



Evaluation of the Behavior of Cashew Genotypes with Regard to Bacterial Disease in Agroforestry Farms in Northern Côte d'Ivoire

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Authors' contributions

This work was carried out in collaboration among all authors. Author BKG designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors DN and DD managed the analyses of the study. Author OZF managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Notwithstanding the inflows of currency for the populations who practice it, the cultivation of the cashew tree contributes to strengthening the forestry agrosystem in Côte d'Ivoire. However, this culture, with multiple interests, is confronted with attacks from parasites including bacterial disease. Bacterial disease causes extensive damage to vegetative organs as well as fruits and causes yield

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losses. The objective of this study is to assess the behavior of cashew genotypes in relation to the severity and incidence of bacterial disease in the context of agroforestry production in Côte d'Ivoire. To achieve this objective, 1.200 branches were observed to assess the tolerance or sensitivity of 30 cashew trees in agroforestry orchards of cashew trees in the localities of Korhogo, Sinématiali and Boundiali. These cashew genotypes were selected and geolocated. The factor studied is the cashew genotype, composed of 30 cashew genotypes, with 6 modalities which are the severity index of bacterial disease on leaves, twigs and nuts, the incidence of bacterial disease. bacterial disease on leaves, twigs and nuts. The data collected that are the index of severity of bacterial disease on leaves, twigs and nuts, the incidence of bacterial disease on leaves, twigs and nuts have made the subject to descriptive analysis and PCA. The ascending hierarchical classification (HAC) and multivariate analysis completed the data analysis. The results obtained revealed three groups of cashew trees. Those of group 3 made up of twelve (12) genotypes, namely SYDN, SDYY, SDYN and KBSD coming from the localities of Sinématiali and Korhogo, differ from the others by a weak infection of the nuts (8.67 ± 2.74). The genotypes in this group are more resilient and their development in an agroforestry system could help promote agroecological management of bacterial disease, improve and intensify agroforestry practices in C. d'Ivoire.

Keywords: Cashew tree; agroforestry system; resilient; bacterial blight; incidence; severity.

1. INTRODUCTION

The cashew tree (*Anacardium occidentale*) was introduced to Africa in the 16th century by Portuguese navigators [1]. Originally, this plant was developed in Côte d'Ivoire as a species for reforestation [2,3]. Nowadays, the cashew tree has become a cash crop for this country and is among others developed there following an agroforestry system as in Tanzania, Mozambique, Nigeria, Guinea Bissau and Benin [4,5,6,7]. As a result, this crop is experiencing tremendous development for the Ivory Coast [4] due to the marketing of cashew nuts [5].

Even if the associated crops can be hosts of the bacterial disease [6,7] and present themselves as a potential source of natural inoculation of cashew trees, this agricultural system associating the cashew tree with other crops on the same agricultural holding contributes to the reduction of the atmospheric carbon rate, promotes an environment conducive to agricultural and human development [8]. Consequently, this crop has aroused real enthusiasm which is reflected in the increase in the areas sown (1.350.000 ha in 2018 against 8,200 ha in 1970) [4,9]. Thus, Côte d'Ivoire has managed to rank first among cashew nut producing and exporting countries since 2015 with 702,510 tonnes of raw nuts [10].

However, the average yield of orchards remains low (350 to 500 kg / ha) [10] and lower quality nuts with an outturn of between 46 to 48 lbs [11] due to growing environment favoring the distribution of diseases including bacteriosis in

farms. The farms are characterized by unimproved planting material and vulnerable to pests including bacterial diseases caused by *Xanthomonas* sp. Which causes enormous losses in production and product quality [12-14]. The management of these plagues raises several issues.

Indeed, producers have always practiced chemical control, which is not only expensive but proves inaccessible, non-resilient and dangerous for the health of populations and for the environment [15-17]. Therefore, knowledge of the behavior of genotypes on the basis of their sensitivity or tolerance to bacterial disease and their use in agroforestry for agroecological management of this disease could sustainably slow down the damage of bacterial disease. bacteriosis. In addition, it would help build a more resilient, less costly and environmentally friendly farming system.

To achieve this, the authors propose to evaluate, at different stages of development (vegetative and fruiting stages), the behavior of cashew genotypes developed in an agroforestry system and to structure them according to the severity and incidence of bacterial disease.

2. MATERIALS AND METHODS

2.1 Experimental Sites

The peasant orchards of the departments of Korhogo, Sinématiali and Boundiali were the sites of the study. These departments were located in the north of the Ivory Coast. The

climate is marked by two seasons including a short rainy season which starts from May to October and a long dry season which extends from November to April with a dry wind from November to March. The average annual rainfall varies between 1000 and 1400 mm in these departments. The vegetation consists of wooded savannah. The soils are ferralitic, moderately to strongly denatured [5].

2.2 Planting Materials

The planting material used in this study is composed of 372 twigs or individuals, from 30 cashew genotypes, with 40 twigs per cashew genotypes were the observation material. These twigs are composed of leaves, flowers and fruits depending on the stage of development of the cashew tree. The 30 cashew genotypes were identified during surveys in peasant orchards in the localities of Korhogo, Sinématiali and Boundiali. The cashew trees of these peasant orchards have a planting period of 10 years and have the particularity of being developed, in an agroforestry system, in cultural association with the shea tree and the mango tree (Table 1).

2.3 Méthods

2.3.1 Orchard prospection and choice of genotypes

The prospecting was carried out in the peasant orchards of the localities of Boundiali, Korhogo and Sinématiali. During this prospecting, high-producing genotypes of cashew trees (between 20 and 50 kg), with a planting period of 10 years and developed in an agroforestry system which combines them with mango and shea, were sought. These trees populations were surveyed using the traveling inventory method combined with the diagonals and medians method. Each tree or individual has been marked / colored, numbered and geo-referenced using GPS. This approach was inspired by the strategies developed by Maxted et al. [18] to conduct ecogeographic surveys following methods by Diouf et al. [19] to carry out ethnobotanical surveys.

2.3.2 Data collection

According to the Dangneli principle of experimentation (2003 and 2012), in this study, each tree or cashew genotype represents the plot. The twigs are the individuals of the plot.

Table 1. Different cashew genotypes and the geographic location of the orchards

Locality 1: Boundiali		Locality 2: Korhogo		Locality 3: Sinématiali	
Genotypes	Geographic Coordinates	Genotypes	Geographic Coordinates	Genotypes	Geographic Coordinates
BKKY	N: 09°33.136' O: 06°26.243'	KTY1	N: 09°29.984 O: 05°43.309'	KBSD	N : 09°35'154' O : 005°21'019
BBY	N: 09°27.798' O: 06°29.779'	KTY2	N: 09°30.168' O: 05°34.716'	KOMC	N : 09°36.505' O : 005°20.710'
BKA	N: 09°38.382' O: 06°21.127'	KTYT	N: 09°31.162' O: 05°38.626'	KLYN	N : 09°36.354' O : 005°20.627'
SST	N: 09°37.438' O: 06°20.229'	KKSN	N: 09°31.674' O: 05°38.783'	KT3	N : 09°33'721' O : 005°25'396
SYD	N: 09°24.467' O: 06°21.871'	KKSS	N: 09°17.491' O: 05°32.697'	BAK	N : 09°34'751 O : 005°25'302
SFA	N: 09°27.935' O: 06°25.350'	KBT	N: 09°19.103' O: 05°34.223'	SSS	N : 09°34.751' O : 005°28.030'
SWSZ	N: 09°31.733' O: 006°25.921'	KSCK	N: 09°23.008' O: 05°33.643'	STSL	N : 09°36.669' O : 005°22'204'
SLLC	N: 09°32.295' O: 006°30.356'	KC3	N:09°19.033' O: 05°38.441'	SGYM	N : 09°29.800' O : 005°20.414'
SDYY	N: 09°28.861' O: 06°32.693'	KCP2	N: 09°19.643' O: 05°39.207'	SYDN	N : 09°33.345 O : 005°24.330'
SDYN	N: 09°39.932' O: 06°29.327'	KCP1	N: 09°29.919' O: 05°48.486'	STSB	N : 09°32.789' O : 005°23.864'

Thus, at the level of each plot, 40 branches were marked in the North-South and East-West axes at the rate of 20 branches in each axe. These branches or individuals were observed according to the parameters of severity and incidence. These observations were carried out during a cropping year comprising the rainy and dry seasons and at three stages of development of the branch which are the vegetative, flowering and fruiting stages in order to evaluate the behavior of cashew genotypes with respect to the bacterial disease. Thus, data collection focused on the incidence and severity of bacterial disease on leaves, twigs and fruits.

2.3.2.1 Evaluation of the severity index (SI) of bacterial disease

Severity was assessed every two weeks on the leaves, fruits and panicles of the ten branches marked on either sites of the N-S and E-W axes.

The evaluation approach resulted in a visual rating scale ranging from 0 to 24 [20] (Groth et al., 1999; in Silué et al., 2018).

The bacterial disease severity index was determined according to the formula of Kranz [21] cited by Dianda et al. [22] according to the following formula.

$$I_s = \sum \left(\frac{X_i \times n_i}{N \times Z} \right) \times 100$$

I_s : severity index; X_i : severity i of the disease on the organ; n_i : number of organ of severity i ; N : total number of the organ observed; Z : highest severity scale (9).

2.3.2.2 Assessment of the incidence (Ic) of bacterial disease

The incidence was determined as the ratio of the number of infected individuals to the total number of individuals observed as a percentage. The impacts were determined according to the following formula [11,12]:

$$I_c = \frac{\text{Number of organs attacked on the date of observation}}{\text{Total number of organs in the plot orbit}} \times 100$$

A scale adapted to that used by Bhagwat et al. [23] for the discrimination of mango varieties infected with anthracnose allowed to qualify the level of incidence of bacterial disease. This six-grade scale (0-5) is defined as follows: 0 (no symptoms); grade 1 (1-10%: low incidence);

grade 2 (11-20%: moderate incidence); grade 3 (21-30%: medium or intermediate incidence); grade 4 (31-50%: high incidence); grade 5 (> 50%: very high incidence).

This evaluation focused on ten branches marked on each side of the N-S and E-W axes to be seen and carried by hand.

2.3.3 Statistical analysis of the data collected

Data entry and graphs were performed with Excel 2013 software. Statistica 7.1 software was used to perform descriptive analyzes. These analyzes were carried out to test the hypothesis according to which the behavior of the cashew genotypes would vary according to whether this genotype is sensitive or tolerant to the bacterial disease. Thus, if this hypothesis is verified, the ascending hierarchical classification (CAH), following the orientation of the principal component analysis (PCA) will be carried out to structure the genotypes. Subsequently, the multivariate test will complete these analyzes to characterize according to the sensitive or tolerant homogeneous groups established by the CAH.

3. RESULTS

3.1 Descriptive Profile of the Severity and Severity Index of Bacterial Disease

The mean severity indices presented in Fig. 2 revealed a strong variability between the genotypes. The infections range from moderate to very severe, including severe infections (Table 2, Fig. 1). Mild severe infections (between 11 and 25%) were observed in ten genotypes which are SST, KSCK, SYD, KVSS, KBT, STSB, KKS, SDYN, SDYY and SYDN (Fig. 1).

Nineteen genotypes presented severe infections (between 25 and 50%); these were SWSZ, KTY1, KTY2, KTY3, KCP1, KCP2, KCP3, STSL, BKA, SSS, SGYM, SFA, BBY, BAK, KLYN, KTY, KBSD, BK, SLLC.

3.2 Descriptive Analysis of the Incidence of Bacterial Disease

The histogram and the table of incidence values (Fig. 2 and Table 3) shows a significant difference between the genotypes ($P < 0.05$). All trees exhibited mean incidences greater than 10%. Eight genotypes (STSL, BKA, SGYM, BBY, KBSD, SDYN, SDYY and SYDN) presented incidences of between 11 and 25%. Twenty genotypes, namely, SST, SSS, SFA, KOMC, BAK, KSCK, KLYN, SYD, KTY, KVSS, KBT,

STSB, BKKY, KKSJ, SWSZ, SLLC, KCP3, KTY1, KTY2 and KTY3. Only KCP1 and KCP2 presented incidences greater than 50%.

3.3 Principal Component Analysis (PCA) of the Incidence and Severity of Bacterial Disease

Principal component analysis PCA was defined by the first two axes which explained 56.72% of the total variability observed. Axis 1 which

returned 32.59% of the variability was defined on the negative side by the bacterial disease Severity Index on Walnuts (ISNx) with the KOMC. Axis 2 explained 24.13% of the observed variability. This axis was formed on the positive side by the incidence of bacterial disease on leaves (IsFe) and on the negative side by the incidence of bacterial disease on walnut (IsNx) and the severity index of bacterial disease on twigs (ISR) with the SFA and STSL genotypes.

Table 2. Bacterial disease severity index following the evolution of the cashew tree

Genotypes	Vegetative stage		Fruiting stage
	(IsFe)	(IsRam)	(IsFr)
SST	26,25±2,52ab	11,09±2,43a	29,00±0,50c
STSL	59,05±6,15efg	13,35±3,25ab	52,01±5,31e
BKA	41,12±5,35c	17,17±2,21abc	44,00±0,30de
SSS	57,10±2,35efg	19,45±2,05abc	53,20±0,05ef
SGYM	43,81±5,32cd	14,55±3,21ab	42,25±2,50d
SFA	67,24±2,30fgh	17,25±5,60abc	51,51±3,25e
BBY	39,25±3,45bcd	18,75±5,46abc	35,33±2,50cd
KOMC	80,51±0,22g	25,44±6,45bc	74,15±8,35f
BAK	39,25±5,60bcd	22,05±1,33b	35,23±2,35cd
KSCK	30,49±2,41b	16,15±5,12ab	20,50±0,30b
KLYN	65,29±10,25fg	30,25±8,45c	55,15±1,75ef
SYD	20,01±9,33a	10,25±4,33a	15,05±0,30ab
KTTY	40,50±1,30c	24,56±7,30bc	33,15±0,00cd
KVSS	30,03±0,15b	14,00±6,05ab	22,02±10,5b
KBSD	41,15±0,50c	25,10±0,50bc	33,00±1,05cd
KBT	31,51±0,30b	16,01±2,14ab	25,05±0,00bc
STSB	24,05±2,45ab	14,56±3,25ab	18,51±4,55abc
BKKY	41,12±0,33c	23,55±0,10b	32,01±0,20c
KKSJ	31,00±0,00b	17,05±2,15abc	24,00±0,50bc
SWSZ	37,20 ± 5,20bcd	17,81±2,11abc	28,01±0,20bcd
SDYN	27,01±9,45abc	15,05±7,44ab	21,05±3,05b
SDYY	29,07±8,44abc	15,08±1,25ab	22,00±1,05b
SLLC	41,15±2,45d	25,00±0,33bc	33,00±1,75c
SYDN	33,01±1,95cb	10,50±9,35a	13,50±0,00ab
KCP1	57,05±5,52efg	23,77± 5,33bc	52,10±2,45e
KCP2	47,50±4,75def	31,25± 9,44c	30,55±0,05c
KCP3	58,51±15,65efg	26,25±4,33bc	35,73±8,50cd
KTY1	38,50±2,33bcd	20,75±9,55b	23,95±9,88b
KTY2	43,15±2,52de	25,55±4,33bc	24,50±0,75bc
KTY3	41,02±4,55d	25,15±2,33bc	33,00±7,33cd
Average	42,03±4,25	19,56±6,42	37,39±5,78
P-value	0,001	0,000	0,000
CV(%)	24,75	6,76	51,62

The numbers assigned the same letters in the columns are not statistically different according to Turkey's HSD tests at the 5% level

CV: coefficient of variability; IsFe: severity index on leaves; IsRam: severity index on twigs; IsFr: severity index on fruits

Table3. Incidence of bacterial disease following the organs of the cashew tree

Genotypes	Vegetative Stage		Fruiting Stage
	(IcFe)	(IcRam)	(IcFr)
SST	46,18±2,05de	28,25±3,33cde	40,00±5,01d
STSL	22,96±6,45ab	25,00±7,25cd	25,00±5,36bc
BKA	22,87±1,45ab	15,00±2,85bc	17,00±2,80abc
SSS	61,01±1,75f	10,83±5,11b	65,00±5,30fg
SGYM	19,89±8,65a	23,00±7,51cd	28,07±6,25bcd
SFA	50,43±0,45e	28,75±2,35cde	48,55±4,33def
BBY	19,32±5,74a	4,16±4,70a	20,00±2,90b
KOMC	68,67±3,35fgh	24,33±8,39cd	52,08±15,35g
BAK	40,84±1,73d	17,08±7,54bcd	45,15±9,20de
KSCK	63,34±5,27fg	23,58±6,45cd	43,00±7,50de
KLYN	66,81±12,45fgh	23,25±17,25cd	56,26±4,35ef
SYD	58,62±6,75efg	30,41±9,65d	50,00±7,85 ^e
KTYT	56,28±11,55efg	34,16±8,45de	45,33±15,35de
KVSS	53,20±7,25ef	23,00±7,61cd	38,47±18,05cde
KBSD	35,30±9,61cd	19,00±13,50bcd	15,00±1,50ab
KBT	49,43±6,75def	34,58±4,56de	19,50±0,30b
STSB	36,02±3,69cd	28,08±5,11cde	17,36±5,71abc
BKKY	50,39±14,35e	39,58±9,45e	28,00±16,05bcd
KKSN	32,91±5,15cd	33,25±6,11de	16,00±0,50ab
SWSZ	39,60±7,43cde	40,41±8,35e	29,00±0,33bcd
SDYN	23,67±9,75ab	18,16±14,23bcd	10,81±6,45a
SDYY	24,55±7,30ab	21,00±9,25c	15,00±0,65ab
SLLC	35,42±7,45cd	32,00±8,50de	20,25±5,33b
SYDN	23,94±11,5ab	17,08±7,55bcd	14,00±8,44ab
KCP1	71,15±6,75g	56,06±13,45fg	48,16±12,51def
KCP2	80,14±15,20h	55,25±8,33fg	35,00±10,87cd
KCP3	62,87±9,45f	40,16±8,20e	43,66±12,33de
KTY1	63,9±17,66fh	25,74±9,44cd	46,69±20,33de
KTY2	58,24±12,05efg	33,41±10,32de	35,00±15,23cd
KTY3	46,3±12,55de	23,51±12,30cd	39,25±12,44d
Average	45,38±14,21	27,60±7,25	33,55±10,62
P-value	0,001	0,025	P= 0,000
CV%	17,97	6,08	45,13

The numbers assigned the same letters in the columns are not statistically different according to Turkey's HSD tests at the 5% level

CV: coefficient of variability; IcFe: incidence on leaves; IcRam: incidence on twigs; IcFr: incidence on fruits

3.4 Structuring and Characterization of Genotypes by ASCENDING Hierarchical Classification (AHC) and Multiple Analysis of Variances (MANOVA)

The Ascending Hierarchical Classification (CAH) made it possible to structure the genotypes studied into 3 groups (Fig. 4).

Group 1 was made up of nine genotypes (KCP1, KCP2, KCP3, KTY1, KTY2, KTY3, KLYN, KTYT and SLLC). Group 2 also contained nine genotypes namely KOMC, SFA, SSS, KBT, KVSS, the SST, SWSZ, BAK, and BKK.

The third group with 12 individuals comprised the genotypes SYD, KSCK, KBSD, KKSN, BBY, BKA, SGYM, STSL, SDYY, SYDN, STSB and SDYN.

3.5 Characteristics of the Groups Obtained

Multiple analysis of variance (MANOVA) (Table 4) showed a significant difference between these groups ($F > 4$ or $P < 0.005$). This significant difference was observed in the incidence of bacterial disease on twigs, leaves and nuts and the level of severity indices in nuts.

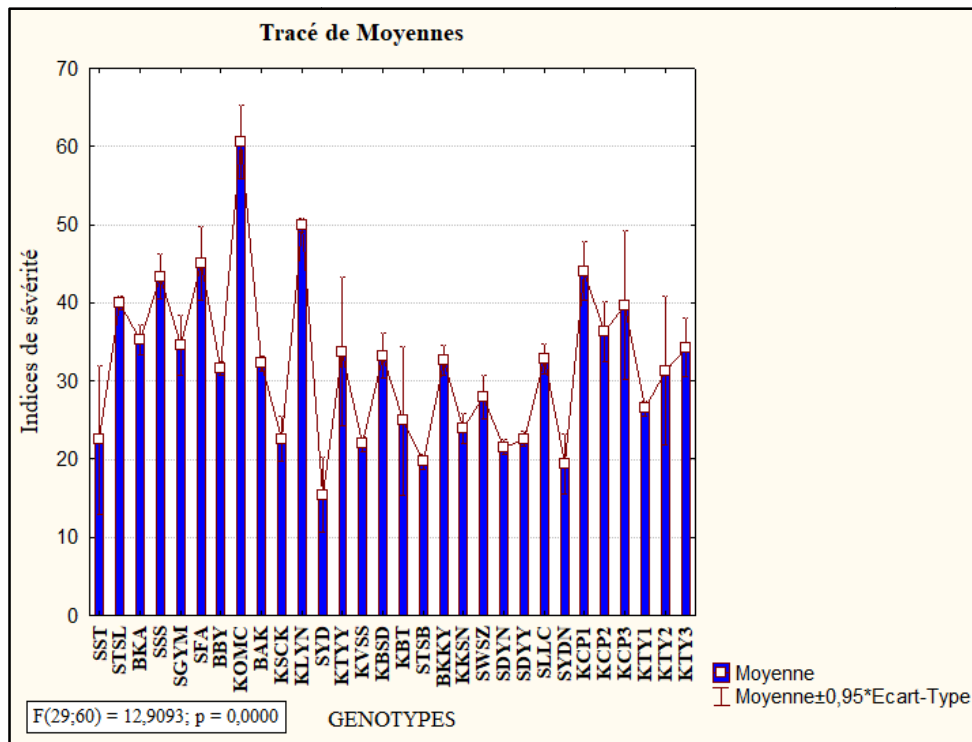


Fig. 1. histogram of the means of the severity indices

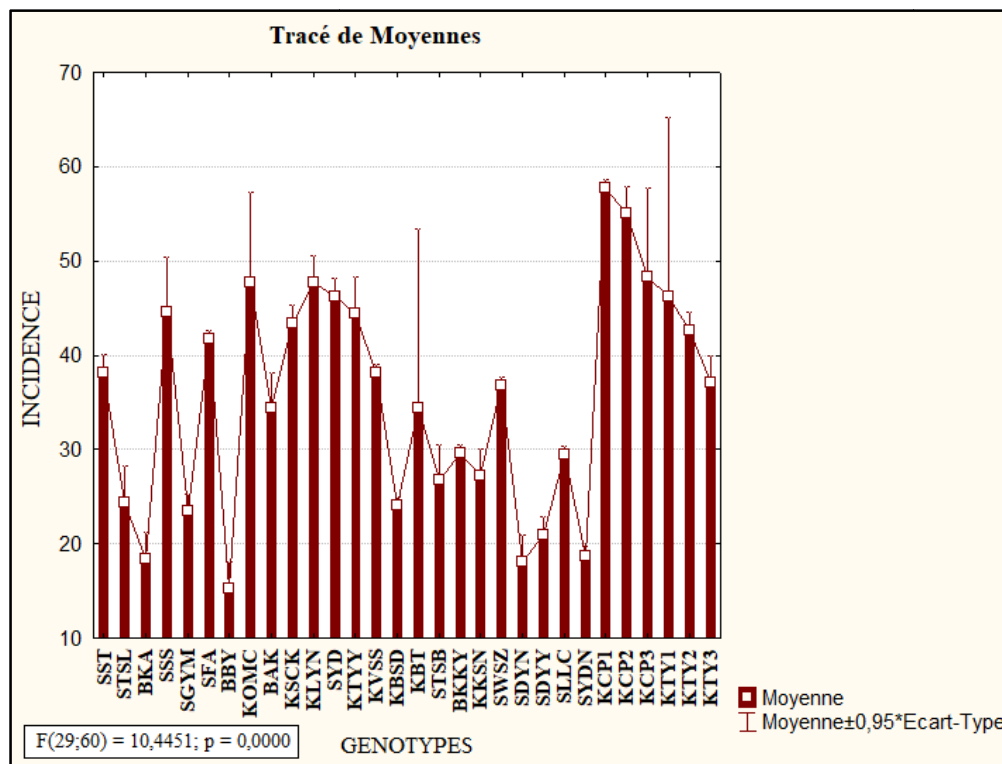


Fig. 2. Histogram of mean bacterial disease incidence values according to genotypes

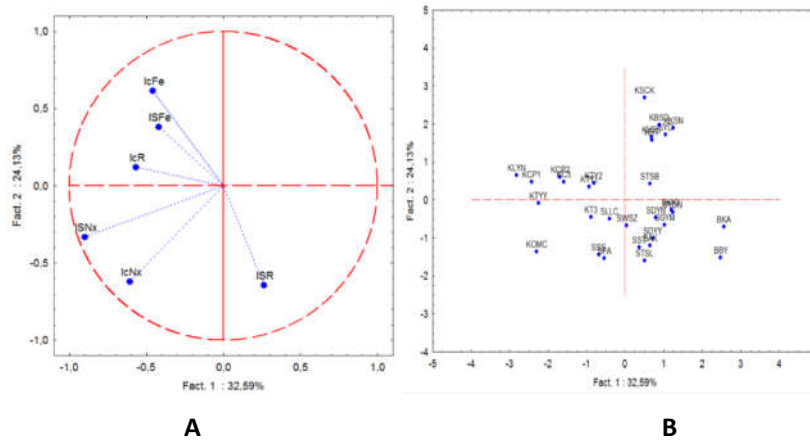


Fig. 3. Projection of variables (A) and genotypes (B) in the factorial plane 1 and 2

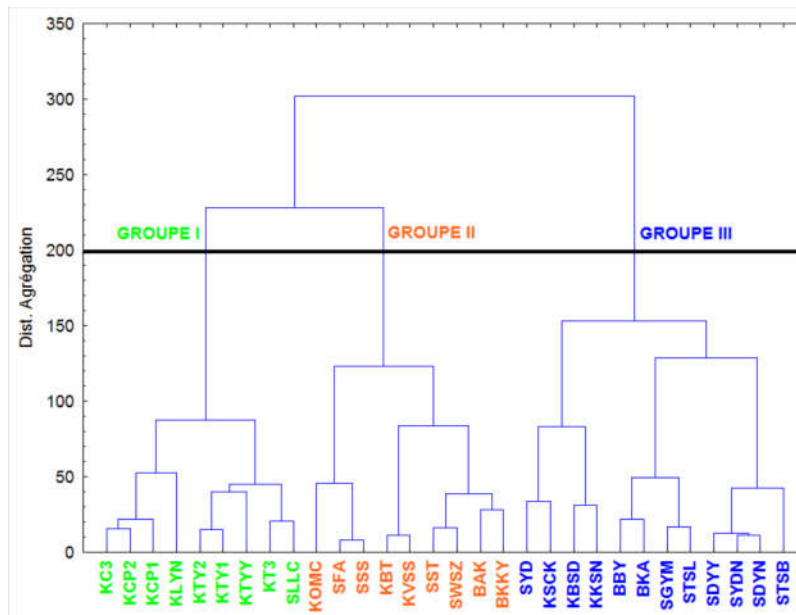


Fig. 4. Structuring of groups of cashew trees by the Ascending Hierarchical Classification (CAH)

Thus, group 1 was characterized by a high incidence of bacterial disease on the leaves (50.98 ± 3.96) and severe infections in the nuts (45.13 ± 4.01). Group 2 was characterized by a high incidence of disease in the twigs (81.1 ± 3.21) and by severe infections in the twigs (47.58 ± 5.82). Group 3 was differentiated from the others by a weak infection in the nuts (8.67 ± 2.74).

4. DISCUSSION

Analysis of the data on the severity of bacterial disease showed a significant difference between

the genotypes. This difference testifies to the existence of great diversity within the genotypes of peasant orchards. In fact, three classes of genotypes were obtained with the severity data. The presence of bacterial disease symptoms in all trees would show the sensitivity of these genotypes to virulent strains of *Xanthomonas sp*, and the existence of a microclimate favorable to the development of the pathogen according to the "triangle of diseases" described by Nasraoui [24]. According to the author, the development of a disease in the plant requires the presence of three components (the host plant, the pathogen and a favorable environment).

Table 4. Descriptive analysis of the different groups of cashew trees formed by the CAH

Variables	Groupes			Statistiques		
	I	II	III	F	P value	Significativité
IcR	64,77 ± 5,89 ^b	81,1 ± 3,21 ^c	33,23 ± 5,03 ^a	25,7	P < 0,0001	OUI
IcFe	50,98 ± 3,96 ^c	20,02 ± 1,74 ^b	33,36 ± 5,72 ^b	10,34	P < 0,001	OUI
IcNx	20,37 ± 1,22 ^b	14,03 ± 3,28 ^{ab}	10,12 ± 2,92 ^a	3,62	P < 0,05	OUI
ISR	28,10 ± 2,93 ^a	47,58 ± 5,82 ^a	38,89 ± 6,59 ^a	2,62	P > 0,05	NON
ISFe	36,28 ± 3,29 ^a	34,45 ± 4,70 ^a	29,91 ± 3,97 ^a	0,69	P > 0,05	NON
ISNx	45,13 ± 4,01 ^c	21,71 ± 6,76 ^b	8,67 ± 2,74 ^a	17,04	P < 0,0001	OUI
Effectifs	9	9	12	13,95	P < 0,0001	OUI
Pourcentage (%)	30	30	40			

The climate would have impacted the occurrence of bacterial disease, making the plant vulnerable while promoting the dissemination and germination of spores. In fact, this study began at a period corresponding to strong variations in temperature which would have caused physiological instability in these genotypes. This instability would have predisposed the immune system of these plants which recorded strong severities. These results were in accordance with Cardoso et al. [25] who showed that temperature variations create physiological instability in plants and make them more vulnerable to attack.

In addition, this variation in severity in these genotypes would be linked to the difference in their defense mechanisms. According to Desanlis [26] the defense mechanisms put in place by a plant against a pathogen differ from one genotype to another. Indeed each genotype has intrinsic characteristics allowing it to resist its attacker. Thus, slightly infected genotypes have defense mechanisms that allow them to react more quickly to aggression and to limit the progression of the infection.

In general, this first level of defense is easily avoided by the pathogen. As for the defense mechanisms induced, they result from an initial recognition between the pathogen and the host plant. It can be general or specific. In this category, we can distinguish some of the so-called "non-host" mechanisms that provide all host plants with a first level of immunity against all species of a pathogen. On the other hand, so-called "host" mechanisms which provide a particular host with specific immunity against a reduced number of pathogens of a species [26-28].

The ACP performed showed that 3 variables (incidence of bacterial disease on leaves and twigs, severity index on twigs) out of the six studied contributed the most to the total variability. The CAH carried out made it possible to classify the genotypes studied into four groups of phytopathological diversity (incidence and severity index). This screening confirms the phenotypic diversity observed within these genotypes.

5. CONCLUSION

The present study notes the existence of a strong phenotypic diversity within the genotypes studied. The diagnosis of bacterial disease revealed a strong variation in incidence and

severity depending on the genotypes and the organ attacked.

The hierarchical ascending classification made it possible to screen the studied genotypes into three groups on the basis of the mean values of incidence and severity index. Thus, group 1 (made up of 9 genotypes) was characterized by a high incidence of bacterial disease on the leaves (50.98 ± 3.96) and severe infections in the nuts (45.13 ± 4.01). Group 2 (9 genotypes) was characterized by a high incidence of disease in the twigs (81.1 ± 3.21) and by severe infections in the twigs (47.58 ± 5.82). Group 3 (composed of 12 genotypes) differentiated from the others by a weak infection in the nuts (8.67 ± 2.74). Ce groupe 3 présente donc des génotypes résilients pour la tolérance de leur comportement au niveau de la noix.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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