difference for p<0.05 (test REGW-*F*).

2.1.3.3. Correlation matrix

A Pearson bivariate test Analysis was computed to assess the relationship among PhTs and AF. Results of correlation matrix associated those parameters are showed in Table 26. Absolute values of association detected among traits are ranging from 0.124 to 0.991. Almost 81% of tested PhTs were related. The strongest correlations were detected between GY and EW (ρ= 0.991, p<0.001) followed by MFD and FFD (ρ= 0.942, p<0.001) while the less associated of all PhTs was PH. GY is strongly (p<0.001) and directly associated with NRG (ρ= 0.260) and EW (ρ= 0.991). Strong (p<0.001) and negative associations were detected for GY and MFD (ρ= -0.214), and FFD (ρ= -0.245). No significant correlation was observed between GY and SD (ρ= 0.107, p>0.05) and GY and TL (ρ= 0.055, p>0.05). Strong and positive correlation was observed between GY and NLP (ρ= 0.283, p<0.001). Moderate and direct relationship was showed between GY and PH (ρ= 0.192, p<0.01). Strong, moderate and inverse associations were also detected between LA and NLP (ρ= -0.198, p<0.001) and LA with SD (ρ= -0.202, p<0.01) respectively. Moreover EL and NLP were associated with all the other traits (Table 26).

Table 26. Correlation between phenotypic traits and aflatoxin registered from the five maize varieties evaluated in Haiti during 2016-17 cropping season

AF	AF	GY	EW	NRE	NGR	EL	LA	TL	NLP	MFD	FFD	SD
GY	-0.347***											
EW	-0.344***	0.991***										
NRE	-0.032^{NS}	-0.007^{NS}	0.006^{NS}									
NGR	-0.004^{NS}	0.260***	0.275***	0.137*								
EL	-0.029^{NS}	0.164**	0.191**	0.169**	0.608***							
LA	-0.048^{NS}	-0.178**	-0.154*	-0.042^{NS}	0.171**	0.244***						
TL	-0.017^{NS}	0.055NS	0.075^{NS}	0.052^{NS}	0.651***	0.755***	0.328***					
NLP	-0.119^{NS}	0.283***	0.301***	0.201**	0.295***	0.348***	-0.198***	0.238***				
MFD	0.180**	-0.214***	-0.198**	0.159**	0.665***	0.599***	0.276***	0.632***	0.229***			
FFD	0.128*	-0.245***	-0.229***	0.137*	0.669***	0.604***	0.238***	0.606***	0.182**	0.942***		
SD	0.052^{NS}	0.107^{NS}	0.124*	0.299***	0.404***	0.279***	-0.202**	0.170**	0.485***	0.382***	0.425***	
PH	-0.106^{NS}	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}

***: 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**.**

2.1.3.4. Comparison between introduced maize varieties and landraces

Student t-test was performed for completing ANOVA and established a comparison between INMVs and landraces. Results are showed in Table 27. The analysis revealed significant difference between landraces and INMVs (ranging from (0.05 to 0.001) for all traits. Seventy five percent of PhTs were highly differed (p<0.001) and almost 17% were moderately differed (p<0.01). The most static was PH (p<0.05). Seventy-five percent of PhTs showed better performance in landraces compared with INMVs. INMVs showed better performance for LA and were also earlier flowering both MFD and FFD compared with local MVs. The average GY obtained from INMVs and landraces were respectively 4.37 and 6.22 t/ha. Landraces NGR was almost four times greater compared with INMVs with respectively 25 and 105. Average NLP detected in landraces was almost 12 compared with 9 registered in INMVs (Table 27).

Table 27. Comparison between introduced varieties and landraces evaluated in Haiti during 2016-17 cropping season

Source	Introduced varieties	Landraces	P-value
Aflatoxin(µg/kg)	2.411	2.083	0.001
Plant height (cm)	137.355	153.130	0.03
Steam diameter(mm)	4.844	6.225	0.001
Male flowering days(u)	55.852	60.722	0.001
Female flowering days(u)	60.247	64.065	0.001
Number of leaves per plant(u)	9.426	11.602	0.001
Tassel length(cm)	35.062	38.944	0.005
Leaf angle	53.555	45.490	0.001
Ear length (cm)	14.747	16.417	0.002
Number of rows per ear(u)	9.858	11.148	0.001
Number of grains per row(u)	25.377	105.124	0.001
Ear weight(g)	72.815	105.124	0.001
Grain yield (t/ha)	4.370	6.221	0.001

2.1.3.5. Clustering analysis

All PhTs recorded from tested MVs were submitted to Clustering analysis using Hierarchical Cluster Method with Euclidean distance test and results are sowed on Figure 17. Local MVs formed two separate clusters and all the three INMVs are in one cluster. INMVs were subdivided into two subclusters: variety R4271x4185 formed one and the other two varieties the other subcluster. Chicken corn was more associated with INMVs compared to Maquina. Chicken corn was also closer with R4271x4185 in comparison with R4865 and R4185. The two more separate clusters were Maquina and R4185 and, Maquina with R4865. Distance between the two local MVs was greater compared with that detected among INMVs (Figure 17).

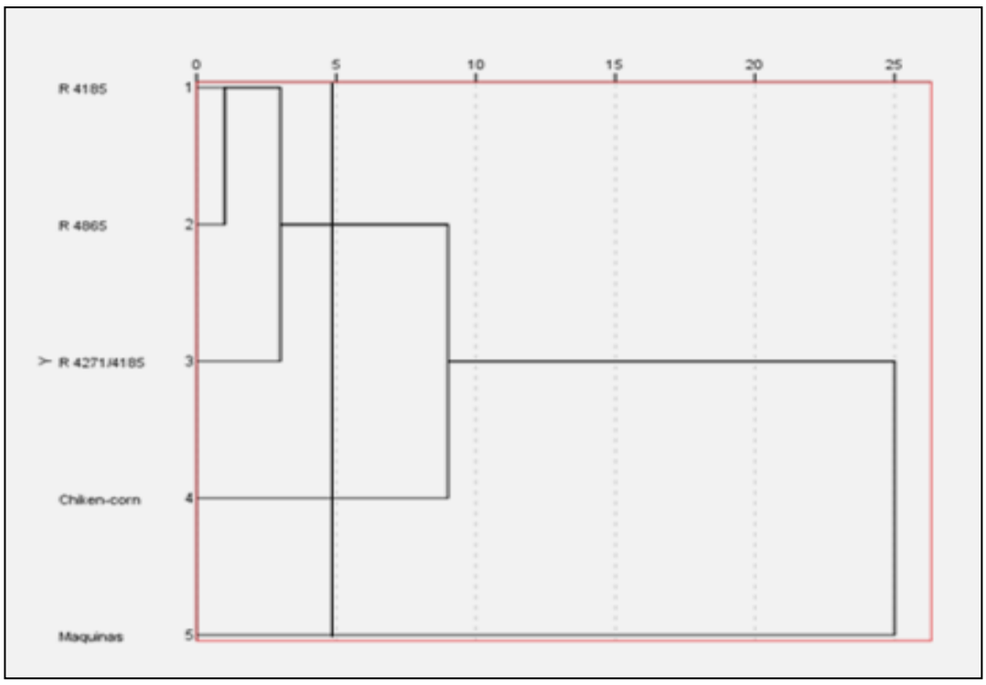

Figure 17. Maize varieties dendrogram resulted from Clustering Analysis using Hierarchical Cluster Method with Euclidean distance test with the twelve phenotypic traits.

2.1.3.6. Discriminate Function Analysis

A discriminate analysis (DA) was performed beyond ANOVA and the Clustering Analysis using Hierarchical Method. Also DA was performed for identifying the principal predictors and provided reliable information to the contribution of each trait to the variability detected among tested MVs (Figure 18 and Table 28). GY, EL, EW, NRE, NGR, LA, TL, NLP, MFD, FFD, PH and SD were predicted variables. The Tests of Equality of Group Means showed strong statistical significant differences between means of MVs groups for all predictors. GY, EW, NLP, NRE and SD shown very high Fisher's values equivalent respectively: 49.14, 46.51, 32.874, 23.298 and 20. High ($p<0.001$) and moderate ($p<0.01$) significant mean differences were obtained respectively for 75% and 17% of all predictors. The Pooled Within-Group Matrices also revealed very low correlation among predictors. The most important logarithm determinants were ranging from 16.585 to 29.983, R4865 and Maquina showed the maximum and minimum values.

Also Box's M was 2965.641 with F= 8.859 with a highly significant difference $p<0.001$. The four first discriminating functions were used during the test. Wilks' lambda value obtained for the four first discriminating functions was ($\Lambda=0.144$) and was highly significant different ($p < 0.001$). Only data relative to the two first discriminate

functions were taking in account. The two first discriminating functions explain 89.20% of variability between groups. Principal component one (PC1) explain 74% of the variability whilst principal component two (PC2) explain 15.20%.

Structure matrix associated with the traits revealed that more than fifty-eight percent (seven out of the 12) were positively associated with PC1 and PC2. All showed greater than 0.30 absolute coefficient correlations values and could be consisted with the most important predictors associated with the variability detected among tested MVs. Those traits are EW (0.530), GY (0.516), NLP (0.442), SD (0.356), NGE (0.337), FFD (-0.336) and NGR (0.310). Male and Female flowering days were positively and negatively associated with PC1 and PC2. None of the traits showed inverse correlation with the PC1and PC2 simultaneously (Table 8).

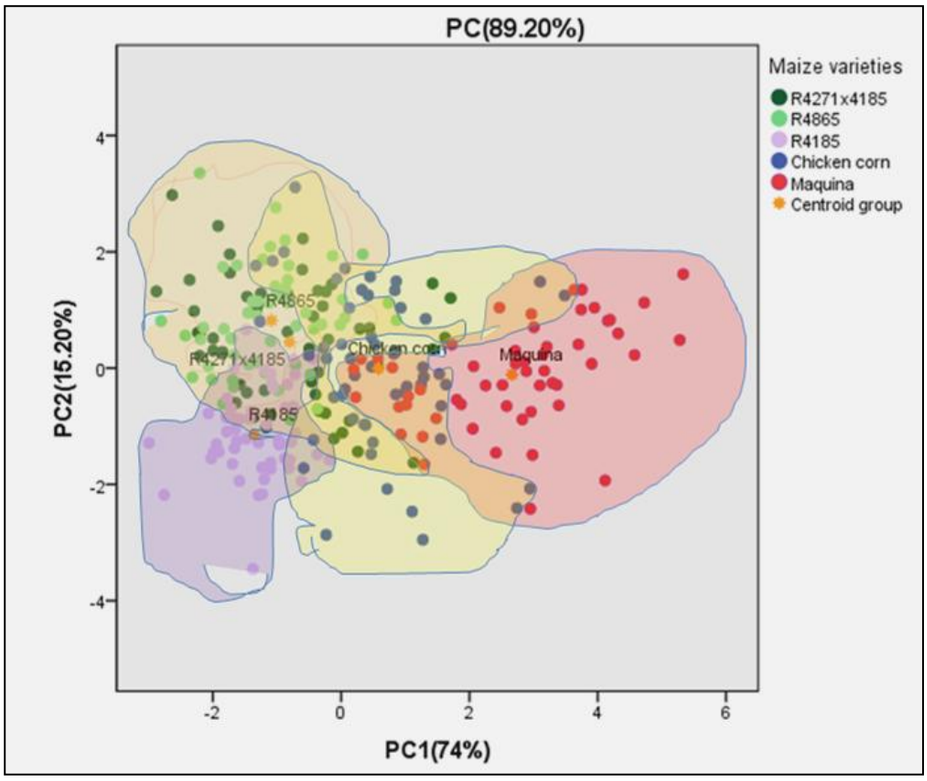

Figure 18. Canonical plot computed with the 12 phenotypic traits registered from the five maize varieties.

Table 28. Structure matrix of phenotypic traits resulted from the five maize varieties evaluated in Haiti during 2016-17 cropping season

Phenotypic traits	Function I	Function II
Ear weight(g)	0.530	0.280
Grain yield(t/ha)	0.516	0.291
Number of leaves per plant(u)	0.442	-0.037
Stem diameter(mm)	0.356	0.020
Plant height(cm)	0.082	-0.073
Male flowering days(u)	0.173	-0.336
Tassel length(cm)	0.135	0.157
Number of rows per ear(u)	0.337	-0.107
Leaves angle(u)	-0.141	0.066
Number of grains per row(u)	0.241	0.310
Female flowering days(u)	0.129	-0.215
Ear length(cm)	0.201	0.164

2.1.3.7. Aflatoxin

Similarly to PhTs, AF was submitted to one way analysis of variance and Student t-test, Pearson correlation and, results are showed in (Tables 23,24, 25,26, and 27). S, G and SxG affected strongly ($p<0.001$) AF content. AF quantified in S varied from 2.10 to 2.48 µg/kg. MVs were more AF contaminated in E_2 compared with E_1 and E_3 (not showed data). AF content of MVs was ranging from 2.033 to 2.733 µg/kg taking G as fixed (Table 24). Eighty percent of MVs showed less than 2.50 µg/kg. Chicken corn with an average of 2.033 µg/kg was significantly less contaminated compared with R4185. Maquina with 2.133 µg/kg was the second less contaminated MV. No significant difference among Chicken corn, R4271x4185 and R4865 was detected (Table 24). Results of Pearson rank correlation showed in (Table 26) revealed that AF was correlated with 25% (four out of the 12) of evaluated traits. Moderate, weak and direct relationships were detected between AF and MFD ($\rho= 0.180$, $p=0.003$) and AF with FFD ($\rho= 0.128$, $p= 0.036$). Strong and inverse correlations were detected between AF and GY ($\rho= -0.347$, $p= 0.001$) and AF with EW ($\rho= -0.344$, $p= 0.001$).

Student t-test results showed that INMVs were significantly more AF contaminated compared to landraces. Means AF content of INMVs and landraces were respectively 2.411 and 2.083 µg/kg (Table 27).

Chapter 2.1.4. Discussion and conclusions

Reducing malnutrition exposure by raising agricultural productivity through breeding program to improve crop varieties is one of the major challenges faced by international programmes focussing on agriculture and development. This approach is still limited in public as well as private sectors of developing countries. In this context the present study attempts to address the issue of testing novel maize varieties utilizing local resources. Five novel varieties kindly provided by UNIMI (Table 21) were tester for yield and AF compared to two Haitian landraces, Maquina and Chicken corn. Climate parameters such a rainfall and temperature were recorded and compared to 25 years average historical weather data. Both precipitations and temperature trend were differed from 25 years average (i.e. precipitation was almost two times the 25 years average), and great variations among FTs was recorded. Nevertheless, weather patterns i.e. mean air temperature and rain were suitable for maize cultivation (18 to 27 ^{0}C and 600 to 1100 mm). Thus, findings confirm that Haitian climate is favorable for growing maize all year long (i.e. during rainy season as well as dry season). Most of phenotypic traits (92%) were affected by S and SxG. Results suggested that tested MVs responded differently to the location. Those results were expected because S were characterized by great dissimilarity such as soil types and relief (hill/plain), precedent culture, season and harvesting. The variation of precipitations and their distribution during the plant live cycle could also explain the role of S on evaluated traits. This study is the first conducted in Haiti recording precipitations and temperature at different S. Studies conducted on Haitian maize cultivation are mainly focus on agroforestery (Isaac and Woods, 2004) and/or fertilization in only one agroecological zone (Clermont-Dauphin et al., 2003). Therefore it is very difficult comparing present data. Because of that, others investigation might be conducted for confirming present findings.

Tadesse et al (2014) did a similar study with six MVs in three agro ecological zones in the northwest of Ethiopia. Even if Ethiopia shows similar wheatear conditions with Haiti, the team reported that PhTs were not influenced by sites. This could be due to Ethiopian climatic parameters which are differently distributed compared with those of the present study. The team did not take in account differences due to altitude, temperature and precipitation which could vary across sites.

Moreover, different genetic materials were used. All those parameters could explain the difference between findings of the two studies.

Maize is cultivated for its productive potential and the ability to colonize different reliefs and agro- ecology. In most part of Haiti, the open pollinated maize cultivated under different agro-ecology, yield about 0.90 t/ha (Timothy et al., 1988). Even if Clermont-Dauphin et al (2003) report an average production of 0.71 t/ha since a large part of the plots was destroyed by animals. Twenty five years after the first work conducted by Timothy's team, in 1988, Ray et al (2013) reported that maize yield constantly decrease in Haiti. This trend is supported by Molnar et al (2015) who evaluated at 0.825 t/ha the maize yield in the Northern Haiti.

Landraces as well as INMVs tested gave high grain yield compared to National average even if cultivated according to traditional farmer's practices. Similar results could be explained by the good AGPs applied. Optimum conditions were maintained during all plant cycle, this could explain higher yield (Drury et al., 2009).

Maquina and Chicken corn varieties, are the most popular landraces used by farmers over several generations (Harlan, 1992), and are the most cultivated in southern department. In experimentations those two varieties yielded more than 7 and 5 t/ha respectively (8 and 6 times higher than National average) respectively. These findings confirm farmers' behavior, indeed they asserted that Maquina is more productive than Chicken corn and it can be cultivated in mountain, even during dry season. Chicken corn yield confirm findings of Aloys (2014) for this variety in South, Haiti.

Means GY of this survey were higher than 1.41 t/ha reported in Senegal (Okuyama et al., 2017) and 1.24 t/ha in Center Africa (Nin-Pratt et al., 2011) both places with similar Haiti climate and maize grow conditions. Data reported by Tadesse et al (2014) (around 5-7 t/ha) about six cultivated maize varieties in rainfed condition were consistent with present findings. Similar data were also reported by Baretta et al (2016) who worked on yield potential and genetic variability in nine landraces and four commercial maize varieties from Brazil. Beretta and colleagues report a GY ranged from 5 to 10 t/ha. The great variation detected in this study could be due to the presence of hybrids in the experimentation.

Most of PhTs (81%) of tested MVs were correlated. Those findings could be very useful for setting a breeding program baseline. GY was strongly

and directly associated with EW, EL and NGR of tested MVs. This means that the increasing of one of those traits correspond to GY increasing. This could be due because requirements of GY are similar to the needs of those parameters. Irrigation and fertilization contribute simultaneously to higher yield, increase ear length and number of grains per ear (Grant et al., 1989; Aydinsakir et al., 2013; Tovihoudji et al., 2017). Findings of this survey are consistent with data reported by Bavec and coworker (2002) who found that GY and NLP were associated strongly in tested FAO maize varieties. Likewise (Kayaga et al., 2016) analyzed thirty six commercial hybrids and two local maize open-pollinated populations noted the strong association between NLP and GY. Also, Pavlov et al (2012) reported strong correlation between those agronomic traits. Moderate correlations between GY and PH, is not in agreement with Yousuf and Saleem (2001) who reported negative correlation between those phenotypic traits in Pakistan maize.

Plants with longer and great number of leaves are, preferred by breeders, therefore data detected associated with GY and NLP is remarkable. Strong relationships found between EW, EL and NGR with EL, are supporting data already reported by Pavlov et al (2012), who have found ear length strongly and positively correlated with NGR and weakly related with EL and NGR. These results are also in concordance with positive correlations between EW and EL, but negative correlation between EW and NGR reported by (Srećkov et al., 2011).

Negative association was detected between GY with FFD and MFD. So, decreasing MVs shelf life in field corresponds to an increasing of GY. This is also a good and promising result because in countries with low income and where maize is mostly used for subsistence as Haiti, earlier /precocity is a character often required /demanded by farmers beyond GY. Male and female flowering days were strongly and positively associated. Similar results were earlier reported by (Malik et al., 2005); Chase and Nanda, 1967). Male and female flowering days after sowing and their associations with number of grains per row are also consistent with data reported by (Altenbas and Algan, 1993).

Significant and strong association among PhTs are partly agree with data reported earlier by Malik et al (2005) who found positive association between plant height firstly and ear length and number of grains per row secondly. Moreover, the great variability detected in the relationship observed among evaluated traits may be due to the presence of phenotypic plasticity, which is the amount of change in the

expression of traits under different environments (Bradshaw, 1965; Vidal-Martínez et al., 2004).

A large amount of results obtained from analysis of variance (91.67%) and the Student t (75%) tests performed for all traits were highly significant. Similar results suggested the presence of genetic divergence among tested genotypes and between INMVs and landraces (Wietholter et al., 2008). Highly significant differences of GY, EW, NLP, NGR, NRE and SD, MFD and, FFD detected among tested MVs are also reported by (Baretta et al., 2016; Vidal-Martínez et al., 2004). The unresponsiveness of PH to G and SxG mean that tested MVs responded similarly to different environment and the genotypes conserved their trait ranks. This could be due because PH is one of the most resistant trait to plasticity (Locatelli et al., 2002; Vidal Martínez et al., 2001).

Most of variability detected during analyzes of variance and student t test were fully confirmed by the Dendrogram built on the divergence observed in the phenotypic MVs characters. Clustering Analysis using Hierarchical Cluster Method with Euclidean distance plays important role because it gives information about divergence of genotypes that in turn could be used in increase precision and reduce costs in breeding program (Wietholter et al., 2008). The Clustering Analysis revealed that the five tested MVs formed three main clusters. Those results confirmed also findings relative to Analysis of variance and Student t test. Local MVs formed two separate clusters and INMVs formed one and, thus confirm also the important genetic divergence between landraces and INMVs. Distance between landraces was broader compared to that detected among INMVs. Similar findings suggest that MVs could be constituted an important reserve of interesting traits and could be used as parents in breeding program. The large distance observed between the two local MVs could be suggested a great genetic variability within the two most important MVs gown in South, Haiti. Even so, similar results were not surprising because landrace populations are often genetically diverse (Dreisigacker et al., 2005; Pressoir and Berthaud, 2004).

The short distance observed among IMNVs could be due by fact they were prior crossed among them in Italian or in African breeding program in (Cantaluppi et al., 2017**).** Chicken corn was closer INMVs compared with Maquina, suggesting that they share some common genetic traits. Similar results are not unexpected because most of the INMVs were developed in breeding program in Italy where Chicken

corn enters as a donor of traits (not showed data). Moreover, the largest distance among tested MVs varieties was observed between Maquina and two of the three INMVs. This supposed the great genetic divergence between Maquina and INMVs. Those could be important point because maize breeders are emphasizing the importance of diversity among parental genotypes as an important prerequisite for obtain heterotic hybrids (Ahloowalia and Dhawan, 1963; Saxena et al., 1998).

Characterization of genetic diversity of maize germplasm is of great importance in maize breeding (Xia et al., 2005). For better understand, which were the most important predictors of MVs variability, a discriminate Analysis was performed using the phenotypic traits as predictors. Findings obtained from the DA revealed that the two first components analysis explained almost 90% of the divergence observed among tested MVs (Figure 19), in agreement with Kayaga et al (2016) but not with Jafarzadeh et al (2016) who reported less than 65% of variation explained by the two first PC. Precision of the test was confirmed by Wilks' lambda, $p<0.001$.

Correlation Matrix findings revealed also that more than 50% of traits contribute positively to the two PCs. These findings confirmed their contribution to the primary and secondary modification in maize varieties divergence. Five showed more than 30% (limit of minimal contribution of predictor) and constitute the most important vectors responsible of the divergence. The loading vectors responsible of the divergence detected among tested MVs were EW, GY, NLP, SD, NGR, and MFD. The presence of genotypic variation for all those measured traits confirmed genetic differences between genotypes (Vidal-Martínez et al., 2001). Participation of NGR to the divergence among tested MVs is consistent with findings of (Suryanarayana et al., 2017). Suryanarayana and colleagues evaluating the genetic variability of thirty maize genotypes reported that GY and NGR as the most important traits responsible of primary variation in agreement with (Ganesan et al., 2010; Azad et al., 2012).

It is the first study indicating that Haitian climate is favorable to AF production in field condition. It was observed that S of FTs influenced considerably AF concentration of maize grains at harvesting moment. Thus, similarly to PhTs, whether trend could explain the role of S on AF contamination as earlier reported. In Alabama Bowen et al (2014) reported that S affects AF levels of maize in field condition. Even Bowen's team utilized different maize materials and a more sensitive

method for quantifying AF, (enzyme-linked immunosorbent assays); results are quite similar. S with particular traits such as altitude and temperature, with climate change could easily contribute to accumulation not only AF but other mycotoxins (Mox) such as Ochratoxins and Fumonisins (Magan et al., 2011). In Uganda, it was showed that altitude was linked to Mox contamination of maize in field condition (Kaaya et al., 2006) and in Tanzania precipitation variation was responsible of the same toxin accumulation (Nyangi et al., 2016).

It is know that AF contamination of grains in field condition depends on some key environmental factors (Cotty and Jaime-Garcia, 2007). In this study, MVs were more AF contaminated in E_2 compared with other sites. E_2 was conducted in hill at 295 m altitude and the maize grow water stress during flowering and early milk vegetative phases, followed by a pick of precipitation just before harvesting.

It has been reported that drought during grains maturation as well as rains occurred few days before the harvest are environmental favorable condition for fungal attacks and AF accumulation (Doohan et al., 2003 and Blandino et al., 2009; Bowen et al., 2014; Hawkins et al., 2008; Widstrom et al., 1990). Moreover, AF accumulation in field condition could be also a result of birds, mammals, insects, mechanically wounding or hot dry stress conditions (Cotty and Lee, 1990; Guo et al., 2004; Ni et al., 2014). Climate condition similar to that detected in site environment (26 ^{0}C and variation in rainfall distribution) of FTs could be favorable to insect proliferation and damage that end up in AF accumulation. In this study, the impact of insects was evaluated.

Quite low AF level was quantified in all MVs and results are not in agreement with previously data (Castor et al., 1987; Schwartzbord and Brown, 2015, Aristil et al., 2017). Most of the reported level of AF contamination in Haiti was higher than the US Food and Drug Administration limit (20 µg/kg). In the current investigation, all tested MVs showed less than 5 µg/kg, for AFB1, the most potent mutagenic and carcinogenic metabolite known (Ahsan et al., 2010 and CAST, 2003). In all articles cited here, AF detection has performed using storage products. Castor and colleagues used maize grains samples collected from several markets spread on divers departments, Schwartzbord and Brown used maize samples collected in Port-Au-Prince and Cap Haitian markets, and Aristil and coauthors (2017) investigated AF from storage maize grains of Les Cayes department. Aristil et al (2017) found that cultural cycle is correlated with TF and AF. Relatively, low level of AF

in MVs at harvest could be due firstly to the good AGPs applied and secondly to the short storage duration of the grains since all MVs were AF tested less than one week after harvest. Castor et al (1987); Schwartzbord and Brown (2015) support that AF accumulation in Haitian maize grains is link to storage duration and post harvest management.

In the current survey, AF found at harvesting in MVs was negatively associated with GY and EW. Those inverse relationships detected between production quality and quantity components suggest that an increasing of GY and/or EW correspond to a decreasing of AF at harvest. This imply that the factors involved in reduction AF contamination in Haitian maize grains in field condition are the same involved in productivity. Those could be explained by the fact that the requirements for production increasing, are against the requirement for AF production in field condition: plant stress conditions.

AGPs such as adequate irrigation, control of weed, fertilization, harvesting grains at the right humidity (<15%), no grains damage during transportation and drying, reduce considerably toxigenic fungal growth and AF contamination (Iken and Amusa, 2004; Prandini et al., 2009, Waliyar et al., 2014, Wu et al., 2016, Giorni et al., 2016, and Agol et al., 2017). Even the drying is not an AGP; it is advisable that practice of drying in high tide field favorites the attacks of insects and increase humidity in grains, that in turn the development filamentous fungi and Mox.

Negative correlation between GY and AF was earlier reported. Bowen et al (2010) working on maize Bt in Alabama, reported the consecutively and strongly inverse relationship between GY and AF over 3 years with respectively: ρ= -0.78, year 2010, ρ= -0.71, year 2011, and (ρ= -0.49, year 2012 ($p<0.001$), similar data were reported by (Abbas et al., 2007).

Direct relationship was observed between AF and male and female flowering days. Similar results suppose that an increasing of maize grain shelf live in field condition corresponds to an increasing of AF accumulation at harvest. Similar results were reported in countries with similar Haiti climate characteristic and overall where maize grain is dried on stalk, in field (Degraeve et al., 2016; Mutiga et al., 2015).

In conclusion, this study provides for the first time Haitian maize landraces and INMVs reliable information. Findings revealed that Maquina and Chicken corn have great grain yield potentiality and could be used for AF reduction in Haiti along with good agricultural practices.

Also R4865 has very important yield potentiality which is highly linked with EW and inversely related with AF. Therefore, those maize varieties could be used in breeding program aims to increase yield and reduce AF. Deeply studies using the F_1 generation obtained by crossing Chicken corn with INMVs should be tested in order to evaluate yield and AF potential of novel improved varieties under Haitian tropical climate.

Chapter 2.1.5. References

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Correlation analysis of five improved maize (Zea mays) varieties traits with grain yield and Aflatoxin content at harvest in Haiti

Summary. Five improved maize varieties (IMVs) resulted from the introgression of exotic germplasm into landrace were tested for grain yield (GY) and aflatoxin (AF) content. The contribution of 11 phenotypic traits to the two last variables was also evaluated. The IMVs were obtained crossing: R4865xChicken corn, R4865xR4543, R4543xChicken corn, R4777xChicken corn and (R4777xR4185) x R4865. Two field trials were conducted in South, Haiti at agricultural farm of the University Notre Dame of Haiti (Torbeck $18^0$10'N, $73^0$49'W, 13 m altitude), and Bourjolly ($18^0$16'N, $73^0$47'W, 30 m altitude) during cropping season 2016-2017. Experiments were laid out in a three-replicate randomized complete block design. Each experimental plot was 3.50 m per 10.50 m with a gross area of 36.75 m^2.Each plot consisted of fifteen subplots of 3 rows (13 plants each) equivalent to a global density of 60 000 plants/ha. One-way analysis of variance was carried out and the Ryan-Einot-Gabriel-Welsch-*F* test was applied to detect significant differences among means. Pearson's rank correlation coefficients (ρ) were calculated to identify relationships among measured traits using SPSS. Results showed that 80% of IMVs give more than 6 t/ha and the significantly ($p<0.05$) less yielded is R4777xChicken corn with 5.579 t/ha. Also 40% of IMVs have AF > 20 µg/kg, limit fixed by US Food and Drug Administration. Comparing with parents, IMVs were more yielded and AF contaminated. GY was positively associated with ear weight ($\rho = 0.998$) and AF ($\rho = 0.198$). AF was negatively related with tassel length ($\rho = - 0.241$). Thus, ear weight and tassel length are the two selection criteria that could be taking in account in the further steps of breeding program to increase GY and minimize AF in Haiti.

Keywords. Improved maize, grain yield, aflatoxin, correlation, Haiti

Chapter 2.2.1. Introduction

Mycotoxins (Mox), secondary metabolites produced by saprophytic fungi, are responsible of 25% of crops contamination worldwide (Smith et al., 1994; Granados-Chinchilla et al., 2017). Along with Ochratoxins and Fumonisins, Aflatoxins (AFs) mainly produced by *Aspergillus flavus* and *Aspergillus parasiticus* constitute the most important Mox group responsible of food safety problems. Those are usually found in oleaginous and cereal crops including wheat, walnut, maize, cotton, peanuts and tree nuts and can lead to serious threats to human and animal health by causing various complications such as hepatotoxicity, teratogenicity, and immunotoxicity (Lewis et al., 2004, Kachapulula et al., 2017; Wagacha et al., 2008; Susca et al., 2016; Mo et al., 2016; Beukes et al., 2017; Ncube et al., 2017).

Even if food safety is an important issue in temperate and subtropical areas, it is dramatic in tropical country with low income as Haiti where Mox management is rare (Filbert and Brown, 2012; Gerding et al., 2015; Schwartzbord and Brown, 2015; Schwartzbord et al., 2014; Aristil et al., 2017; Emmanuel et al., 2009; Wampler and Sisson, 2011; Dowell et al., 2011).

Mox contamination could occur in field as well as during post-harvesting managements. Even if no control strategy is completely effective when environmental conditions are extremely favorable to fungal growth and Mox accumulation (Windham and Williams, 2002; Brown et al., 1999), good agricultural practices (GAPs), utilization of adapted varieties, avoiding harvest during raining period, rapid drying after harvesting, pest control could contribute to decrease of contamination (Brown et al., 1999; Lisker and Lillehoj, 1991).

Plant tolerance or resistance to aflatoxigenic fungal strains is considered as a highly desirable approach for reducing or eliminating AF contamination (Williams, 2006). Develop maize breeding program (MBP) focusing on reduction of AF contamination requires important materials, which are often scare in developing country and is time consuming (Williams et al., 2003; Scott and Zummo, 1994; SC212M and SC343, 1989).

Taking in consideration climate parameters (rainfall and air temperatures) is evident for effectiveness of MBP in Haiti where majority of maize is cultivated in rainfed system. In developing country with limited breeding materials, statistic tools are often used for

predicting desirable traits such as yield. One of the most largely used is correlation analysis. Genetic correlation analysis is a useful technique which elaborates the degree of association among quantitative traits (Malik et al., 2005). Degree and sense of the relationships among traits could give appropriate information relative to decision taking (Nemati et al., 2009; Bello et al., 2010). Correlations between grain yield (GY) and other traits were widely documented (Ho et al., 2001; Umakanth et al., 2000; Saleem et al., 2007). GY was reported positively related with several traits such as plant height, number of grains per row, and ear length (Saleem et al., 2007; Pavlov et al., 2012; Dewey and Lu, 1959; Rafiq et al., 2010; Alvi et al., 2003; Nemati et al., 2009; Betran et al., 2002; Robertson-Hoyt et al., 2007). Days to flowering, plant and ear height, ear weight were reported as the most important selection criteria in improving open pollinated maize varieties and hybrids for high GY (Bello et al., 2010). GY was reported negatively related with flowering days and AF contamination of maize genotypes in controlled conditions (Bowen et al., 2014; Betran and Isakeit, 2004).

Nevertheless, until now, data relative to AF contamination and agronomic traits in rainfed condition remain rare. Yet, most of maize cultivated in subtropical and tropical areas as Haiti is developed in rainfed systems. This preliminary study was designed to (i) compare five improved maize varieties developed in Haiti for GY and AF content at harvest and (ii) evaluate its relationships with eleven agronomic traits.

Chapter 2.2.2. Materials and methods

2.2.2.1. Fields trials location and plants management

Two field trials (FTs) were carried out in South Haiti during winter and spring 2016-17 (Figure 19). One was performed at agricultural farm of the University Notre Dame of Haiti (UNDH), (Torbeck $18^{0}10'$N, $73^{0}49'$W, 13m altitude), and the other at Bourjolly, close to the international airport *Antoine Simon* of Les Cayes ($18^{0}16'$N, $73^{0}47'$W, 30 m altitude). Maize was the previous crop tested in both sites. Five improved maize varieties (IMVs) developed in FT/ E_1 (Tables 29 and 30) were evaluated until third generation (F_3). From F_1 to F_3 the crosses were performed pooling the pollen of nine plants belong to the same varieties as decrypted in (2.1.2.2 section). All IMVs were submitted to similar agricultural practices (AGPs) already decrypted in (2.1.2.1 section).

2.2.2.2. Aflatoxin quantification

The screening for aflatoxin (B1, B2, G1 and G2) contamination and concentration were performed according with Reveal® Q+ aflatoxin test (Neogen®) supply instruction as decrypted in (2.1.2.3 section).

2.2.2.3. Data collection

Grain yield (t/ha) was calculated according to Sesay et al (2016). Number of row/ear (NRE), number of grains/row (NGR) and ear length (EL) were collected at harvest. Leaf angle (LA), tassel length (TL), number of leaves per plant (NLP), female flowering days after sowing (FFD), male flowering days after sowing (MFD), plant height (PH) and stem diameter (SD) were evaluated at flowering. These data were collected on 18 plants per subplot. In addition, precipitations, maximal and minimal air temperatures were registered using a pluviometer and a max/min thermometer for each site. The temperature was record twice a day (9 AM; 05.30 PM). The precipitation was registered after each raining.

2.2.2.4. Statistical analysis

The SPSS statistical package for Windows, v. 23.0 (SPSS Inc.), was used for all statistic analyses (Corp, 2013). Combined analysis of variance using genotypes (G), sites (S) and SxG as fixed effects was performed for evaluating impact of each fixed point on PhTs. Normal distribution and homogeneity of variances were verified using the Shapiro–Wilk test and the Levene's test, prior analysis of variance, for all data collected except climate parameters (Brown and Rothery, 1993). One-way analysis of variance (ANOVA) was carried out and the Ryan-Einot-Gabriel-Welsch-

F test (REGW-*F*) was applied to detect significant differences among means for each collected datum. ANOVA was conducted with genotypes (G) as fixed, and replication was considered random. Pearson's rank correlation coefficients (ρ) were computed for all recorded traits and AF quantifying at harvest for evaluating the relationship among phenotypic traits according to (Kwon and Torrie, 1964).

Figure 19. Maize field trials conducted at Bourjolly.

Table 29. Improved maize varieties background

Improved variety	Male	Female
G1	R4865/ Argentina Salta	Chicken-corn/ Haiti
G2	R4543/ Peru	Chicken-corn/ Haiti
G3	R4865/ Argentina Salta	R4543/ Peru
G4	R4271x4285/ Haiti	R4865/ Argentina Salta
G5	R4777/ Argentina	Chicken-corn/ Haiti

Table 30. History of improved maize varieties

Field trials	Crossing	Location	Sowing month	Harvesting month
E_1	F_1(parents)	-	-	-
F_2	F_1XF_1	Torbeck	22 December 2016	11 March 2017
F_3	F_2XF_2	Bourjolly	4 April 2017	6 June 2017

E_1: first field trial; F_2: second generation and F_3: third generation.

Chapter 2.2.3. Results and discussion

2.2.3.1. Sites study. Averages air temperature and total precipitations registered during each FT are showed in Table 31. Significant variations were detected among sites. Means air temperature recorded at Torbeck and Bourjolly were respectively 27.48 and 25.38 ^{0}C. The maximum and min air temperatures were recorded at Torbeck, in February, 28.26 ^{0}C and December, 26.93 ^{0}C. Air temperature values seem more constant at Bourjolly site compared to Torbeck. Amplitude temperature at Torbeck site was 1.33 ^{0}C in comparison with 0.41 ^{0}C registered at Bourjolly. The overall mean air temperature recorded during FTs is 26.43 ^{0}C, which had 2.03 ^{0}C greater than 25 years data (WBG, 2017). Total precipitation registered at Bourjolly (418 mm) corresponds to more than three times of that of Torbeck site (132 mm). Cumulative precipitation recorded during FTs (550 mm) was low compared to 625.33 mm registered during the last 25 years for the same period. Similar findings suggest significant variations in temperature and precipitation in Haiti during the last 25 years. May be this difference observed is due to climate change. The effect of climate change was mentioned prior by (Barnett, 2001; Alexander et al., 2006). More robust data are needed to confirm present findings. The average of temperature at Torbeck (27.48 ^{0}C) was near the limits of climatic variation range of maize cultivation (18-27 ^{0}C). Total precipitation recorded at Torbeck (132 mm) and at Bourjolly (418 mm) were both not in the range of the requirement of maize cultivation (600-1100 mm). Thus, it seems that variation of precipitation could affect more genotypes compared to temperature.

More than 83% of PhTs showed variation in regard with S. Fifty percent was positively affected by SxG interactions simultaneously. This could be due to plastic effect (PhTs revealed great dependence on environment), similar results were reported by (Kayaga et al., 2016; Gami et al., 2017). MFD was not affected by any of fixed effect. Findings reveal that all tested IMVs conserve their rank and response similarly to environment effects (Table 32).

2.2.3.2. Variance analysis. Obtained results of variance analysis are showed in Table 33. All tested IMVs were more productive in F_3 (at Bourjolly) compared to F_2 (at Torbeck). GY obtained during F_2 (at Torbeck) and F_3 (at Bourjolly) were ranged respectively from 3.908 to 6.029 t/ha and 6.7817 to 8.349 t/ha, with a delta (amplitude) of about 3 t/ha. The most significant variation was observed in G3, with 3.691 t/ha. Maximum GY obtained during experimentation was showed by

G5 and G2 with 8.349 t/ha and 8.114 t/ha during F_3 (at Bourjolly). The overall GY obtained for F_2 (at Torbeck) and F_3 (at Bourjolly) is more than 6 t/ha for 80% of tested IMVs. The less productive was G4 with an average of 5.579 t/ha. Thus, findings indicate the great importance of S environments on GY since the most important variation was between F_2 (at Torbeck) and F_3 (at Bourjolly). Additional investigations are needed to better understand the right genotypes selection for specific location exploiting data already presented by (Stuber et al., 1992; Giauffret et al., 2000; Signor et al., 2001; Fan et al., 2010). Tested IMVs were more yielded (6 t/ha) compared to (3.611 to 5.043 t/ha) obtained from parents. Results are very promising especially for Haiti, where more than 30% of Gross Domestic Brut depending on agriculture and particularly on maize cultivation. Increasing GY could be an important step in malnutrition reduction (Lundahl, 2015; Iannotti et al., 2014; Albert, 2016).

Similarly to GY, EW maximum and minimum values were recorded in G4 and G3 with 92.976 g and 113.13 g, which were significantly different. G5 was not differing from G3. The other IMVs tested were not differing from G4. Ear lengths were significantly differing in F_2 compared with F_3. The Maximum value obtained was registered in G2 with an average of 17.444 cm. Overall EL obtained for tested IMVs are ranging from 15.37 to 16.95 cm. Overall NGR seems more constant in tested IMVs compared with EL. This trait was showed more variation in F_3 (at Bourjolly) in comparison with F_2 (at Torbeck). All tested IMVs showed almost 30 grains per row. Only G4 was significantly differing from the other tested IMVs ($p<0.05$). NRE showed similar variation in comparison with NGR. NGR per generation is ranging from 10 to 13. Only G4 showed value significantly differing from the others. Minimum and maximum values registered for PH were detected in G1 and G5 respectively with 164.278 and 184.333 cm even if this character showed no significant difference ($p>0.5$).

MFD and FFD were not significantly differing for all the five IMVs. Means MFD and FFD of tested G were 46 and 51 days. The NLP mean was about 11. Those PhTs showed quite similar variation in F_2 (at Torbeck) than F_3 (at Bourjolly). LA was more varied in F_2 (at Torbeck) compared with F_3 (at Bourjolly) and 80% of IMVs had less than 46^0 as values of formed LA, which is consistent with findings of (Marlina et al., 2017; Wietholter et al., 2008).

2.2.3.3. Correlation. Pearson's rank correlation matrix results associated PhTs and AF content recorded from all five tested IMVs are showed in Table 34. Significant variations were observed among evaluated traits. Fifty-six percent of data were positively related with values ranging from 0.165 to 0.998. This large proportion of direct correlation is important and is often taking in account for effective breeding (Nemati et al., 2009; Bello et al., 2010). The strongest correlation was detected between EW and GY (ρ= 0.998, $p<0.001$) whilst the lowest relationship was between PH and NRE (ρ= 0.165, $p<0.05$). Positive and strong ($p<0.001$) correlations were observed among GY with SD (ρ= 0.403), LA (ρ= 0.439), NGR (ρ= 0.303), and EW (ρ= 0.998). Similar findings suggested that 99% of variation detected among GY is due to EW. This is consistent with findings of (Reddy et al, 2013; Pandey et al., 2017). Negative correlations at different levels were detected among GY and TL (ρ= -0.248, $p<0.01$), and EL (ρ= -0.203, $p<0.01$) and PH (ρ= -0.494, $p<0.001$). Similar findings mean that increasing of one of those traits correspond to a decreasing of GY. Also significant and positive correlations were detected among LA and EW (ρ= 0.438, $p<0.001$) and FFD with MFL (ρ= 0.445, $p<0.001$). Similar findings were earlier mentioned by (Ghosh et al., 2014; Kumar, et al., 2014; Reddy et al., 2013).

2.2.3.4. Aflatoxin. Similar to PhTs traits, AF content of tested IMVs were submitted to three tests and results are presented in (Tables 32, 33 and 34). Genotype and SxG affected considerably AF content of all the five IMVs whilst S independently didn't have any effect on AF concentration (Table 4). Overall AF quantified among the five IMVs tested is ranging from 2.135 to 30.871 µg/kg and 40% had value greater than the US Food and Drug Administration (USFDA) limits (20 µg/kg). AF showed significant variation across generations. It is ranging from 1.99 to 59.10 µg/kg during F_2 (at Torbeck) and, from 2.28 to 51.079 µg/kg during F_3 (at Bourjolly). The two maxima AF concentrations values were recorded in G3 (59.10 µg/kg) during the second generation (at Torbeck) followed by G2 with 51.079 µg/kg in F_3 (at Bourjolly). Findings suggest that IMVs AF contamination is an important issue to take in account for further program breeding even if more investigation needs to be carried out in this field. Mean AF concentration obtained from IMVs (13.841 µg/kg) represented almost six times of (2.317 µg/kg) detected from parents.

Correlation matrix associated traits of IMVs with AF is showed in Table 6. AF is positively related with GY (ρ= 0.198, $p<0.01$), EW (ρ= 0.199, $p<0.01$), NGR (ρ= 0.154, $p<0.05$) and NRE (ρ= 0.149, $p<0.05$).

Consequently, increasing of one of those traits correspond to an increasing of AF accumulation in genotypes. AF content is negatively associated with TL (ρ= -0.241, $p<0.05$). Similar findings suggest that maize with long tassel could have ability to reduce AF concentration in field condition. Other studies must be conducted for confirming the present results. Positive correlation detected between GY and AF is conflicting with findings of (Betran and Isakeit, 2004; Betran et al., 2002). In conclusion, present study gives for the first time information relative to GY and AF content at harvest of improved varieties developed with landrace (Chicken corn) in South Haiti. IMVs mean GY is 6.311 t/ha that represent up to six times of National grain yield (0.96 t/ha). From first to third generation, mean GY of genotypes increase of 39%. More than 99% of the variation observed in GY is due to ear weight. Conversely, 40% of IMVs showed AF content higher than USFDA limits 20 µg/kg. GY is also responsible of 4% of AF content, suggesting the necessity to break this correlation in future steps of this breeding. Genetic correlation among evaluated traits indicating EW and TL as the most important indicator on which focus in the choice of parents for increasing grain yield and reducing aflatoxin.

Table 31. Air temperatures and rainfall registered in field trials

Year	Field trials	Location	Month	Average air Temperature (°C)		Average precipitation (mm)	
				FTs	**25years**	**FTs**	**25years**
2016	**F2**	Torbeck	December	26.93	23.55	8	55.91
			January	27.25	22.90	28	41.32
			February	28.26	23.12	96	52.17
	Average/total			**27.48**	**23.19**	**132**	**149.4**
2017	**F3**	Bourjoly	April	25.39	24.60	141	152.66
			May	25.58	25.34	195	192.42
			June	25.17	26.11	82	131.05
	Average/total			**25.38**	**25.61**	**418**	**476.13**
	Overall	-	-	**26.43**	**24.40**	**550**	**625.53**

Table 32. Mean square resulted from analysis of variance of phenotypic traits and aflatoxin

Sources	Maize varieties		
	Sites	**Genotypes**	**Sites x Genotypes**
Aflatoxin(µg/kg)	36.929^{NS}	6754.942***	12691.629***
Grain yield (t/ha)	310.642***	7.300***	16.081***
Ear length (cm)	91.350***	19.764***	1.507NS
Ear weight(g)	85926.946***	2049.809***	4435,187***
Number of rows per ear(u)	18.689**	29.203***	4.425^{NS}
Number of grains per row(u)	1456.356***	121.769**	32.731^{NS}
Leaf angle(°)	2205***	299.658***	23.347^{NS}
Tassel length(cm)	938.404***	1120.990***	234.989***
Number of leaves per plant(u)	0.672NS	6.436NS	15.269***
Male flowering days(u)	29.606NS	19.186NS	20.231^{NS}
Female flowering days(u)	88.200**	40.203**	46.464**
Plant height (cm)	39807.288***	1880.768***	1461.668**
Steam diameter(mm)	58.927***	13.726***	2.327^{NS}

: p<0.05; **: p<0.01;: p<0.001. NS: not significant p>0.05**

Table 33. Analysis of variance of phenotypic traits and aflatoxin

Sources	Genotypes	F_2	F_3	Overall means
Aflatoxin(µg/kg)	G1	1.99a	2.28a	2.135a
	G2	1.99a	51.079b	26.534b
	G3	59.1b	2.643a	30.871b
	G4	6.4b	2.965a	4.683a
	G5	1.99a	7.974a	4.982a
Grain yield(t/ha)	G1	6.029c	6.7817a	6.406ab
	G2	4.439b	8.114bc	6.277ab
	G3	5.948c	7.628abc	6.788b
	G4	3.908a	7.249ab	5.579a
	G5	4.658b	8.349c	6.504ab
Ear weight(g)	G1	100.49c	113.028a	106.759ab
	G2	73.997b	134.118bc	104.058ab
	G3	99.131c	127.129abc	113.13c
	G4	65.137a	120.815ab	92.976a
	G5	77.551b	139.704c	108.627bc
Ear length (cm)	G1	16.25	14.49a	15.37a
	G2	17.444	16.456b	16.95c
	G3	17.417	15.883ab	16.649bc
	G4	16.6	14.758a	15.679ab
	G5	17.433	16.434b	16.934c
Number of rows per ear(u)	G1	36.667b	30ab	33.333b
	G2	37.389b	32.722b	35.083b
	G3	37.444b	29.50a	33.444b
	G4	31.5a	28.556a	30.028a
	G5	36.333b	30.111ab	33.222b
Number of grains per row(u)	G1	12.2778b	11.611ab	11.944b
	G2	13.111b	12b	12.556b
	G3	12.889b	12.056b	12.472b
	G4	10.056a	10.611a	10.333a
	G5	12.667b	11.50ab	12.083b
Plant height (cm)	G1	177.5a	151.056a	164.278a
	G2	189.889ab	152.5ab	171.194ab
	G3	181.222a	165.955b	173.589ab
	G4	185.722a	163.556bc	174.639ab
	G5	208.056b	160.611abc	184.333b
Steam diameter(mm)	G1	7.611a	7.989a	7.8a
	G2	7.722a	9.411b	8.5667b
	G3	8.333ab	9.255b	8.7942bc
	G4	8.333ab	9.672b	9.003bc
	G5	8.778b	10.172b	9.4750c
Male flowering days(u)	G1	46.722	47.667	47.194
	G2	45.778	45.333	45.556
	G3	48.333	46.556	47.444
	G4	46.889	46.944	46.917
	G5	48.222	45.389	46.806
Female flowering days(u)	G1	54.611b	51.611	53.111c
	G2	50.222a	51.722	50.972ab
	G3	54.167b	51.667	52.917bc
	G4	50.611a	51.167	50.889a
	G5	53.389b	49.833	51.611abc
Number of leaves per plant(u)	G1	10.278a	10.778a	10.528a
	G2	10.556a	11.444ab	11ab
	G3	10.556a	12.222b	11.389ab
	G4	11.944b	10.556a	11.25ab
	G5	12.167b	11.111ab	11.639b
Leaf angle(°)	G1	39.889abc	50.979b	42.778ab
	G2	37.167a	42.031a	40.472a
	G3	44.667c	40.698a	47.389b
	G4	42.056bc	51.122b	45.944ab
	G5	37.167ab	59.448b	41.861a
Tassel length(cm)	G1	54.611ab	45.667a	52.795b
	G2	49.167a	43.778a	45.599a
	G3	49.333a	50.111b	45.016a
	G4	58.5b	49.833b	54.811bc
	G5	55.5ab	46.556a	57.474c

G1:R4865 x Chicken corn; G2: R4865XR4543; G3: R4543xChicken corn; G4: R4777xChicken corn and G5: (R4777xR4185) XR4865. For each trait values with same letters indicate there is no significant difference for $p<0.05$ (test REGW-*F*).

Table 34. Correlation between phenotypic traits and aflatoxin recorded from tested improved maize varieties

	PH	SD	MFD	FFD	NLP	LA	TL	EL	NRE	NGR	EW	GY
SD	-0.141^{NS}											
MFD	0.046^{NS}	-0.033^{NS}										
FFD	0.045^{NS}	-0.108^{NS}	0.445***									
NLP	0.075^{NS}	0.081^{NS}	-0.092^{NS}	-0.198**								
LA	-0.298***	0.320***	-0.092^{NS}	-0.125^{NS}	0.004^{NS}							
TL	0.143^{NS}	-0.014^{NS}	0.056^{NS}	-0.080^{NS}	-0.017^{NS}	-0.105^{NS}						
EL	0.206**	-0.003^{NS}	-0.021^{NS}	-0.048^{NS}	0.043^{NS}	-0.321***	-0.043^{NS}					
NRE	0.165*	-0.074^{NS}	-0.065^{NS}	0.089^{NS}	-0.056^{NS}	-0.119^{NS}	-0.116^{NS}	0.163*				
NGR	0.294***	-0.331***	-0.021^{NS}	0.231**	-0.019^{NS}	-0.268***	-0.023^{NS}	0.296***	0.410***			
EW	-0.496***	0.402***	-0.084^{NS}	0.015^{NS}	-0.037^{NS}	0.438***	-0.242**	-0.203**	0.036^{NS}	-0.307***		
GY	-0.494***	0.403***	-0.082^{NS}	0.015^{NS}	-0.033^{NS}	0.439***	-0.248**	-0.203**	0.032^{NS}	-0.303***	0.998***	
AF	-0.113^{NS}	0.056^{NS}	0.011^{NS}	0.125^{NS}	-0.080^{NS}	0.061^{NS}	-0.241**	0.128^{NS}	0.149*	0.154*	0.199**	0.198**

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. *: p<0.05; **: p<0.01;*: p<0.001. NS: not significant p>0.05**

Chapter 2.2.4. References

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THIRD PART:

Study and Characterization of *Moringa oleifera* Cultivated in Haiti as Alternative Row Materials For Reducing Malnutrition Exposure

Summary. Malnutrition is one of the most preoccupant problems of developing countries including Haiti. A large majority of infants and pregnant women are suffering from malnutrition in Haiti. Found adequate issues for reducing exposure is one of the most important challenges for the Country. Since 1990s, internationally, scientific start to increase importance accord to *Moringa oleifera* (*M. oleifera*) for the properties of its cake seeds in water purification. In 2013 year, Haitian government, some nongovernmental organizations among all "Associazione Volontari Servizio Internazionale" (AVSI) and private institutions such as University Notre Dame of Haiti, promote *M. oleifera* leaves and derived products as alternative ingredients or basic foods against malnutrition. Campaigns of Moringa cultivation and transformation are conducted in all departments of Haiti. Moringa is planted in the capital as well as in the South, Les Cayes. Leaves and oils of Moringa are used in cosmetic industry as well as in nutritional program in Haiti. Fresh and dried Moringa leaves as well as Moringa oil are using in program aiming to reduce vitamins and mineral deficiencies of Haitian infants and pregnant women. One of the most popular in South department is MAMA+, which is a fortified flour specifically designed to fight dietary deficiency in children aged from 6 to 24 months. It was developed by AVSI Foundation, in collaboration with AVSI Canada, and in partnership with World Food Programme, and the University Notre Dame of Haiti. Conversely, since *M. oleifera* is a basic food in a large part of Haitian malnourished programs, very few scientific data are available for its genetic background, cultivation and transformation in Haiti. So, during the last two years, data relative to Moringa were documented and two reviews have been published. One was focusing on cultivation, genetic, ethnopharmacology, phytochemistry and pharmacology of *M. oleifera* leaves and the second was axed on the characteristic and utilization of Moringa seeds and oils for human health. In this PhD dissertation section focusing on the evaluation of potentiality of uses of *M. oleifera* as alternative solution against malnutrition exposure in Haiti, some fragments of texts taking from those two papers are using there and additional information relative to consumption, cultivation and genetic background of Moringa cultivated in Haiti are also summarized.

Keywords. Haiti, Malnutrition, *Moringa oleifera*

3.1. Moringa generality

In the monogeneric genus Moringa of Moringaceae family there are 13 species (namely, *M. arborea*, indigenous to Kenya; *M. rivae* indigenous to Kenya and Ethiopia; *M. borziana*, indigenous to Somalia and Kenia; *M. pygmaea* indigenous to Somalia; *M. longituba* indigenous to Kenia, Ethiopia and Somalia; *M. stenopetala* indigenous to Kenya and Ethiopia; *M ruspoliana* indigenous to Ethiopia; *M. ovalifolia* indigenous to Namibia and Angola; *M. drouhardii*, *M. hildebrandi* indigenous to Madagascar; *M. peregrine* indigenous o Red sea and Horn of Africa, *M. concanensis*, *Moringa oleifera* indigenous to sub-Himalayan tracts of Northern India (Paliwal et al.,2011), among which *M. oleifera* has so far become the most used and studied. This species is a fast growing soft wood tree that can reach 12 m in height and is indigenous to the Himalayan foothills (northern India Pakistan and Nepal) (Sharma et al.,2011). Its multiple uses and potential attracted the attention of farmers and researchers in past historical eras. Ayurvedic traditional medicine says that *M. oleifera* can prevent 300 diseases and its leaves have been exploited both for preventive and curative purposes (Ganguly, 2013). The leaves, which are rich in protein, minerals, ß-carotene and antioxidant compounds, are used not only for human and animal nutrition. Moreover, a study in the Virudhunagar district of Tamil Nadu India reports Moringa among the species utilized by traditional Siddha healers (Mutheeswaran et al., 2011). Ancient Egyptians used *M. oleifera* oil for its cosmetic value and skin preparation (Mahmood et al., 2010); even if the species never became popular among Greeks and Romans, they were aware of its medical properties (Fahey, 2005). The seeds, instead, have attracted scientific interest as *M. oleifera* seed kernels contain a significant amount of oil (up to 40%) with a high-quality fatty acid composition (oleic acid > 70%) and, after refining, a notable resistance to oxidative degradation (Anwar et al.,2005). The oil is known commercially as “Ben oil” or “Behen oil”. Its properties make it suitable for both human consumption and commercial purposes. Indeed, Moringa oil could be a good substitute for olive oil in the diet as well as for non-food applications, like biodiesel, cosmetics, and a lubricant for fine machinery. After oil extraction, the seed cake can be used in waste water treatment as a natural coagulant (Ndabigengesere and Narasiah, 1998) or as an organic fertilizer to improve agricultural productivity (Emmanuel et al., 2011). *M. oleifera* has been grown and consumed in its original areas until recently (the 1990s) when a few researchers started to study its potential

use in clarifying water treatments, while only later were its nutritional and medical properties "discovered" and the species was spread throughout almost all tropical countries. In 2001, the first international conference on *M. oleifera* was held in Tanzania and since then the number of congresses and studies increased disseminating the information about the incredible properties of *M. oleifera*. Now this species has been dubbed "miracle tree", or "natural gift", or "mother's best friend". *M. oleifera* grows in any tropical and subtropical country with peculiar environmental features, namely, dry to moist tropical or subtropical clime, with annual precipitation of 760 to 2500 mm (it requires less than 800 mm irrigation) and temperature between 18 and 28 °C. It grows in any soil type, but heavy clay and waterlogged, with pH between 4.5 and 8, at an altitude up to 2000 m (Palada, 1996; Nouman et al., 2014). A study on local uses and geographical distribution of *M. oleifera (*Popoola and Obembe, 2013) that covers the major agro-ecological region in Nigeria, clearly established that "though considered a not indigenous species, *M. oleifera* has found wide acceptance among various ethnic Nigeria, who have exploited different uses (e.g., food, medicine, fodder *etc.*). Nowadays, *M. oleifera* and its derivatives are distributed mainly in Middle East, African and Asian countries and are still spreading to other areas (Pandey et al., 2011; Sajidu et al., 2005; Tesfay et al., 2011).

3.2. Moringa cultivation

M. oleifera development is achieved in two main ways: sowing and cutting. Traditionally in Sudan the seeds are preferred while vegetative propagation is common in India, Indonesia and in some areas of West Africa (Palada, 1996). Sowing requires selection of the seeds, when they are easily available and human labor is limited, while the possibility to transplant seedlings allows flexibility in field planting even if it requires extra labor and costs. Seeds germinate within two weeks, at a maximum 2 cm depth. When sowing is planned in nursery, the seedlings can be transplanted when they reach about 30 cm (3–6 weeks after germination) (Ojiako et al., 2011). The number of seeds per kilogram ranges from 3000 to 9000, depending on the variety, with a germination rate of 80%–90% for ideal storage conditions (3 °C, 5%–8% moisture). However, the viability decreases if seeds remain at ambient temperature and high relative humidity, their germination rate dropping to 7.5% after three months (Morton, 1991). Cutting is preferred when seeds availability is scarce and/or when labor is not a limiting factor.

Ramachandran et al (1980) reports that plants raised from seeds produce fruits of poorer quality, while Animashaun et al (2013) suggest that trees grown from seeds develop longer roots (an advantage for stabilization and access to water) compare to that grown from cuttings that have much shorter roots. When hard woodcuttings (1–2 m long 4–16 cm diameter (Palada, 1996; Animashaun et al.,2013) from adult trees are planted during the rainy season burying one third in the soil, they readily develop roots that in few months reach a considerable size (Jahn et al.,1986). *M. oleifera* is an exceptionally fast growing tree, in three months it can be 3 m high and in few years reaches 12 m if it is left to growth naturally. Since the tree vigorously re-sprouts after cutting, pruning or pollarding are usually practiced to enhance lateral branching and give the tree a bush shape in order to facilitate the harvest. Nevertheless, since literature reports about the good practice management of *M. oleifera* are scant, practical trials are needed (Ojiako et al., 2011). Leaves and seeds are the parts of the plant of interest. Accordingly, the spatial distribution in planting *M. oleifera* trees are designed to facilitate the relevant harvest and the management practices. For production of leaves, *M. oleifera* plantation can be designed as follows:

(i) intensive production with spacing ranging from 10 cm × 10 cm to 20 cm × 20 cm, harvest interval between 35 to 45 days, irrigation and fertilization are needed;

(ii) semintensive production with spacing about 50 cm × 100 cm, harvest interval between 50 to 60 days, irrigation and fertilization suggested;

(iii) integrate in an agroforestry system with spacing distance of 2–4 m between rows, harvest interval around 60 days, fertilization and irrigation not strictly necessary.

Production decreases from intensive production to less dense spacing (agroforestry system), although a tremendous variability can be observed for a given spatial distribution and the same cultivation management. For example, the yield of an intensive plantation can range from 580 to 40 m/ha/year (Animashaun et al., 2013), being season dependent with the largest yield in wet or cold season. There is a need for further studies to assess optimum spacing and harvest intervals that comply with the different climates and production systems (Gadzirayi et al., 2013; Goss, 2012). Harvest can be mechanical or manual. Shoots are cut at 0.5–1 m height above the ground; but leaves can be picked directly off the tree; this practice, however, albeit quicker, leads to a less

vigorous re-growth. For the production of seed a low density plantation has a positive effect on yields: typically 2.5 × 2.5 m or 3 × 3 m triangular pattern (Sánchez et al., 2006). Fruits (trilobite capsule), referred as pods (brown color and dry and split longitudinally), ripen about three months after flowering and must be harvested as soon as possible. Each pod usually contains about 26 1-cm diameter seeds lined by three whitish papery leaflets on the edge. Like for leaves, also the production of seed shows a tremendous variability. A single tree can produce from 15 000 to 25 000 seeds with an average weight of 0.3 g per seed (Ayerza, 2011); moreover early flowering varieties produce pods in six month, while other varieties require more than one year. After pruning, branches develop new pods within 6 months (Paliwal et al., 2011).

3.3. Moringa genetic diversity and breeding

The major *M. oleifera* producer is India with an annual production of 1.1 to 1.3 million tons of tender fruits from an area of 380 km² (Rajangam et al., 2001). Information about the production in other countries is scarce. The great interest in *M. oleifera* does not concern its commercial value, being mainly related to its multipurpose uses and its ability to guarantee a reliable yield, while other crops cannot, in countries where people are mostly at risk of suffering from nutritional deficiencies. Indeed its cultivation is localized in developing countries where different parts of the plant are utilized: seeds for oil and water purification; leaves, seeds and fruits for their high nutritional value (nutritional integrator); leaves and seeds for biomass and animal feeding; different parts in traditional medicine. Moreover, Moringa has been planted around the world and is naturalized in many areas (*i.e.*, almost the entire tropical belt) increasing the variability of the species. As Moringa is a cross-pollinated tree, high heterogeneity in form and yield is expected. Several works indeed report variability in flowering (Raja et al., 2003) (from annual type to perennial type), tree nature (from deciduous to evergreen), tree shape (from semi spread to upright), resistance to hairy caterpillar (Rajangam et al., 2001; Mgendi et al., 2011), flowering time (*i.e.*, some tree flowering throughout the year while others flower in two distinct season) (Ramachandran et al., 1980). Although *M. oleifera* shows diversification in many characters and high morphological variability, which may become a resource for its improvement, the major factors that limit productivity are the absence of elite varieties adapted to local conditions and the use of seeds obtained through open pollination from plants in the planted area.

Furthermore, despite the various uses of *M. oleifera* and its morphological differentiation, the number of accessions to collections and active germplasm banks are incipient across the world.

3.4. Moringa seeds and leaves studies

3.4.1. Seeds and oil characteristics

M. oleifera seeds are globular, about 1 cm in diameter. They are three-angled, with an average weight of about 0.3 g, 3-winged with wings produced at the base of the seed to the apex 2–2.5 cm long, 0.4–0.7 cm wide; the kernel is responsible for 70%–75% of the weight (Ramachandran et al., 1980). Oil is the main component of the seed and represents 36.7% of the seed weight. The oil can be extracted almost entirely by solvent extraction, generally n-hexane, whereas less yield is obtained by cold press extraction. In fact, only 69% (on average) of the total oil contained in seeds can be extracted by cold press (Tsaknis et al., 1998; Ogunsina et al., 2014). Among rural dwellers, the edible oil is extracted by boiling de-husked seeds with water, and collecting the oil from the surface of the water (Lalas and Tsaknis, 2002). Apart from the oil, the seed has high protein content, on average 31.4%, whereas carbohydrate, fiber and ash contents are 18.4%, 7.3% and 6.2%, respectively. Thus, the defatted seeds of *M. oleifera* could provide an economical source of protein for use as a food supplement to traditional diets to increase protein intake. Furthermore, like the protein fraction, *M. oleifera* seeds have a high content of methionine and cysteine, close to that reported for milk and eggs (Oliveira et al., 1999). Therefore, they can be consumed together with legumes which are deficient in sulphur amino acids. Moreover, *M. oleifera* seeds seem to be free of trypsin inhibitor and urease activity, confirming the high protein digestibility (93%) of *M. oleifera* seeds (Oliveira et al., 1999; Santos et al., 2005). Furthermore, like the protein fraction, *M. oleifera* seeds have a high content of methionine and cysteine, close to that reported for milk and eggs (Oliveira et al., 1999). Therefore, they can be consumed together with legumes which are deficient in sulphur amino acids. Moreover, *M. oleifera* seeds seem to be free of trypsin inhibitor and urease activity, confirming the high protein digestibility (93%) of *M. oleifera* seeds (Oliveira et al., 1999; Santos et al., 2005).

Moringa oil is liquid at room temperature and golden yellow in color. The extraction method does not in any way affect the density and refractive index of the oil, and both are similar to those of olive oil (Gunstone, 2011). Instead, the smoke point is approximately 11 °C

higher than that of olive oil (Tsaknis et al.,1998; Tsaknis et al.,1999) , suggesting a greater stability during the frying process. The oil obtained by cold pressure extraction is higher in viscosity and acidity than that obtained by solvent-extraction. This higher viscosity is due to the water bound in the oil during extraction (Tsaknis et al., 1999), while the higher acidity is attributed to the water added during the milling of the seeds prior to cold pressing. Indeed, the water addition enhances the lipolytic enzyme action (Sengupta and Gupta, 1970) and prolongs the contact of the seed (milled before cold pressing) with air and temperature. Nevertheless, the acidity of the cold-pressed oil is generally moderate, indicative of its good resistance to hydrolysis. The iodine number is lower than that of olive oil as Moringa oil is less unsaturated than olive oil (Gunstone, 2011). There is a very low content of polyunsaturated fatty acids, on average 1.18%, and the content of linoleic and linolenic acids is 0.76% and 0.46%, respectively. In addition, the oil's fatty acid composition does not seem to be particularly affected by the extraction method. Only one study reported a small increment of the stearic and myristic acid content in solvent-extracted oil compared to oil obtained by cold pressure (Ogunsina et al., 2014). On the other hand, the agro-climatic characteristics of the cultivation area and the *M. oleifera* variety cultivated could be the reason for some differences in the fatty acid composition of the oil. Nevertheless, the present fatty acid composition shows that *M. oleifera* seed oil falls in the category of high-oleic oils, and contains a high monounsaturated to saturated fatty acids ratio (MUFA/SFA). The MUFA/SFA ratio is characteristic of several oils, particularly olive oil, and has been associated with a reduced risk of all-cause mortality, cardiovascular mortality, cardiovascular events, and stroke (Schwingshackl and Hoffmann, 2014). Therefore, *M. oleifera* seed oil could be an acceptable substitute for olive oil as the main dietary fat in countries where the tree grows including Haiti.

3.4.2. Leaves compounds

Several bioactive compounds were recognized in the leaves of *M. oleifera*. Vitamins, carotenoids, phenolic acids and flavonoids documented on *M. oleifera*.are presented on (Figure 20).

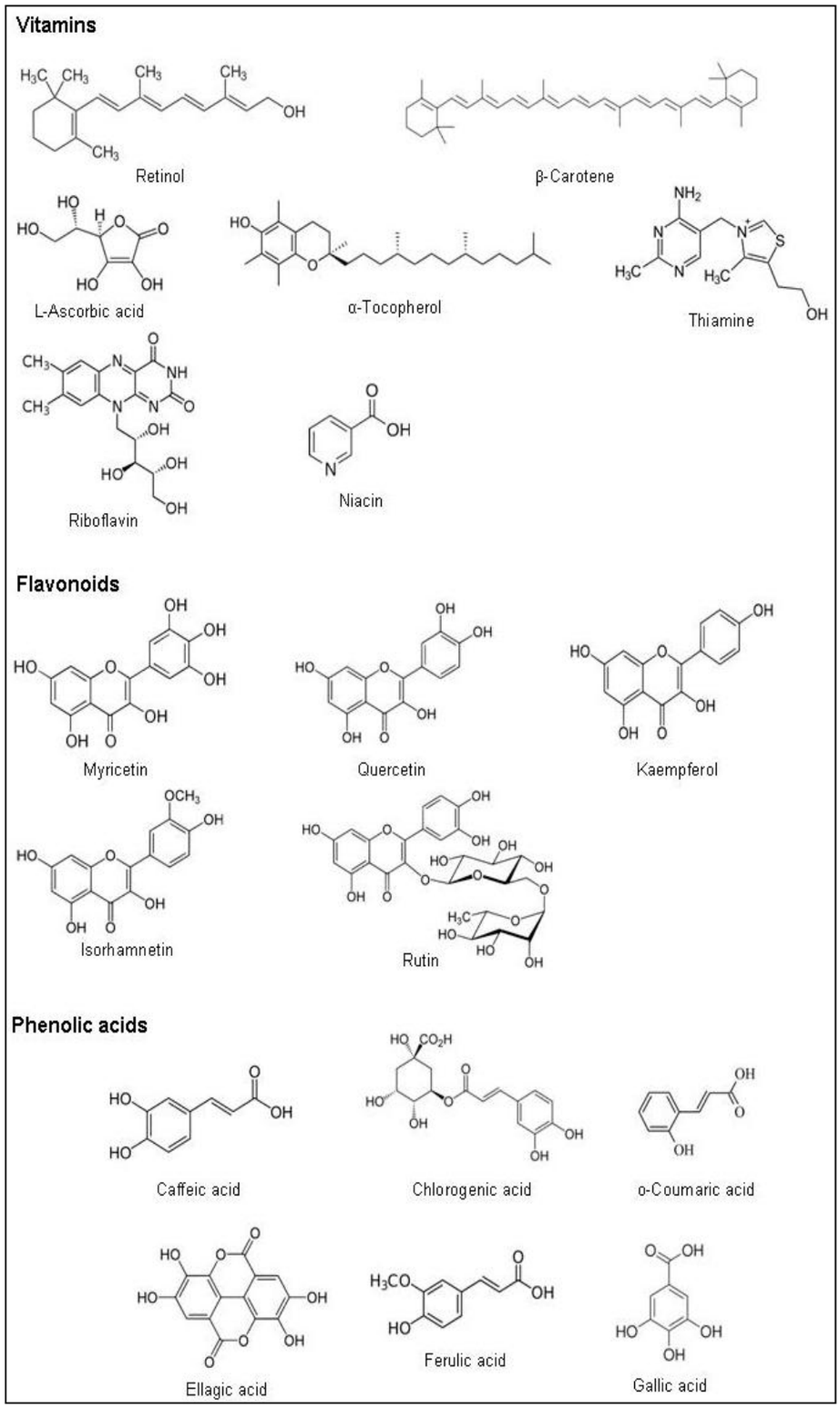

Figure 20. Chemical structure of some bioactive compounds found in *Moringa oleifera* **leaves.**

3.4.2.1. Vitamins

Fresh leaves of *M. oleifera* are reported to contain 11 300–23 000 IU of vitamin A (Ramachandran et al., 1980; Ferreira et al., 2008). Vitamin A plays key roles in many physiological processes such as vision, reproduction, embryonic growth and development, immune competence, cell differentiation, cell proliferation and apoptosis, maintenance of epithelial tissue, and brain function. Its deficiency is still prevalent in many developing countries, and considered responsible for

child and maternal mortality (Alvarez et al., 2013). Fresh leaves of *M. oleifera* are also a good source of carotenoids with pro-vitamin A action. They contain 6.6–6.8 mg/100 g (Price, 1985; Kidmose et al., 2006) of β-carotene, greater that carrots, pumpkin and apricots (6.9, 3.6 and 2.2 mg/100 g, respectively) (Salvini et al., 1997). β-carotene is more concentrated in the dried leaves, with amounts ranging from 17.6 to 39.6 mg/100 g of dry weight (DW) (Price, 1985; Joshi and Mehta, 2010). This wide range may be explained by the different environmental conditions existing among different origin countries, genetic of the plant, drying method (Joshi and Mehta, 2010) and the different extraction and analysis methods employed as well. Freeze-drying seems to be the most conservative dehydration method. In freeze-drying leaves the β-carotene content is approximately 66 mg/100 g (Zhang, 2011). *M. oleifera* is an interesting source of vitamin C. Fresh leaves contain approximately 200 mg/100 g (Ramachandran et al.,1980), greater than orange (Salvini et al.,1997). These amounts are of particular interest, as the vitamin C intervenes in the synthesis and metabolism of many compounds, like tyrosine, folic acid and tryptophan, hydroxylation of glycine, proline, lysine carnitine and catecholamine. It facilitates the conversion of cholesterol into bile acids and hence lowers blood cholesterol levels and increases the absorption of iron in the gut by reducing ferric to ferrous state. Finally, it acts as antioxidant, protecting the body from various deleterious effects of free radicals, pollutants and toxins (Chambial, 2013). However, being vitamin C sensitive to heat and oxygen, it is rapidly oxidized, so much so that its concentration in the *M. oleifera* dried leaves is lower than in the fresh leaves, dropping to 18.7 to 140 mg/100 g of DW (Iqbal and Bhanger, 2006; Price, 1985).

Difference in (i) environmental conditions in the various origin countries; (ii) genetic of the plant; (iii) drying method (Joshi and Mehta, 2010) and (iv) different extraction and analysis methods, may explain the wide range of vitamin C content in Moringa leaves reported in literature. Freeze-drying seem to better preserve vitamin C from oxidation, so much so that greater amounts of this vitamin were found in leaves undergone to freeze-drying soon after the collection. In these latter, vitamin C concentration ranges between 271 and 920 mg/100 g of DW (Siddhuraju and Becker, 2003).

M. oleifera fresh leaves are a good source of vitamin E (in particular α-tocopherol) and contain approximately 9.0 mg/100 g (Ching and Mohamed, 2001) of this compound, similarly to nuts (Salvini, 1997).

Vitamin E acts mainly as liposoluble antioxidants, but it is also involved in the modulation of gene expression, inhibition of cell proliferation, platelet aggregation, monocyte adhesion and regulation of bone mass (Borel et al., 2013). Drying procedure determines a concentration of vitamin E up to values of 74.45–122.16 mg/100 g of DW (Price, 1985). Among vitamins of group B, only thiamine, riboflavin and niacin seem present in *M. oleifera* leaves. These vitamins mainly act as cofactors of many enzymes involved in the metabolism of nutrients and energy production, and their concentration in fresh leaves ranges between 0.06 and 0.6 mg/100 g, 0.05 and 0.17 mg/100 g and 0.8 and 0.82 mg/100 g for thiamine, riboflavin and niacin, respectively (Price, 1985; Ramachandran et al., 1980), similarly to fruits and vegetable (Salvini, 1997). Only one study reported the contribution of vitamin B1, B2 and B3 of dried leaves of *M. oleifera (*Price, 1985). Their concentrations were 2.85, 22.16 and 8.86 mg/100g of DW, respectively. However, the amount of riboflavin in dried leaves seems very high compared to that of fresh leaves. Further studies are needed to confirm these values. Finally, Girija and coauthors showed an appreciable physiological availability of these three vitamins in leaves of *M. oleifera* (61.6%, 51.5% and 39.9%, respectively) (Girija et al., 1981).

3.4.2.2. Antioxidant properties

M. oleifera leaves are a rich source of antioxidant compounds (Chumark et al., 2008). Many *in vitro* studies on antioxidant activity of *M. oleifera* leaves are available in literature (Shih et al., 2011; Santos et al., 2005; Vongsak et al., 2013; Siddhuraju and Becker, 2003; Iqbal and Bhanger, 2006). Siddhuraju and Becker (2003) examined the radical scavenging capacities and antioxidant activities of the aqueous, aqueous methanol, and aqueous ethanol extracts of freeze-dried leaves of *M. oleifera* from different agro-climatic regions. The authors found that different leaves extracts inhibited 89.7%–92.0% peroxidation of linoleic acid and had a scavenging activity on superoxide radicals in a dose-dependent manner (EC50 within the range of 0.08–0.2 mg/mL, with the exception of water extract from Indian leaves which has an EC50 > 0.3 mg/mL). All of the solvent extracts of leaf samples had a very high radical scavenging activity, however better results were obtained in methanol and ethanol extracts. Both methanol and ethanol extracts of Indian origins showed the highest antioxidant activities, 65.1% and 66.8%, respectively, in the β-carotene-linoleic acid system. Nonetheless, increasing concentration of all the extracts had significantly increased reducing power (FRAP). The

authors concluded that both methanol (80%) and ethanol (70%) were the best solvents for the extraction of antioxidant compounds from *M. oleifera* leaves. Similar results were obtained by Vongsak et al (2013) who measured antioxidant activity using various extraction methods on Thailand *M. oleifera* leaves. The authors found that the extract obtained macerating dried leaves with 70% ethanol exhibited high DPPH-scavenging activity (EC50 = 62.94 g/mL) and the highest FRAP value (51.50 mmol FeSO4 equivalents/100 g extract). Iqbal and Bhanger (2006) showed that season and agroclimatic locations have profound effect on the antioxidant activity of *M. oleifera* leaves. In this study, the authors collected the leaves in the months of December, March, June, and September from five different areas of Pakistan, and found that the overall antioxidant efficacy was greater in December or March depending upon location, and least in June. The authors suggested that the environmental temperature and, most likely, the soil properties have significant effects on antioxidant activity of *M. oleifera* leaves. The effect of season on antioxidant activity of *M. oleifera* leaves was also found by Shih et al (2011) in leaves collected in Taiwan. The results of this study showed that the winter samples (DPPH-radical scavenging EC50 = 200 µg/mL) of *M. oleifera* leaves had a significant stronger antioxidant activity than summer samples (DPPH-radical scavenging EC50 = 387 µg/mL). Finally, Sreelatha and Padma (2009) evaluated the antioxidant activity of leaves collected in India in two stages of maturity. The analysis revealed only minor, but significant; differences in the two maturity stages, mature and tender leaves, for the DPPH scavenging activity (EC50 = 18.15 *vs.* 19.12 µg/mL), superoxide scavenging activity (EC50 = 12.71 *vs.* 15.51 µg/mL), nitric oxide scavenging activity (EC50 = 56.77 *vs.* 65.88 µg/mL) and for lipid peroxidation inhibition (EC50 = 25.32 *vs.* 30.15 µg/mL). Verma et al (2009*)* examinated the effects of administration of 50 and 100 mg/kg bw/day of ethylacetate/polyphenolic extract of *M. oleifera* leaves for 14 days on markers of oxidative stress in mice treated with CCl4. The authors observed that the supplementation with *M. oleifera* leaves extract in CCl4-intoxicated rats prevented the increment of lipid peroxide oxidation (LPO) levels, the decrement of glutathione (GSH) concentration and in the activities of superoxide dismutase (SOD) and catalase (CAT) antioxidant enzymes in liver and kidney compared to negative control.

Interestingly, the effects obtained in the group treated with 100 mg/kg bw/day of leaves extract were comparable to those obtained in the standard group treated with 50 mg/kg bw/day of vitamin E. Similarly, Luqman et al (2012) studied the effects of administration of increasing amounts of both aqueous and alcoholic extract of *M. oleifera* leaves on markers of oxidative stress in Swiss albino mice. The authors observed an enhancement of GSH concentration in mice erythrocytes treated with the aqueous extract of *M. oleifera* leaves. The observed rise is consistent with the increase in the dose of the extract. Similarly, the dose-responsive effect on LPO was also observed. Moreover, the basal value of malondialdehyde (MDA) concentration was maintained in case of the aqueous extract of *M. oleifera* leaves. Milder, but appreciable, results were obtained using ethanolic extract. Moyo et al (2012) studied the antioxidant properties of diet supplemented with *M. oleifera* dried leaves in goats model. The authors found a significant increment of the activity of GSH in goats supplemented with Moringa leaves as compared with the control group. In comparison, the activities of catalase (CAT) and superoxide dismutase (SOD) of diet supplemented with *M. oleifera* were increased appreciably than the goats fed with ordinary feed. Finally, the supplementation of *M. oleifera* leaves inhibited the amount of MDA generated in liver homogenate. Therefore, also the consumption of *M. oleifera* leaves could protect the animals against diseases induced by oxidative stress. Finally, the administration of *M. oleifera* leaves extract seems to prevent also the oxidative damages caused by high-fat diet. Das et al (2012) reported a lower MDA concentration, a higher GSH concentration and hepatic FRAP in mice fed with high-fat diet and supplemented with *M. oleifera* leaves extract compared to mice only treated with high-fat diet. Only one study reported the effects of *M. oleifera* leaves extract on oxidative modification of LDL using an "*ex vivo*" assay (Chumark et al., 2008). In this study, human plasma was collected and pre-incubated at 37 °C for 1 h with various concentrations of freeze-dried *M. oleifera* leaves extract (1, 10, 30 and 50 µg/mL). Control group was treated with vitamin E as standard antioxidant. Oxidation reaction of LDL was initiated by adding freshly prepared 10 µM CuSO4 to the samples. This experiment showed that in the presence of *M. oleifera* leaves extract the oxidative modifications of human plasma LDL were significantly inhibited in a dose-dependent manner. In the presence of the leaves extract at a final concentration of 1, 10, 30, and 50 g/mL, the lag-time of conjugated diene formation was increased to 45.0,

151.66, 251.67, and 340.33 min, respectively with an IC50 at 2 h of 5 µg/mL. Moreover, in the presence of *M. oleifera* leaves extract at a final concentration of 1 and 10 g/mL, the thiobarbituric acid reactive substances (TBARS, products of lipid peroxidation) formation was reduced to 104.16 and 2.80 nmol/mg LDL protein, respectively. Interestingly, the incubation with higher concentrations of the leaf extract completely blocked TBARS formation. These findings indicate that *M. oleifera* leaves extract could suppress the initiation and propagation of lipid peroxidation also in humans.

3.5. Characteristics of Moringa cultivated in Haiti

Even *M. oleifera* is used in Haiti in different forms and for multiples purposes (Figure 21), history of Moringa cultivation in Haiti is not clear. No data are available on the exact period of introduction of this plant in Haiti. Majority of Haitian people working with Moringa agreeing with the idea that this tree was started to receive importance of Haitian government since the year of 2013. Moringa is cultivated in almost all districts of Haiti. Majority of Moringa cultivated in Haiti is from subsistence traits and agroforestery. Yield of Moringa cultivated in Haiti is around 1.5 t/ha and 2-4 t/ha for leaves and seeds, respectively. More than 500 000 persons worked in Haitian Moringa food chain (APEMH, 2016). It seems that there are not articles and reviews online about Moringa in Haiti. If even not published researches on Haiti are available, some general articles and reviews mentioned data relative to Haitian *M. oleifera*. One of the most important review documented Haitian *M. oleifera* leaves was writing by (Shahzad et al., 2013). This study was focused on genetic diversity and population structure of *M. oleifera*. Samples were from trees growing in different locations representing 10 districts of Punjab province in Pakistan and, 30 accessions of *M. oleifera* from ECHO (Educational Concerns for Hunger Organization) in Florida. ECHO accessions from nine countries including different Haitian regions (see Table 35). This research gives important and constructive pictures of Haitian Moringa. Even if the aim of the research was not to focused on Haitian Moringa population it gives an appropriate idea regarding genetic background of Haitian population comparing with American continent. Results revealed that Haitian Moringa leaves samples clustered in the same ECHO collection group and were completely differing from those of Pakistan. Haitian Moringa accessions are similar to those of Florida, Mexico and Belize. So, authors had been suggested that ECHO accessions could be derived

from a common population or few populations. In addition, for explaining the narrow genetic background of Moringa, the team suggests that, an Englishman carried Moringa from Jamaica in 1784 as report by (Fawcett and Rendle, 1910). Now, Moringa is in cultivation throughout the tropics of West Indies, Bermuda (Britton, 1918) and in Latin America, including Cuba, Haiti, Jamaica, Puerto Rico, Panama, Belize, Mexico, Brazil, and Venezuela (Correll et al., 1982). Subsequently, the United States Department of Agriculture (USDA) obtained seeds from both Cuba (Bur.Pl. Indus 1915) and Nicaragua (Bur. Pl. Indus 1923), and grown them as ornamentals in South Florida as well as California.

More studies regarding Haitian Moringa population is needed since the team had not investigated on difference among the 13 Haiti samples accessions. Moringa cultivated at Les Cayes could be different from those cultivated at Port-Au-Prince, Titayen or La Gonave in term of mineral, compounds and other elements content as documented worldwide (Iqbal and Bhanger, 2006; Anwar et al., 2006). Two years later (2015 year), another study with aims to evaluate the nutritional proprieties and phenolic profiling of *M. oleifera* leaves grown in Chad, Sahrawi Refugee Camps, and Haiti was carried out by (Leone et al., 2015). Samples from Haiti were collected in the area around Port-au-Prince. All samples were subjected to proximate analysis, trolox equivalent antioxidant capacity (TEAC), total phenols, and Salicylic (SA) and Ferulic Acids (FA) contents and phenolic profile were determined three times (Tables 36 and 37). Comparing Haitian Moringa results with others two places, some similarity traits were mentioned by the authors. Yet, regarding protein content, with around 20.80g/100g of dried mater, Haitian Moringa leaves were poor compared with those of Chad and Sahrawi camps, but similar to data reported in the leaves from Mexico, Niger, and Thailand (Jongrungruangchok et al., 2010; Sánchez-Machado et al., 2010). The total amount of crude proteins found in Haitian Moringa leaves is greater compared to 6.70 and 16.70 reported by (Elkhalifa et al., 2007; Verma et al., 1976). Also significant difference was detected among Moringa leaves samples regarding total fiber. The Haitian leaves were richer in fiber compared to the other two places. These amounts are similar to the fiber content of *M. oleifera* leaves collected in Mexico (Sánchez-Machado et al., 2010), but greater than amounts found in the leaves collected in other countries (Jongrungruangchok et al., 2010; Yaméogo el al.,2011; Oduro et al.,2008).

It was observe similarity in term of protein content and total fiber of Moringa leaves from Mexico and Haiti. Those similarities could be explained by the climate or in some extent by the genetic improvement. Genetic similarity between Moringa cultivated in Haiti and Mexico was already mentioned (Shahzad et al., 2013). Sugar content was very different among the samples. It was higher in the leaves collected in the Sahrawi camps, while the leaves collected in Haiti were the richest in glucose and fructose. Haitian sugar content of Moringa leaves is greater compared with that reported by (Yaméogo et al., 2011). The predominant minerals in the leaves were calcium and magnesium. The sodium content found in the leaves collected in Haiti and Chad was similar to the amounts reported in previous studies (Sena et al., 1998; Aslam et al., 2005). The iron content found in the leaves collected in the Sahrawi camps was in agreement with the amounts found in the leaves collected in some provinces of Pakistan and Thailand (Aslam et al.,2005; Jongrungruangchok et al., 2010), while the iron content of the leaves collected in Haiti and Chad was about three- to four-fold lower. Finally the total amount of calcium quantified by the team from Haitian Moringa leaves is quite similar to (Verma et al., 1976) but greater than that reported by (Aslam et al., 2005).

Samples presented detectable amounts of salicylic and ferulic acids i.e. leaves collected in Haiti were richer in both salicylic and ferulic acids compared with Chad and Sahrawi camps. Salicylic acid concentration found in *M. oleifera* leaves was comparable with amounts presented in nectarine, pineapple, tomato, and asparagus, but higher than amounts found in other fruit and vegetables (Wood et al., 2011). Only spices present a concentration of salicylic acid 10-fold higher (Wood et al., 2011). Similarly, ferulic acid amounts found in *M. oleifera* leaves were comparable with the amounts found in several fruits and vegetables, such as orange, eggplant, spinach, red cabbage, and peanuts, but widely lower than amounts found in cereals (Zhao and Moghadasian, 2008). These findings confirm that leaves of *M. oleifera* could be used as functional food in fight against malnutrition in Haiti (Abbaspour et al., 2014; Wilhelm et al., 2014; Kirkpatrick et al., 2002; Ayoya et al., 2013).

3.6. Perspectives relative to Moringa cultivated in Haiti

M. oleifera tree is cultivated in almost all tropical countries including Haiti where malnutrition is an important issue. Climate condition (rainfall and temperature) are suitable for Moringa grown all year long in all districts. Since Haiti has, less than 2% of vegetal cover, this species

could be utilized for reforestation and the use of leaves and fruits as functional food are strongly suggested, even if to improve Moringa production and properties more studies on its cultivation practices and genetic diversity are needed.

Table 35. List of 13 Haitian Moringa oleifera accessions along with their corresponding origin and accession IDs, obtained from ECHO (Educational Concerns for Hunger Organization), Florida, USA

Code no.	Accession #	Source
1	18 01046	011A K. Flanagan, Haiti
2	19 02055	021H Bohoc, Haiti
3	20 02073	021H La Gonave, Haiti H
4	21 02057	021H Les Cayes, Haiti
5	22 02056	021H Port Au Prince, Haiti
6	23 03064	031H C. Thede, Haiti
7	24 03065	031H C. Thede, Haiti
8	25 03067	031H C. Thede, Haiti
9	26 03068	031H C. Thede, Haiti
10	27 03069	031H C. Thede, Haiti
11	28 03070	031H Archai, Haiti H
12	29 03071	031H C. Thede, Haiti
13	30 02058	021H Titayen, Haiti

Table 36. Nutritional characterization of *M. oleifera* leaves from Haiti, expressed as dry matter

Nutrients		HAITI
		Mean ± sd
Proteins	g/100 g	20.80 ± 0.01
Lipids	g/100 g	7.05 ± 0.11
Total fibre	g/100 g	37.63 ± 1.00
Insoluble fibre	g/100 g	30.09 ± 1.40
Solubre fibre	g/100 g	7.54 ± 0.40
Starch (*estimated by difference*)	g/100 g	13.75 ± 0.31
Glucose	g/100 g	4.57 ± 0.16
Fructose	g/100 g	4.81 ± 0.31
Sucrose	g/100 g	1.77 ± 0.08
Maltose	g/100 g	ND
Ashes	g/100 g	9.62 ± 0.02
Sodium	mg/100 g	262.50 ± 5.45
Calcium	mg/100 g	2150.26 ± 56.07
Iron	mg/100 g	11.91 ± 0.82
Zinc	mg/100 g	2.18 ± 0.06
Magnesium	mg/100 g	533.51 ± 23.87
Copper	mg/100 g	0.66 ± 0.00
Phytates	g/100 g	2.55 ± 0.19
β-carotene	mg/100 g	10.01 ± 0.07

Table 37. Bioactive compounds content of *M. oleifera* leaves grown in Haiti, expressed as dry matter

Compounds		HAITI
		Mean ± sd
TEAC	µmol trolox/g	335.61 ± 7.37
Total polyphenols	g/100 g	2545 ± 194
Salicylic acid	g/100 g	0.33 ± 0.04
Ferulic acid	g/100 g	9.69 ± 0.26

Figure 21. Transformation and utilization of Moringa in Haiti.

3.7. References

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Annexes

Annex A

UNIVERSITY NOTRE DAME OF HAITI SOIL ANALISYS

pH $_{H2O}$		7.97	**Limits Italian Law n. 152/06***
Cation Exchangeable Capacity	(cmol^{+} kg DM^{-1})	32.26	
Organic Carbon	(% DM)	2.46	
Organic Matter		4.24	
Total Nitrogen		0.26	
C/N ratio		9.46	
P_2O_5 Olsen	(mg kg DM^{-1})	171	
Sand	(% DM)	31.24	
Silt		43.84	
Clay		24.92	
Texture (USDA)		Loamy	
As	(mg kg DM^{-1})	12.9	20
Cd		0.21	2
Co		18.5	20
Cr		137.8	150
Cu		51.9	120
Ni		80.9	120
Pb		32.1	100
Zn		144.8	150

*for heavy metals

Comments

The soil has a balanced texture. For USDA classification it is a loamy soil. The pH is slightly alkaline. The organic matter content is very good. The Cation Exchangeable Capacity is very high, in agreement with organic matter and clay content. The C/N ratio is equilibrate. The phosphorus content is high.

It's recommended to limit the addition of nutrients only to the removal by the crops that will be practiced. The content of heavy metals is below to the specified limits.