

Genetic Variability of Sugarcane Clones as Affected by Endemic Diseases Under Natural Infection at One-Row Screening Stage in Ferké, Northern Ivory Coast

Comment [F1]: Not necessary here. It is instead making the title look so long. Just capture it in materials and methods

Abstract

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The overall study objective was to contribute to sugarcane yield improvement in Ivory Coast. Specific objective was to evaluate the diversity of susceptible sugarcane genotypes mainly in first ratoon crop to three major endemic diseases under natural infection, namely leaf scald, smut and pokkah boeng. The study was carried out over 2 seasons (2016-18) as plant and first ratoon cane at Ferké 1 experimental station under full covering sprinkler irrigation in northern Ivory Coast. Treatments were composed of 863 sugarcane genotypes split into 39 families planted at single row density. Planting was done per genotype in rows of 3 m long depending on families, without replication and compared to the check variety SP70/1006. That check was replicated every five rows to ease comparison with the clones. Phytosanitary observations regarding the three endemic diseases made at the age of five months were subjected to a series of multivariate analyses. The study showed that most relevant diseases determining the diversity of susceptible sugarcane genotypes were, in descending order, pokkah boeng, smut and leaf scald. Increase in clone infestations on first ratoon cane compared with plant cane was observed regarding the three endemic diseases but more importantly for smut by 51 %. The dendrogram deduced from cluster analysis showed that infected genotypes were split into six groups with same families belonging often to different clusters so that no family investigated specifically susceptible or resistant to any disease was determined. In other words, each family investigated was composed of disease-free as well as susceptible genotypes in certain proportion which varied from one family to another. Therefore, all families investigated were relevant to maintain the diversity required for the breeding process underway.

Key words: leaf scald, smut, pokkah boeng, susceptibility, resistance, agro-ecology, multivariate analysis,

1. Introduction

Sugarcane is a major commercial crop grown in tropical and subtropical regions of the world, including West and Central Africa. During the last 100 years, many sugarcane producing countries such as Mauritius, Reunion Island, Java-Indonesia, New Guinea, India and South Africa, have experienced epidemics of various diseases like red rot, smut, wilt, rust leaf scald gummosis and yellow leaf (Viswanathan and Rao, 2011; Viswanathan *et al.*, 2011; Singh *et al.*, 2011). The damage caused during each epidemic would vary depending on the nature of disease and spread of affected varieties. Number of sugarcane varieties were replaced because of their breakdown to new diseases or to new pathogenic strains. Propagation of sugarcane through vegetative cuttings enhances spread of diseases through planting materials. Primary transmission of diseases by seed canes causes a serious threat to sugarcane growth and yields. Therefore, disease resistant varieties play a key role in controlling numerous biotic constraints in sugarcane and several varieties were developed to manage diseases in the past. In parallel, different agronomic practices and physical methods like hot water therapy are being effectively used to control diseases transmission in sugarcane. More recently, propagation of sugarcane through tissue culture is being used in some advanced countries to produce virus, phytoplasma and bacteria disease-free planting materials. Use of disease resistant or tolerant varieties along with healthy seed nursery programs would form the basis to successfully manage diseases in sugarcane. Across West and Central Africa, smut, leaf scald and pokkah boeng used to be considered as endemic diseases in sugarcane. The first two (smut and leaf scald) were revealed economically important compared to pokkah boeng. Still, severe symptoms of pokkah boeng with top-rotting damages were often observed on highly susceptible cane genotypes which needed to be identified and eliminated in the crop breeding process. That's why the three diseases used to be considered as one of the key criteria in variety selection of sugarcane carried out in Ivory Coast (Zadi *et al.*, 2016; Péné *et al.*, 2016). Pokkah boeng is a fungus caused by *Fusarium moliniforme*. Injury varies from slight chlorosis and splitting of the base of young unfolding leaves to top rotting, which may kill the growing point. While common in certain susceptible varieties during warm and rainy weather, it is seldom of commercial importance. Leaf scald, caused by a bacterium (*Xanthomonas albilineans*), is considered among sugarcane major diseases and therefore of commercial importance. It is primarily a vascular disease with streaks produced on cane leaves. Sometimes, instead of definite stripes, the entire shoot is chlorotic to nearly white. Diseased plants have a characteristic stunted appearance and the terminal whorl of leaves curves inward at the tips, which are often dried or withered (Rott, 1995). Sprouting of the lateral buds beginning at the base of stalk is characteristic and may occur when there is no

apparent injury to the top. In the acute stage, some shoots or the entire stool may suddenly wilt and die (Rott, 2000). The disease causes marked reductions in growth, tillering and ratooning ability of susceptible varieties. It is highly infectious and spreads through infected seed cane, knife cuts and probably by other means of physical contact (Royer *et al*, 2018). However, aerial transmission and epiphytic survival have also been reported for this pathogen (Autrey, 1992; Daugrois 2003; Champoiseau, 2009). Smut, caused by a fungus namely *Ustilago scitaminea* or *Sporisorium scitamineum* is characterized by the production from the growing point of a long whiplike shoot (Piepenbring *et al*, 2002; Que *et al*, 2014). Smut teliospores are scattered when the membrane covering this shoot bursts and carried by wind or rain/irrigation water (Sundar *et al*, 2012). Infection takes place through seed pieces and through axillary buds of the growing plant. Germination of buds from infected cuttings may be seriously reduced, the plant is stunted and ratooning ability is weakened (Peters *et al*, 2017). Managing these endemic diseases through variety selection in the targeted African region is of crucial importance regarding the context of growing interest for early stage crop improvement. That breeding program is based on cross hybridization and selection of genotypes at early stages where numerous plant materials investigated are often highly susceptible (Béhou *et al*, 2018). Having knowledge of crosses responsible for susceptible or highly susceptible genotypes would help in the choice of parental material for location-oriented hybridizations.

The aim of the study was to characterize the genetic variability of susceptible sugarcane clones mainly in first ratoon crop to three endemic diseases at one-row screening stage, namely smut, leaf scald and Pokkah Boeng.

2. Material and methods

Site characteristics

The study was carried out at Ferké 1 experimental station in northern Ivory Coast (9°20' – 9°60' N, 5°22' – 5°40' W, 325 m). Prevailing climate is tropical dry with two seasons: one is dry which occurs from early November to April and the other, wet, from May to late October. The dry season is marked by a northern and warm trade wind (*Harmattan*) taking place from mid-November to late January. Rainfall pattern is unimodal and centered over August and September which totalize almost half of annual average rainfall (1 200 mm) with an average daily temperature of 27 °C, maximum and minimum values yielding 32.5 and 21.0 °C, respectively. Irrigation water requirements for sugarcane growth and yield performance to

about 650-700 mm/yr (Péné *et al*, 2013; Konan *et al*, 2017a,b). Main soil units (ferralsol or hydromorphic type) are characterized by shallow to moderate depths (30-80 cm) with sand-clay as predominant soil texture where the experiment was located.

Sugarcane crop material

The crop material investigated which comprised 863 sugarcane genotypes, was grown over two years as plant crop and first ratoon at one-row screening stage. All clones were planted with stem cuttings following families and compared to a check commercial variety (SP70-1006) which was moderately susceptible to smut and resistant to leaf scald and pokkah boeng. Genotypes derived from the second generation of sugarcane hybrid seeds were provided in November 2015 by Reunion Island sugarcane breeding center (eRcane). They resulted from bi-parental crosses of commercial or elite varieties of diversified origins (Reunion, Brazil, Australia, Sudan, Florida, Colombia, South Africa, etc.).

Experimental design

The experimental design used at one-row screening stage was a randomized block design comprising 863 clones, each planted in a single-row plot of 3 m long without replication apart from the check variety. That one was replicated many times (173) every 5 rows of clones subjected to visual screening. Clones split into 39 families (or crosses) as well as the check variety were planted separately in single row plots with 1.5 m between-row spacing (4.5 m²/plot) in November 2016 following 11 blocks of 7 m wide and 30 m long with 3 m spacing. Within each block, there was a 1 m spacing between adjacent clones to allow distinction of individual clones during disease ratings, growth vigor evaluation and selection. To prevent edge effects the field trial was surrounded by a buffer zone of 3 m wide and 30 m long planted with a commercial variety (R579) rather resistant to the three diseases.

Epidemic disease observations

Symptoms, based on natural infection of smut, leaf scald and pokkah boeng as epidemic diseases with high pressure in West and Central African agro-ecology, were observed on sugarcane genotypes at the age of five months. Disease ratings were based on percentage of cane shoots or stools infected by fungus or leaf scald.

Comment [F2]: Is it randomised complete block or incomplete block ? How many blocks?

Comment [F3]: Of the 11 blocks, each block had how many single row plots? OR Were the 40 families (inclusive of the check) repeated in each of the 11 blocks ?? Please clearly clarify this so that we can know whether this was a RCBD or IBD otherwise the readers will be left guessing

The susceptibility scale of smut was provided by Rao et al (1996) as follows: Free of symptom (0 %); Resistant (0.1 - 5 %); Moderately resistant (5.1 - 15 %); Moderately susceptible (15.1 - 30 %); Susceptible (> 30 %).

That of pokkah boeng was provided by Gulya *et al* (1980) as follows: Free of symptom (0 %); Resistant (0.1 - 1 %); Moderately resistant (1.1 - 10 %); Moderately susceptible (10.1 - 25 %); Susceptible (25.1 - 50 %); Very susceptible (50.1 - 100 %).

The leaf scald incidence scale as described by Rott et al (1997) is the following: Free of symptom (0 %); Susceptible (0.1 - 10 %); Very susceptible (11 - 100 %).

Statistical analysis

Data processing was conducted using Excel 2013, Statistica 7.1 and R 2.2 software packages which was based on clone phenotypic traits observed in the experiment. To do so, data were firstly recorded as a database and processed on Excel following a dynamic crossed table. Percentage of disease infestations and qualitative assessment of infestations (ratings) were used in data processing. A series of 3 multivariate analyses using R software, i.e. principal component analysis (PCA), cluster analysis (CA) and corresponding factor analysis (CFA), were made. The data were computed in application of Mahalanobis D^2 statistics among all possible combinations of genotypes grouped into different clusters following canonical root method described by Rao (1952).

3. Results

Estimates of disease free or infected clones in plant and first ratoon cane

Clone phytosanitary profile regarding the three endemic diseases ~~which-whose~~ symptoms were observed is shown in Table 1. As shown in Figure 1, number of disease-free genotypes in plant cane decreased significantly ($P<0.05$) from leaf scald to smut and pokkah boeng with, respectively, 98, 86 and 80%. Number of naturally infected genotypes increased significantly ($P<0.05$) in first ratoon cane for the three diseases compared to plant cane, with 13.4, 65 and 39.5 %, respectively, for leaf scald, smut and pokkah boeng. Smut was the most infectious disease in first ratoon cane whereas leaf scald was the less infectious one with, respectively, 50 and 12-% increase compared to that of plant cane.

Table 1. Phytosanitary profile under natural infection of sugarcane genotypes in plant cane and first ratoon at one-row stage in Ferké (Ivory Coast).

Comment [F4]: And what deduction can you make from Table 1 ?

Families	Parents		Leaf scald				Smut				Pokkah boeng				Total
			Heathy		Infected		Heathy		Infected		Heathy		Infected		
	Female	Male	R0	R1	R0	R1	R0	R1	R0	R1	R0	R1	R0	R1	
F01	R98/4009	R95/4065	14	12	1	3	10	0	5	15	10	3	5	12	15
F02	N42	R96/6422	4	4	0	0	4	3	0	1	3	2	1	2	4
F03	R92/2401	R97/6375	42	33	0	9	39	16	3	26	32	29	10	13	42
F04	NCo 310	R99/6153	28	27	0	1	28	13	0	15	24	19	4	9	28
F05	R03/4018 (e)	R04/8052	17	17	0	0	17	8	0	9	16	15	1	2	17
F06	R01/0277	R95/2100	27	23	0	4	26	14	1	13	21	13	6	14	27
F07	RB83/5486 (e)	R575	160	137	1	24	142	44	19	117	134	127	27	34	161
F08	R81/0833	SP70/1143	12	8	2	6	13	2	1	12	13	12	1	2	14
F09	N14 (e)	R585	45	45	1	1	41	11	5	35	36	30	10	16	46
F10	H32/8560	R585	52	41	3	14	30	6	25	49	39	40	16	15	55
F11	R03/4018 (e)	N14	39	36	2	5	26	9	15	32	36	35	5	6	41
F12	R584	R99/6153	19	17	0	2	17	13	2	6	17	16	2	3	19
F13	M1042/86	PR83/1248	17	17	0	0	15	10	2	7	14	13	3	4	17
F14	R98/0814	R585	13	13	1	1	13	6	1	8	13	11	1	3	14
F15	R96/2569	R585	13	12	1	2	14	7	0	7	8	12	6	2	14
F16	H72/8597 (e)	R585	24	22	2	4	19	10	7	16	19	19	7	7	26
F17	R582	R585	20	18	0	2	18	6	2	14	14	16	6	4	20
F18	R83/0444	N14	5	4	0	1	5	1	0	4	2	5	3	0	5
F19	R575	N6	4	2	0	2	4	1	0	3	4	4	0	0	4
F20	R575	CP81/1384	6	6	0	0	6	4	0	2	6	4	0	2	6
F21	R93/2351	R99/6254	17	13	0	4	15	9	2	8	15	13	2	4	17
F22	R90/2992	R97/2332	3	2	0	1	3	1	0	2	3	2	0	1	3
F23	R579	R92/0804	6	5	0	1	6	4	0	2	6	4	0	2	6
F24	R91/4188	R00/2460	10	10	0	0	8	0	2	10	6	8	4	2	10
F25	R94/6113	R93/6769	16	4	0	12	15	5	1	11	15	11	1	5	16
F26	R92/6545	R93/6683	11	8	0	3	11	6	0	5	5	5	6	6	11
F27	R96/2569	R97/2332	2	2	0	0	1	1	1	1	2	1	0	1	2
F28	R582	R570	35	30	0	5	34	20	1	15	31	25	4	10	35
F29	R01/2072	VMC71/238	7	6	0	1	6	3	1	4	5	7	2	0	7
F30	R93/0136	R00/2460	14	13	0	1	13	4	1	10	10	12	4	2	14
F31	R89/2042	R97/2332	10	9	0	1	9	6	1	4	7	10	3	0	10
F32	R11/7003	N27	2	2	0	0	2	2	0	0	2	2	0	0	2
F33	R93/0136	SP80/3280	2	1	0	1	1	0	1	2	2	1	0	1	2
F34	R579	R94/6447	12	11	0	1	12	6	0	6	10	2	2	10	12
F35	R98/6095	HoCP85/845	10	10	0	0	10	2	0	8	8	8	2	2	10
F36	TC9	R95/4065	33	33	0	0	32	19	1	14	26	21	7	12	33
F37	R00/4009	R95/4053	43	41	0	2	32	9	11	34	31	17	12	26	43
F38	R98/4009	R98/4001	28	28	0	0	25	16	3	12	20	18	8	10	28
F39	RB83/5054	R97/2335	26	25	1	2	19	4	8	23	24	16	3	11	27
Total			848	747	15	116	741	301	122	562	689	608	174	255	863

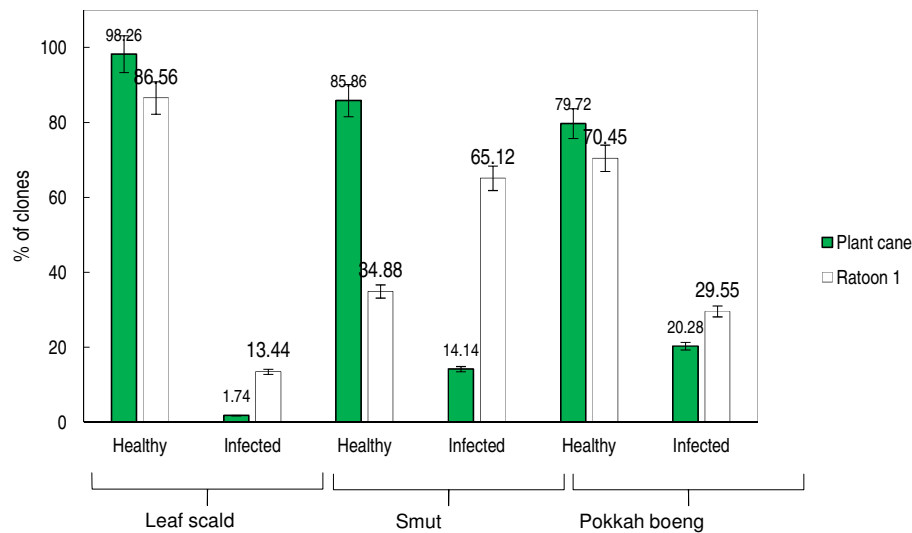


Figure 1. Percentage of disease free and naturally infected clones depending on endemic disease observed in plant cane and first ratoon in Ferké 1, Ivory Coast. Leaf scald (healthy: n = 848, 747; infected: n = 15, 116); Smut (healthy: n = 741, 301; infected: n = 122, 562); Pokkah boeng (healthy: n = 689, 608; infected: n = 174, 255).

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Clone susceptibility to endemic diseases in first ratoon

The number of disease free genotypes in first ratoon cane was significantly high ($P < 0.05$) regarding leaf scald and pokkah boeng with, respectively, 87 and 70 % as opposed to low (35 %) for smut. About 50 and 28-% of cane genotypes were resistant or moderately resistant to smut and pokkah boeng, respectively (Figure 2). In contrast, 13.4, 16.5 and 0.1-% of genotypes were susceptible or highly susceptible to leaf scald, smut and pokkah boeng, respectively. This shows the economic importance of leaf scald and smut.

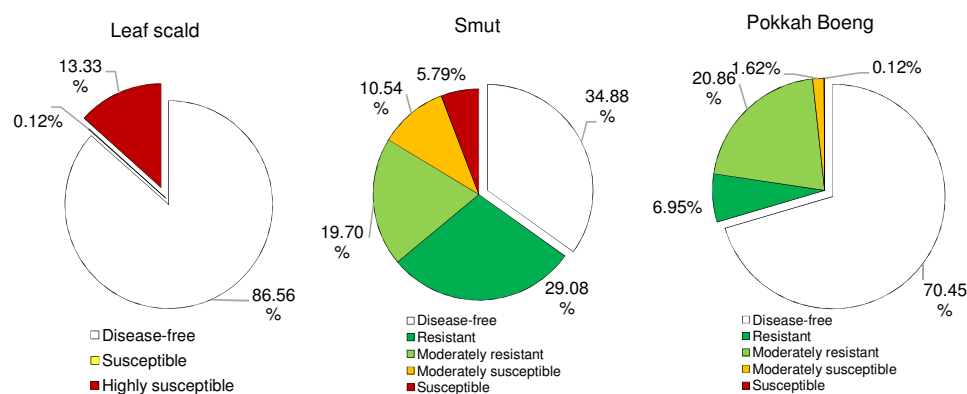


Figure 2. Distribution of sugarcane genotypes in first ratoon crop following their susceptibility to each of the three endemic diseases observed under natural infection at one-row screening stage in Ferké 1, Ivory Coast.

Clone susceptibility in first ratoon depending on endemic diseases observed

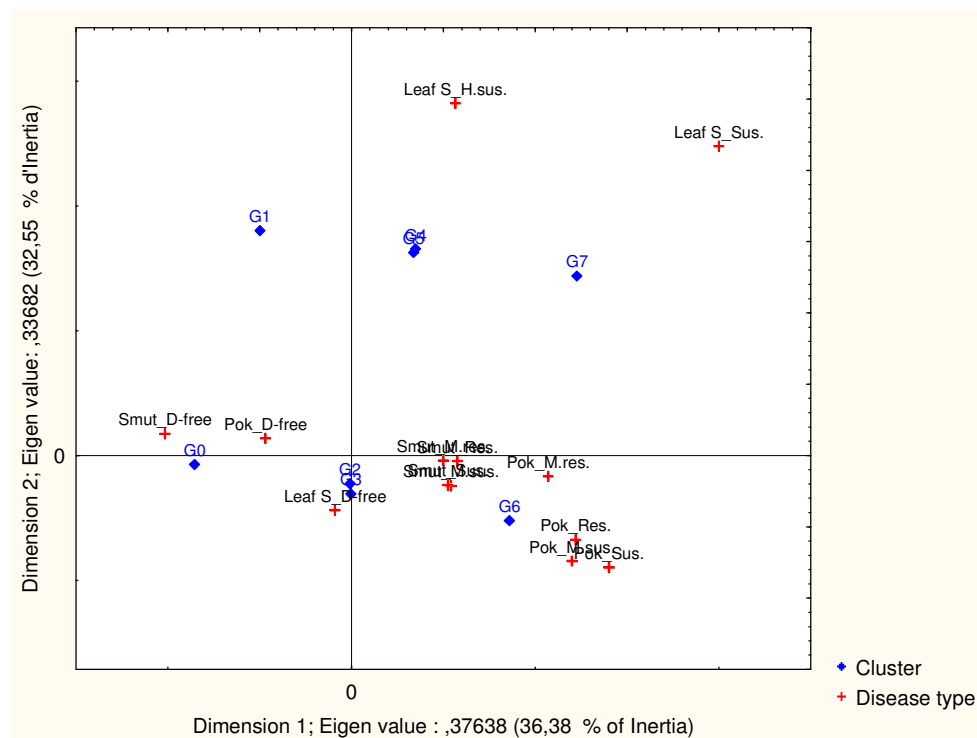
In first ratoon cane, all genotypes studied were split into ~~8-eight~~ different groups depending on endemic disease infestations observed (Table 2). Group G0 was composed of 209 disease-free sugarcane genotypes (24-%) split into 34 families out of 39 (87-%). Groups G1, G2 and G3 were composed of genotypes infected only by leaf scald, smut and pokkah boeng, respectively. Smut was the most infectious disease with a rate of 37-% corresponding to 319 genotypes split into 34 families out of 39 (87 %). Leaf scald was the less infectious one with a rate of 3-% involving 25 genotypes split into 15 families. Infestation rate of pokkah boeng alone gave 6.5-% corresponding to 56 genotypes split into 22 families. Group 7 was composed of genotypes infected by the three endemic diseases with a rate of 3 % which corresponded to 25 clones split into 15 families out of 39 (38.5 %).

~~As shown in Figure 3,~~ Groups G1, G4, G5 and G7 were associated with genotypes susceptible or highly susceptible to leaf scald whereas G0, G2, G3 were associated with disease-free genotypes (Fig. 3). Group G6 was associated with genotypes resistant, moderately resistant or moderately susceptible to pokkah boeng and smut.

187 Table 2. Grouping of sugarcane clones following their susceptibility or not to endemic
 188 diseases observed under natural infection at one-row stage in first ratoon, Ferké 1 (Ivory
 189 Coast).

Group	Characteristics	Number	Families represented (Number of clones)
G0	Disease free clones	209	F02 (2), F03 (12), F04 (10), F05 (7), F06 (9), F07 (34), F08 (1), F09 (8), F10 (2), F11 (7), F12 (9), F13 (10), F14 (6), F15 (6), F16 (7), F17 (5), F18 (1), F20 (3), F21 (3), F22 (1), F23 (3), F25 (2), F26 (3), F27 (1), F28 (13), F29 (2), F30 (3), F31 (5), F32 (2), F35 (1), F36 (12), F37 (5), F38 (11), F39 (3)
G1	Infected clones only by leaf scald	25	F03 (2), F07 (6), F08 (1), F10 (1), F11 (2), F12 (2), F15 (1), F19 (1), F21 (3), F25 (1), F26 (1), F28 (1), F29 (1), F31 (1), F39 (1)
G2	Infected clones only by smut	319	F01 (1), F03 (11), F04 (9), F05 (8), F06 (4), F07 (75), F08 (6), F09 (21), F10 (27), F11 (24), F12 (5), F13 (3), F14 (5), F15 (4), F16 (10), F17 (9), F18 (3), F19 (2), F20 (1), F21 (7), F23 (1), F24 (8), F25 (1), F28 (9), F29 (4), F30 (8), F31 (4), F33 (1), F34 (2), F35 (7), F36 (9), F37 (12), F38 (7), F39 (11)
G3	Infected clones only by pokkah boeng	56	F02 (1), F03 (1), F04 (3), F05 (1), F06 (3), F07 (2), F09 (3), F10 (3), F12 (2), F16 (1), F17 (1), F20 (1), F21 (3), F25 (1), F26 (2), F28 (4), F30 (1), F34 (6), F35 (1), F36 (7), F37 (4), F38 (5)
G4	Infected clones by leaf scald and smut	55	F01 (2), F03 (4), F07 (12), F08 (4), F09 (1), F10 (10), F11 (2), F15 (1), F16 (2), F17 (2), F18 (1), F19 (1), F22 (1), F25 (7), F26 (1), F28 (2), F30 (1), F39 (1)
G5	Infected clones by leaf scald and pokkah boeng	11	F03 (1), F06 (2), F07 (2), F16 (2), F23 (1), F25 (1), F28 (2)
G6	Infected clones by smut and pokkah boeng	163	F01 (11), F02 (1), F03 (9), F04 (5), F05 (1), F06 (7), F07 (26), F08 (1), F09 (13), F10 (9), F11 (5), F12 (1), F13 (4), F14 (2), F15 (2), F16 (4), F17 (3), F20 (1), F22 (1), F23 (1), F24 (2), F26 (3), F27 (1), F28 (4), F30 (1), F34 (3), F35 (1), F36 (5), F37 (20), F38 (5), F39 (11)
G7	Infected clones by the three endemic diseases	25	F01 (1), F03 (2), F04 (1), F06 (2), F07 (4), F08 (1), F10 (3), F11 (1), F14 (1), F21 (1), F25 (3), F26 (1), F33 (1), F34 (1), F37 (2)

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193 Figure 3. Projection of groups of sugarcane genotypes in 1-2 factor plane following
 194 correspondence factor analysis (CFA) based on variance of disease susceptibility observed
 195 under natural infection in first ratoon cane, Ferké 1 (Ivory Coast).

Comment [F6]: This detail should go to materials and methods

196 Cluster analysis of naturally infected cane genotypes

197 The dendrogram deduced from cluster analysis showed that infected genotypes were split into
 198 six groups (Figure 4, Table 3) with same families belonging often to different clusters so that
 199 no family specifically susceptible or resistant to any disease investigated was determined. In
 200 other words, each family investigated was composed of disease-free as well as susceptible
 201 genotypes in a certain proportion which varied from one family to another. Therefore, all
 202 families investigated were relevant to maintain the diversity required for the breeding process
 203 underway.

204 Mahalanobis square distance between clusters displayed in Table 4 shows that they were
 205 significantly different from one another ($P < 0.00001$). Cluster C5, as the most prolific of
 206 infected genotypes, was composed of 286 clones (33 %) split into 36 families over 39 (92 %).

Comment [F7]: $P < 0.001$ is just enough and smarter to show very highly significant

It was followed by clusters C6 and C1 with 125 and 93 infected genotypes split into 29 and 28 families, respectively. Clusters C2, C3 and C4 were the less prolific, with 42, 52 and 56 infected genotypes split into 14, 23 and 21 families, respectively. Means as well as coefficients of variation regarding all clusters were given in Table 5. Extremely high values of coefficient of variation obtained were in line with the high diversity in clone susceptibility or resistance to disease observed with clusters determined (Table 5). It is particularly the case for clusters 1 and 2, cluster 3 and 4 all six clusters regarding, respectively, leaf scald, smut and pokkah boeng.

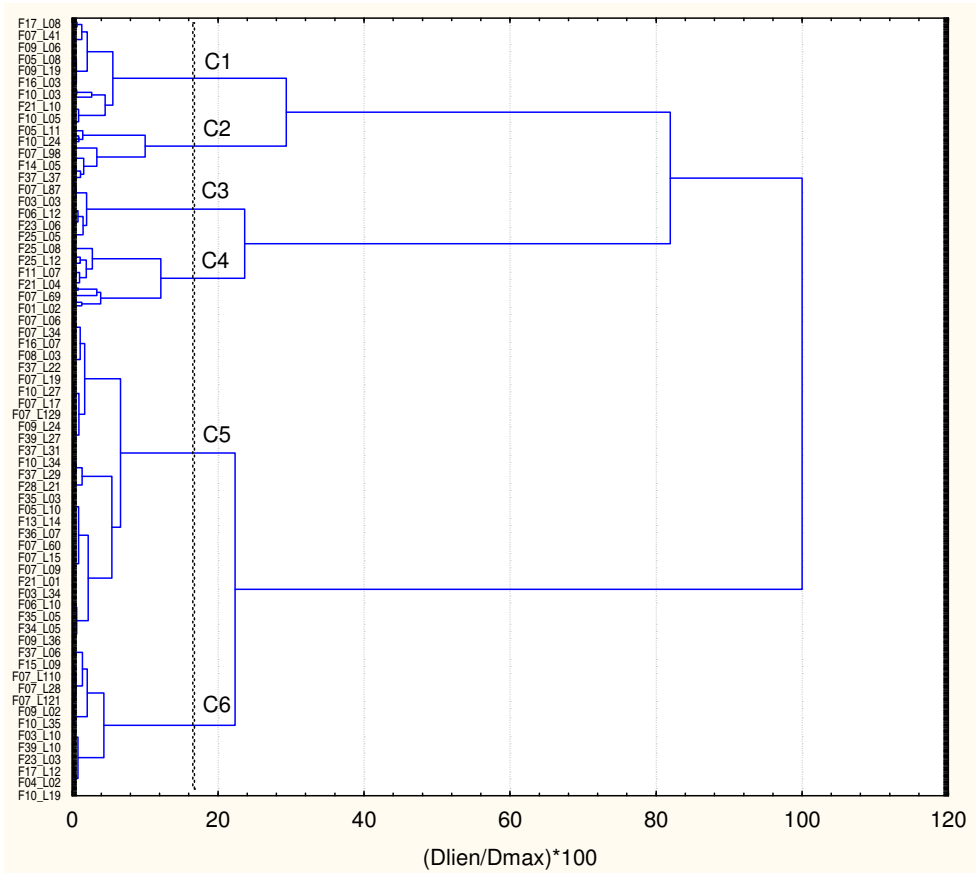


Figure 4. Dendrogram deduced from cluster analysis regarding 654 naturally diseased cane genotypes in first ratoon split into six different clusters.

Table 3. Families composing different clusters of sugarcane genotypes in first ratoon determined by multivariate analysis.

Clusters	Total of clones	Families represented (Number of clones)
C0 (disease free)	209	F02 (2), F03 (12), F04 (10), F05 (7), F06 (9), F07 (34), F08 (1), F09 (8), F10 (2), F11 (7), F12 (9), F13 (10), F14 (6), F15 (6), F16 (7), F17 (5), F18 (1), F20 (3), F21 (3), F22 (1), F23 (3), F25 (2), F26 (3), F27 (1), F28 (13), F29 (2), F30 (3), F31 (5), F32 (2), F35 (1), F36 (12), F37 (5), F38 (11), F39 (3)
C1	93	F01 (4), F04 (3), F05 (2), F06 (2), F07 (17), F08 (2), F09 (9), F10 (11), F11 (5), F12 (1), F13 (1), F15 (3), F16 (3), F17 (2), F18 (1), F19 (1), F20 (2), F21 (1), F24 (1), F25 (1), F26 (1), F27 (1), F28 (1), F29 (1), F30 (3), F35 (2), F37 (7), F39 (5)
C2	42	F05 (1), F07 (10), F09 (1), F10 (5), F11 (4), F14 (1), F16 (4), F24 (1), F29 (1), F31 (1), F36 (2), F37 (6), F38 (3), F39 (2)
C3	52	F01 (1), F03 (7), F04 (1), F06 (4), F07 (9), F08 (2), F09 (1), F10 (4), F11 (2), F12 (2), F15 (1), F16 (3), F17 (1), F18 (1), F19 (1), F21 (3), F23 (1), F25 (1), F26 (1), F28 (3), F29 (1), F33 (1), F39 (1)
C4	56	F01 (1), F03 (2), F07 (14), F08 (4), F10 (7), F11 (3), F14 (1), F15 (1), F17 (1), F19 (1), F21 (1), F22 (1), F25 (10), F26 (1), F28 (2), F30 (1), F31 (1), F34 (1), F37 (2), F39 (1)
C5	286	F01 (4), F02 (2), F03 (15), F04 (11), F05 (5), F06 (9), F07 (57), F08 (3), F09 (20), F10 (11), F11 (6), F12 (6), F13 (4), F14 (3), F15 (2), F16 (6), F17 (7), F18 (1), F19 (1), F20 (1), F21 (7), F23 (1), F24 (5), F25 (2), F26 (5), F28 (15), F29 (2), F30 (4), F31 (2), F33 (1), F34 (11), F35 (5), F36 (15), F37 (16), F38 (12), F39 (9)
C6	125	F01 (5), F03 (6), F04 (3), F05 (2), F06 (3), F07 (20), F08 (2), F09 (7), F10 (15), F11 (14), F12 (1), F13 (2), F14 (3), F15 (1), F16 (3), F17 (4), F18 (1), F21 (2), F22 (1), F23 (1), F24 (3), F28 (1), F30 (3), F31 (1), F35 (2), F36 (4), F37 (7), F38 (2), F39 (6)

Table 4. Mahalanobis square distance (bellow diagonal) between clusters taken 2 by 2 and Fisher values (above diagonal) regarding the first ratoon crop.

	C1	C2	C3	C4	C5	C6
C1	-	F = 300,3497	F = 255,6737	F = 787,0107	F = 349,0343	F = 114,117
C2	31,23867	-	F = 770,4648	F = 1114,496	F = 1085,876	F = 685,3198
C3	23,06915	99,7911	-	F = 212,3803	F = 122,3954	F = 128,7563
C4	67,75786	139,7433	23,70339	-	F = 930,1317	F = 777,3201
C5	14,96656	89,2283	8,37098	59,7696	-	F = 53,39004
C6	6,43988	65,6016	10,55097	60,4845	1,84710	-

$P < 0.001$ for all values

Table 5. Means of cluster genotypes infected by each of the three endemic diseases observed at five months of age at one-row screening stage in first ratoon cane, Ferké 1 (Ivory Coast).

Clusters	Rate of disease infestation (%)					
	Leaf scald		Smut		Pokkah boeng	
	Mean	CV (%)	Mean	CV (%)	Mean	CV (%)
C1 (n = 93)	1.33	362.8	22,65	20.0	1.76	297.9
C2 (n = 42)	0.48	648,1	52,21	30.4	1.69	215.3
C3 (n = 52)	15.76	17.6	2,39	129.5	0.70	158.4
C4 (n = 56)	42.16	41.0	6,74	126.9	0.86	239.1
C5 (n = 286)	0.00	-	2,20	77.8	1.31	188.9
C6 (n = 125)	0.00	-	9,42	27.1	0.87	204.8

Susceptibility of clusters to endemic diseases

As displayed in Figure 5, clusters C1 and C2 were much more associated with genotypes susceptible to smut and pokkah boeng but also with genotypes moderately susceptible to pokkah boeng (Fig. 5). Clusters C3 and C4 were rather associated with genotypes susceptible or highly susceptible to leaf scald. In contrast, clusters C0, C5 and C6 were related to disease-free, resistant or moderately resistant genotypes. The dendrogram showed that same families belonged often to different clusters so that no family was specifically susceptible or resistant to any disease was determined (Fig. 4, Table 3). In other words, each family investigated was

Comment [F8]: Why repeat yourself. See line 197 to 203 above

composed of disease-free as well as susceptible genotypes in certain proportion which varied from one family to another.

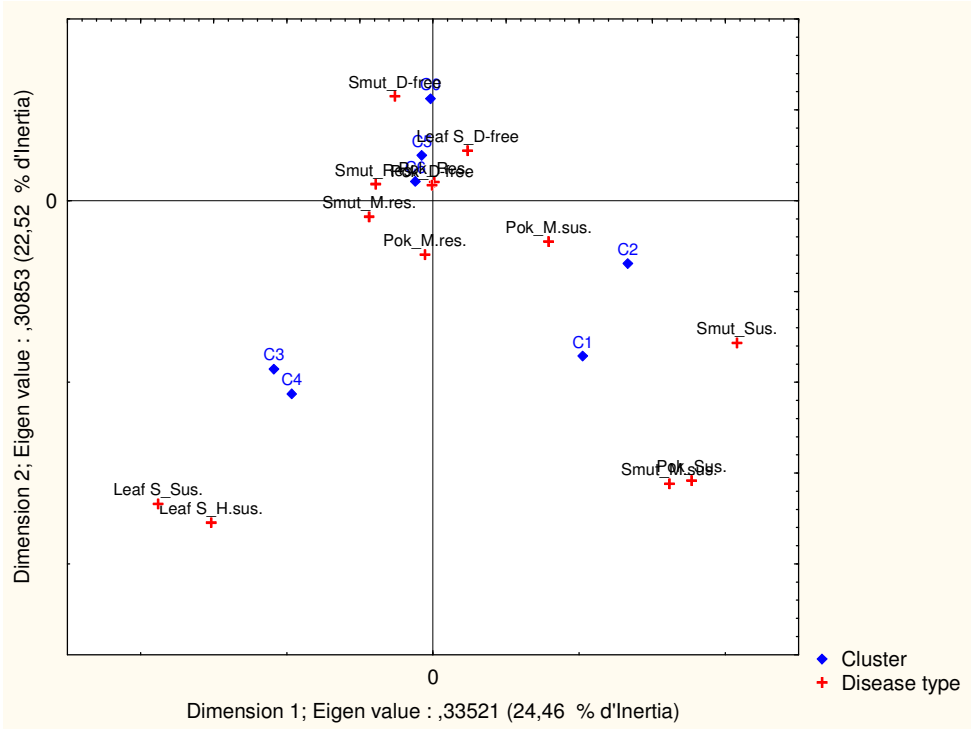


Figure 5. Projection of clusters determined by cluster analysis and susceptibility to endemic diseases in 1-2 factor plane following correspondence factor analysis in first ratoon cane at one-row stage, Ferké 1 (Ivory Coast).

4. Discussion

Diversity of both resistant and susceptible clones

The diversity of both disease-free clones and susceptible clones to smut came from 34 families, not necessarily the same, over a total number of 39 families investigated (87 %) sounds relatively high on the one hand. On the other hand, 25 clones (3 %) from 15 families (38.5 %) were susceptible to the three endemic diseases, which shows their relative genetic diversity. Similarly, clones infested by smut, pokkah boeng and leaf scald came from, respectively, 34, 22 and 15 families, which indicates the genetic diversity of diseased clones.

Comment [F9]: Split sentence

Even clones infested by leaf scald as the less infectious disease were relatively diversified genetically, too. Therefore, the disease susceptibility observed was not only prolific within families (about 3 % with smut as the most infectious disease) but also not specific to a limited number of families or crosses (more than 10 crosses at least, i.e. 25 %). This denotes the complexity of sugarcane breeding in search for resistant or tolerant parental varieties through their progenies while maintaining high genetic diversity for effective selection programs.

Similar screening studies were carried on sugarcane brown rust in Florida (Zhao *et al.*, 2015).

Comment [F10]: Similar findings or just similar screening studies ???

Breeding for high yields and disease resistance

The objective of the study was to characterize the genetic variability of susceptible sugarcane clones to three endemic diseases, namely smut, leaf scald and Pokkah Boeng at early screening stage. This implies also to address the issue of selecting best performing clones in terms of yield traits as well as their resistance or tolerance to endemic diseases and pests. However, It is evident from this study that breeding for disease resistance has been complicated by the frequent emergence of new pathogenic variants. These tend to, which over power the resistant varieties, as witnessed from withdrawal of former ruling varieties from commercial cultivation (Ramesh Sundar *et al.*, 2012).

Considerable success has been obtained in the use of classical plant breeding to control economically important sugarcane pests and diseases. There is consensus that growing genetically resistant varieties is the most cost effective and appropriate means for managing pest and diseases in sugarcane. Therefore, introgression of resistance genes into productive varieties is a key component of sugarcane breeding strategies. It is also generally recognized that a better knowledge of both pest and pathogens and of the host plant defence mechanisms will lead to the development of novel and improved approaches to enhance the durability of resistance. Recently, researchers have realized the biocontrol potential of herbivore insect enemies that are attracted to the site of herbivore feeding in response to chemical signal emissions from the plant when it is being attacked. Similarly, such indirect resistance traits in plants might contribute toward pest control by suppression of insect immunity to entomo pathogens (Rutherford, 2014).

Basically, sugarcane smut resistance mechanism is characterized into bud resistance (infection resistance) and inner tissue resistance (colonisation resistance) (Dean, 1982). It was observed by Singh and Budharaja (1964) that hyphae will not penetrate cells of the scale leaves. Hence buds tightly enclosed with the scale leaves have a better chance of escaping

infection. On this basis, Walter (1970) hypothesized that varietal resistance was determined by bud morphological characteristics. Structural characterization of sugarcane buds could provide clues for classification of test clones according to its smut resistance. Da Gloria *et al* (1995) established an association between the bud structural characteristics and the cultivar resistance. Presence of outer scales were hypothesised to provide protection against bud invasion of the smut pathogen.

Increase in disease infection with age in sugarcane

Increase in clone ~~infestations~~ infections? in first ratoon cane compared with that of plant cane was observed regarding the three endemic diseases but more importantly for smut by 51 %. This is similar to observations made by several authors like Bailey (1979) in South Africa, Croft and Berding (2000) in Australia, Sundar *et al* (2012) in India and Zhao *et al* (2015) in Florida (USA). Increase in infection of susceptible sugarcane varieties with age is the result of increasing pressure of natural infestation from contaminated soil by the crop itself or nearby infected fields with age through whip sheltering teliospores, as far as smut is concerned (Monteiro *et al*, 2018). That's why hot-water and fungicide treatment of seed cane for nurseries and roguing of nursery plantations are key recommendations to reduce disease infections in commercial fields (Gupta, 1978; Bhuiyan *et al*, 2015; Rott and Comstock, 2015). Currently, the use of pre-sprouting seedlings with a phytosanitary certificate and seedlings from micro-propagation methods are alternatives to prevent smut and other diseases affecting sugarcane cultivation (Monteiro *et al*, 2018). Therefore, plant canes which derives from disease free planting material are prone to be much less infected compared to ratoon crops, as far as susceptible genotypes or cultivars in favourable environments are concerned.

5. Conclusion

It came out from the study that most relevant diseases determining the diversity of susceptible sugarcane genotypes were, in descending order, pokkah boeng, smut and leaf scald. Increase in clone infestations on first ratoon cane compared with plant cane was observed regarding the three endemic diseases but more importantly for smut by 51 %. ~~The dendrogram deduced from cluster analysis showed that infected genotypes were split into six groups with same families belonging often to different clusters so that no family specifically susceptible or resistant to any disease investigated was determined. In other words, e~~Each family investigated was composed of disease-free as well as susceptible genotypes in a certain proportion which varied from one family to another. Therefore, all families investigated were

Comment [F11]: Try to relate this to your findings about smut in this study

Comment [F12]: Is this a recommendation you are making which was not captured in your study ? If so, the nit moves to the recommendation section

Comment [F13]: Cross check this in your reference list. There is Irwawan somewhere !

Comment [F14]: This one seems to have studied brown rust and not smut. Please cross check

Comment [F15]: Cross check use of infection verses infestation in disease management

Comment [F16]: Please cross check. Thought it is smut first !

Comment [F17]: Infection ?

relevant to maintain the diversity required for the breeding process underway. Cluster 5 was the most prolific of infected genotypes with 286 clones (33 %) split into 36 families (92 %). It was followed by clusters 6 and 4 with 125 and 93 infected genotypes split into 29 and 28 families, respectively, whereas c-Clusters 2, 3 and 4 were the least prolific ones, with 42, 52 and 56 infected genotypes split into 14, 23 and 21 families, respectively. Clusters 1 and 2 were much more associated with genotypes susceptible to smut and pokkah boeng but also with genotypes moderately susceptible to pokkah boeng. Clusters 3 and 4 were rather associated with genotypes susceptible or highly susceptible to leaf scald. In contrast, clusters 0, 5 and 6 were related to disease-free, resistant or moderately resistant genotypes and which crosses or families would, therefore, be recommended for Ferké agro-ecology.

Comment [F18]: I guess you can mention the most resistant clones (at least five) here in each cluster before finally recommending them for Ferké agro-ecology. This also needs to clearly come out in the abstract

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