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Application of Molecular Markers for the Identification of Resistant Cowpea Varieties Against Fusarium Wilt in the Sudano-Sahalian Zone of Cameroon

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Abstract:- The current study aims to determine four cowpea varieties status under fusarium wilt disease by using Single Nucleotide Polymorphism (SNP) molecular markers potentially associated to cowpea resistant gene. Plant material was constituted by ten cowpea varieties with four varieties developed by the Institute of Agricultural Research for Development and six imported varieties from the International Institute of Tropical Agriculture. The experiment was carried out in completely randomized block with three replications. Infestation was performed by Fusarium oxysporum f.sp tracheiphilum isolate identified as the more virulent in the Far-North Region of Cameroon. The plants' DNA was extracted by FTA Plantsaver cards method. Severity scale of the disease was noted from 0 to 5. The analysis of variance has revealed that significant differences exist among tested varieties as far as disease severity concern. IR15MA33, IT99K-573-2-1, IT82E-18 and IR15MA02 varieties with respectively 4.00, 3.67, 3.33 and 3.00 severity were identified susceptible. TVU109, IT98-503-1, CB46 and TV410 varieties with respectively 1.67, 1.67, 2.00 and 2.33 severities reveal the resistance signs against wilt fusarium. Among twenty-one tested markers, 2_31831, 2_27367 and 2_02374 have shown their potential associability to cowpea wilt fusarium resistance alleles. Potential parent's resistant genes donors against this disease have revealed their resistance and relative information of different cowpea varieties status are henceforth available. Supplementary investigation is therefore necessary to establish genetic map of resistant gene.

Keywords:- *Screening; SNPs; Markers; Cowpea; Far-North Cameroon.*

I. INTRODUCTION

Cowpea (*Vigna unguiculata* (L.) Walp.) is a leguminous crop cultivated worldwide such as in Africa, East Asia, East Europe, South Europe, Australia and New Zealand, in North America, in South America, and in Caribbea [1]. It is an annual, herbaceous, and autogamous plant. It has an allogamy rate that varies between 0.2 to 2 %. Cowpea is a diploid specie with 2n=22 chromosomes and has a small height like most Phasealea species [2]. This leguminous constitutes one of the main leguminous crops in Cameroon [3]. Despite this importance, cowpea faces many abiotic and biotic constraints. Fusarium wilt is one of the major biotic constraints that significantly reduce cowpea production. The symptoms of the disease are characterized by the discoloration that mostly affects just one side of the leaf or the plant. It is followed by defoliation, wilting that starts on one side of the plant before reaching the whole plant and later on the death of the plant [4], [5], [6]. Though the disease is found in almost all cowpea farms cultivated in the Far North Region of Cameroon, the management of the disease by producers is limited which compromises production optimization [7]. In fact, chemical control is less efficient because the resistant form of the pathogen known as chlamydosopres can survive in the soil during several years and in many secondary host plants [8]. Again, chemical control is less recommended not just because of its effect on environment and health, but also because it favors the development of pathogens' resistance [9], [10]. Genetic management becomes in this context the more reliable mean of control. Thus, the goal of the present study is to determine the status of cowpea varieties developed by the Agricultural Research Institute for Development of Cameroon and to evaluate SNP polymorphism which can be used as the potentials markers associated to the resistant genes against *Fusarium oxysporum* f. sp *tracheiphilum.*

II. MATERIALS AND METHOD

A. Plant Material and Pathogen Source

The plant material was constituted of six imported varieties from the International Institute for Tropical Agriculture and four varieties from the Agricultural Research Institute for Development of Cameroon. The pathogen was the more virulent *Fusarium oxysporum* f. sp *tracheiphilum* strain isolated in Far-North Region of Cameroon [7]. Spores developed on Joff culture after seven days of incubation were collected and introduced in the bottle with distilled water. After agitating the content on vortex within thirty seconds, suspensions were filter on Grid paper to return mycelia in the perspective of obtaining solely sporales suspensions.

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Concentration was determined by hemacytometer and adjusted to 10^6 spores /ml [11].

B. Substrate preparation

Substrate was constituted by the mixture of sand and loam soil in 2 : 1 proportion. It has been sterilized at 121 °C during one hour under autoclave. The used plastic pots measure 20.5 cm diameter and 19.5 cm depth filled with sterilized soil.

C. Experimental Design and « root deep method » Inoculation

The experiment was conducted in the screen house of the Agricultural Research Institute for Development of Maroua. The trial was performed in completely randomized block with three replications. Plants were uploaded from soil 14 days after sewing. Therefore, roots were washed with tap water to remove adherent particles and the extremities were trimmed with scissors and sterilized with 90° alcohols and rinsed trice with sterilized distilled water. Each plant root was introduced to 10 ml of sporale suspension during one hour. They were removed after and planted in the pots. Plants reactions to *Fot* were assessed seven weeks after inoculation. It was based on vascular system discoloration of the plant after longitudinal dissection. Disease severity was visually evaluated on the scale of 0 to 5. Severity percentage was estimated according to scale reported by [12] as follow:

- 0= healthy plant with no disease symptom;
- 1= approximately 10 % of the plant present symptoms of the disease;
- 2= approximately 25 % of the plant present symptoms of the disease;
- 3= approximately 50 % of the plant present symptoms of the disease;
- 4= approximately 75 % of the plant present symptoms of the disease;
- 5= approximately 100 % of the plant present symptoms of the disease.

Other measured parameters are pods number per plant, plant height, seeds number per pod. Seeds number per pod were consisted on choosing randomly three pods per plant, count seeds number and calculate the average number using following equation:

$$Seeds\ Number\ per\ Pod = \frac{SNP1 + SNP2 + SNP3}{3}$$

SNP= Seeds Number per Pod

D. Molecular Screening of SNPs

DNA extraction was performed by using the Fast Technology for Analysis (FTA) Plantsaver cards [13], [14], [15]. Thus, fourteen days old leaves after sewing were harvested. Disc of 2 millimeter diameter of Whatmann paper containing cowpea DNA were collected and introduced in the tubes for washing.

Fig 1 DNA Extraction by Fast Technology for Analysis (FTA) Plantsaver cards

Discs were washed three times by alcohol 70 %. Those discs were removed from the tubes and dried on filter paper during 24 hours under laboratory conditions.

The preparation of premix solution was effectuated according to the method described by [17]. Oligonucleotides are kept in lyophilized form and then, solids particles can be attached to tubes lids. Therefore, tubes containing markers were centrifuged at 1000 g during one minute to free oligonucleotides attached to the lid. Buffer solution TE pH 8,0 (10X corresponding to « n » moles marker) was taken and added to each centrifuged tube to obtain stock solution of oligonucleotides. In fact, working solution was obtained by taking 50 µl of stock solution that has previously agitated with vortex and has been introduced to another tube. Ready for use markers were obtained by adding 450 µl of buffer solution and the mix solution is vortexed and centrifuged. The product is kept at 4°C for immediate use and kept in freezer for future use.

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Necessary premix for PCR reaction were produced by General Electric Company (GE) and furnished by Kirkhouse Trust. The content of the kit are: pure Taq DNA polymerase responsible of DNA amplification, activated nucleotids (dATP, dCTP, dGTP and dTTP), BSA stabilizers. At a total reconstituted volume of 25 µl, concentration of those components was: 200µM of each Nucleotide Triphosphate (dNTP) in 10 mM of Tris-Hcl, 50 mM of KCl and 1.5 mM of $MgCl_2$. The designed number of premix tubes for PCR was removed from the packing aseptically the same as the required corresponding lids. Therefore, tubes were separated by sterilized scissors with 70° alcohol. The tube content was verified to be assured that the product is at the bottom before removing the attached lid as preconized by the manufacturer. A disc of DNA was introduced to each tube by sterilized forcep. With a pipette graduated from 20 to 100 µl, 21 µl of biological water was pipetted and introduced to the tube for the solution reconstruction. Next, 1 µl primers of working solution was added to each tube. The content was covered and centrifuged during few second to obtain a limpid mix solution for PCR.

The following table 1 presents primers that were used in this work.

Table 1 List of Primers

Number	Primers' names	nucleotides sequence (5'------>3')
1	2_04477R 2_04477F1 2_04477F2	TGTTAATGGAGCCTGAGTCG (20) ACCATACATTACATATCTTCCCAGAATACGCAACTA(36) ATCAATCTTCCCAGAATACGCAAGTC (26)
2	2_24387F 2_24387R1 2_24387R2	TTTGCAGCAATTGAGAAAACA(21) ACCATACATTACATATCATCCTATTCACCAAGCTCC(36) ATCAATCATCCTATTCACCAAGCACT(26)
3	2_33548R 2_33548F1 2_33548F2	CCATTTTGCAAACAGGATCA(20) ACCATACATTACATACCAGAACTCCTCTCCGA(33) ATCAACCAGAACTCCTCTCCCCT(23)
4	2_04048F 2_04048R1 2_04048R2	TTTTGGGAAAGGCCATGATA(20) ATTACTACTAGACGGGGCAGGTAATGATGCAAAACAA(37) CGAGCGGCAGGTAATGATGCAAAAGAG(27)
5	2_50243F 2_50243R1 2_50243R2	AACTCAACAAATTTGCGATCC(21) ACCATACATTACATATTCACTCCATTGTTTGTGTCTT(37) ATCAATTCACTCCATTGTGTTGTGTGTG(27)
6	2_26364F 2_26364R1 2_26364R2	GCAAGGTGGGCTAGAACGTA(20) ACCATACATTACATATCTAGAAGCAAACAAATCCTCCTCC(37) ATCAATCTAGAAGCAAACAAATCCACT(27)
7	2_09924R 2_09924F1 2_09924F2	TTTGATTTACCAAACCCACCT(21) TTACTACTAGACGGATCAATTTCCATGAGTGCGTT(35) TGCGGTCAATTTCCATGAGTGCCTC(25)
8	2_51968R 2_51968F1 2_51968F2	CAACAGGCTCTGGCTGAAAT(20) ATTACTACTAGACGGCCATCCCTATGTAATGCTTGTG(37) ATCAACCATCCCTATGTAATGCTTCTA(27)
9	2_31831F 2_31831R1 2_31831R2	TGCCTCCAATCTGAACTCAA(20) ACCATACATTACATATAACTACTGTTACGCCCTTGTC(37) ATCCAATAACTACTGTTACGCCCTTCTT(27)
10	2_34044R 2_34044F1 2_34044F2	GTTTTGCGGGGTATGGAAT(19) ACCATACATTACATAAAATGTTCCTTTGGTCGGG(34) ATCAAAAATGTTCCTTTGGTCCGC(24)
11	2_16708F 2_16708R1 2_16708R2	TTTCGGACAGTGAACTGCAT(20) ACCATACATTACATAATGCATAGAAAAGTAGGCTGAA(37) ATCAAATGCATAGAAAAGTAGGCTCAG(27)
12	2_22541R 2_22541F1 2_22541F2	GGTACGTTTTAAAATTGATATGACCA(26) ACCATACATTACATAGATGTTCACAGATGTACTGATG(37) ATCAAGATGTTCACAGATGTACTGTTA(27)
13	2_21262R 2_21262F1 2_21262F2	TCTCCAAATCCAAACAACTCG(21) ATCAAATGATAACGGAATTGAAACCG(26) AACCATACATTACATATGATAACGGAATTGAAAGCA(36)
14	2_04951F 2_04951R1 2_04951R2	TGGGTTAACCAAACCATCTT(20) ACCATACATTACATATCCACTTATCCAAACAGAGAAC(37) ATCAATCCACTTATCCAAACAGAGTAT(27)
15	2_06275R 2_06275F1 2_06275F2	AATCAGTTGTGTCCCGTGCT(20) ATTACTACTAGACGGTGTGAACCCAAGATGAGACAT(36) ATCAATGTGAACCCAAGATGAGAGAC(26)

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16	2_01068R 2_01068F1 2_01068F2	GCAAAAACCCATAGGCACTT(20) GAAGAAGGTTGTAGAGAAAAGTAGATGCGAG(32) TGTAGAGAAAAGTAGATGCCAA(22)
17	2_02374F 2_02374R1 2_02374R2	GGGCATTCGTCGTTCTGTAT(20) ATCAAGCGTTGGAGAACGACTGAAAC(26) GAAGAAAGGTGGGAGGCGTAGGAGAACGACTGATAA(36)
18	2_22099F 2_22099R1 2_22099R2	TTTTTCCCTGCCCTCTTTTT(20) CCATAGATTACATAGGAGTTCGAATTGGTCAGCTA(35) ATCAAGAGTTCGAATTGGTCAGGTG(25)
19	2_27367R 2_27367F1 2_27367F2	TTCTTTCCGATTTCCACCAG(20) ATCAAGGGATGGGAAGAGGTTAGG(24) ACCATACATTACATAGGGATGGGAAGAGGTTTGA(34)
20	2_42732R 2_42732F1 2_42732F2	CGCATTGTGACCACAGAAGA(20) ATCAAAACATTCAGGTCCACTTCCG(25) ACCATACATTACATGAACATTGCT(35)
21	2_0018F 2_0018R1 2_0018R2	TAACCTCGGCTGCGAGATAC(20) AGATAGATAATTTCTCGATCACAGAAAACC(30) TTTCTCGATCACAGAAATCG(20)

E. PCR Running

DNA amplification was executed by Applied Biosystems manufacture' thermocycler according to the program described by [17]. The following table 2 presents the program that has been used.

Table 2 PCR Applied Program

Cycle stages	Temperature	during	Cycling number
Initial denaturation	94°C	5min	1
Denaturation	94°C	30S	35
Annealing	58 à 64°C	30S	
Elongation	72°C	3min	
Final elongation	72°C	5min	1
Stop	4°C	infininite	1

Source : [17]

Gel preparation was realized according to modified protocol of [18]. In fact, 125 ml of polyacrylamide 6 % solution mixed with APS/TEMED was prepared for gel. Thus, 18.8 ml of acrylamide-bis 40 % was taken and introduced into a jar. Then, 2.5 ml of 50X TEA and 53.7 ml of distilled water were added. Subsequently, 50 ml of APS/TEMED solution has been prepared. It has been consisted of introducing 1 ml of APS/TEMED to a 50 ml of distilled water. Particles of APS/TEMED were dispersed and dissolved by the tip scrabbled gently on the side of the sachet after the introduction of distilled water. This has permitted to completely dissolve APS/TEMED in the water. Solution was removed from the sachet and has been introduced to 50 ml of distilled water. Mixture was done by returning and lifting during a couple of time. The whole solution was added to polyacrylamide solution and mixed also by returning and lifting to obtain the gel solution. Therefore, the solution was poured in the tank under where the glass was previously washed with washing solution and deposited on the right face. After one hour, the gel was energetically fixed on the glass and was immediately used.

The glass where acrylamide gel was fixed was deposited in the electrophoresis tank. This tank contents 1X TAE buffer solution. The quantity of buffer solution was regulated in the way it covers slightly gel surface. 2.5 µl of bromothymol blue was added in each tube containing the amplified DNA by PCR to indicate DNA migration in the gel well and to appreciate migration level. Each gel well has received 4 µl of the tube content. Molecular weight markers were introduced in two extreme wells that have received amplified DNA. The apparatus has been connected to a generator where the tension was limited to 120 V. After 1h30 min of migration, the glass was removed and immersed into ethidium bromide solution during a time varying between 45 min to 1 h. This solution was prepared by adding 10 µl of BE into 200 ml of distilled water. Bands observation was done under UV rays by turning the gel face directly toward UV rays.

Twenty-one SNPs markers converted into AS-PCR furnished by Kirkhouse Trust project were screened. Behavior of each molecular marker on different varieties has permitted to identify polymorphic markers from none polymorphic. The sign (+) was used for band presence, the sign (-) for band absence [16] and the sign (++) for codominant situation.

F. Statistical Data Analysis

The R software was used for variance analysis followed by Tukey test to separate significant means differences. Correlation test was equally done by the same software.

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III. RESULTS AND DISCUSSION

A. Tested Varieties Status

The variance analysis of cowpea wilt fusarium severity has revealed the existence of significant differences between varieties. The high index severity was observed in IR15MA33 (4,00) variety followed by IT99K-573-2-1 (3,67) which were susceptible. In contrary, TVU109, IT98K-503-1 and IR16-MA-K varieties with 1.67 of severity index and CB46 and TV410 with respectively 2,00 and 2,33 of severity index seem to be resistant. Similar investigation to the current study conducted by [12] have revealed that, CB46 variety was resistant to race 3 and IT82E-18 was susceptible. Otherwise, those authors reported that, the resistance of CB46 variety is controlled by a single dominant gene. The varieties disparity reaction toward this parasitic fungus can be explained by the fact that, variety response system varies from one genotype to another [19].

The same, variance analysis of pods number per plant, seeds number per pods and plant height have shown significant differences between varieties. The high pod number was observed in TVU109 variety which is 6 followed respectively by CB46 (5) and IT98-503-1 (5) varieties. Those varieties where the pods number was high corresponded to resistant varieties. The IITA IT99K-573-2-1 susceptible variety has presented the lowest pod number per plant which is 0 followed by other susceptible varieties which are IR15-MA02 (1), IT82E-18 (2) and IR15-MA33 (2). Obtained results were similar to [20] and [21] who have worked on the effect of cowpea fusarium wilt disease respectively on the yield production in Brazil and Nigeria. They reported that, the pods number per plant was significantly reduced.

The high seeds number per pod was observed in TVU410 with an average seeds number of 5 per pod followed by IR16MAP (5), dCB46 (5) and TVU109 (5) varieties. The lowest seeds number per plant was obtained in IR15MA33 and IT99K-573-2-1 with an average seed number per pod of 1 followed by IR15-MA02 and IT82E-18 (2) varieties. Those results show that, susceptible varieties have presented a reduced seeds number per pod compare to normal seeds number that each pod should contain.

The highest plant height was obtained in IT82E-18 variety which is 135.00 cm of height followed by TVU410 (122.50 cm) and TVU109 (114.83 cm) varieties. The smallest height was observed in IR15MA33 (38.67 cm) variety followed by IT99K-573-2-1 (40.00 cm), IR16MAP (50.00 cm) and CB46 (51.67 cm) varieties respectively. Those results show that, plant height was less affected by the disease though the smallest height was observed in susceptible variety. Again, results show that, plant height is less influenced by cowpea wilt fusarium disease. This result is in accordance with the works of [21] who have reported that, fusarium wilt disease does not influence enough plant height. Nevertheless, previous works have shown that, fusarium reduces considerably the plant height [22]. This different reaction can be explained by the fact that; environmental conditions can influence the effect of cowpea wilt fusarium disease. According to [12], vascular discoloration varies very little with the environment while the aerial phenotypical variation of the plant is very high. This observation explains the absence of significant correlation between fusarium wilt disease severity and the plant height. This result can be explained by the fact that, generally, the disease symptoms appear during flowering and pod formation [22]. At this period, the plant has reached its major height. It indicates that, disease severity affects more pods number per plant and seeds number per plant than the height. Similar observation is reported by [20]. It suggests therefore that fusarium wilt disease has double reduction effect on cowpea yield production [20], [21].

Table 3 Measured Parameters during « root dip method » Screening

Variety	Severity	Pods number per plant	Seeds number per pod	Height
IR15MA33	4.00 ± 0.00^{a}	$2.33\pm0,00^{ab}$	0.67 ± 0.00^{d}	38.67 ± 12.73^{c}
IT99K-573-2-1	3.67 ± 0.59^{ab}	$0.67\pm1,43^{ab}$	1.00 ± 0.77^{cd}	40.00 ± 12.73^{c}
IT82E-18	3.33 ± 0.59^{ab}	$2.00\pm1,43^{ab}$	2.33 ± 0.77^{bcd}	135.00 ± 12.73^{a}
IR15MA02	3.00 ± 0.59^{ab}	$1.67\pm1,43^{ab}$	2.33 ± 0.77^{acd}	75.00 ± 12.73^{abc}
IR16MAP	2.67 ± 0.59^{ab}	$5.33\pm1,43^{ab}$	5.33 ± 0.77^{a}	50.00 ± 12.73^{c}
TVU410	2.33 ± 0.59^{ab}	$4.33\pm1,43^{ab}$	5.33 ± 0.77^{a}	122.50 ± 12.70^{a}
CB46	2.00 ± 0.59^{ab}	$5.67\pm1,43^{ab}$	5.33 ± 0.77^{a}	51.67 ± 12.73^{bc}
IT98K-503-1	1.67 ± 0.59^{b}	$5.33\pm1,43^{ab}$	3.67 ± 0.77^{abc}	75.00 ± 12.73^{abc}
TV109	1.67 ± 0.59^{b}	$6.67\pm1,43^{a}$	5.33 ± 0.77^{a}	114.83 ± 12.73^{ab}
IR16MAK	1.67 ± 0.59^{b}	$3.33\pm1,43^{ab}$	4.67 ± 0.77^{ab}	81.00 ± 12.73^{abc}

In the same colon of same parameter, means followed by the same letters are not significantly different at the probability rate of 5 %

Results revealed in correlation table (Table4) show negative significant correlation between severity and the pods number per plant, severity and seeds number per pods. In contrary, the pods number per plant is positively correlated with the seeds number per pod. This result indicates that, when the plant is normally developed without the disease constraint, it can produce not just a high number of seeds but also an important number of seeds per pod. Paradoxically, no significant correlation was observed between the height and severity, pods number and the height of the plant and seeds per pod and the height of the plant. Previous researchers in Nigeria have found also the absence of significant correlation of heights between infected plants by *Fusarium oxysporum* f.sp. *tracheiphilum* and the control plants [23]. The same, the pods number per plant are not necessary linked to plant height [24].

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Table 4 Correlation table between measured parameters

Variables	Severity	PNP	SNP	Height
Severity	**1**			
PNP	**-0,385**	**1**		
SNP	**-0,670**	**0,672**	**1**	
Height	-0,248	0,050	0.172	**1**

PNP : pod number per plant
SNP: seeds number per pod

The TVU109, IT98-503-1, CB46 et TV410 varieties were confirmed resistant to the *Fusarium oxysporum* f.sp. *tracheiphilum* strain isolated in Far North Region of Cameroon.

B. Potentials Molecular Markers for Resistance Against Cowpea wilt Fusarium Disease

The screening of 21 Single Nucleotides Polymorphism (SNPs) converted to AS-PCR markers have permitted to identify polymorphic markers. Six among tested markers have presented bands polymorphism after electrophoresis. Two categories of polymorphism were observed. The first polymorphic category was linked to the band presence in resistance varieties and the band absence in susceptible varieties (2_31831, 2_27367, 2_21262) (Table 5).

The second polymorphic category was characterized by the band position variability observed between susceptible varieties and resistant varieties (2_00018, 2_02374, 2_06275). The polymorphism was not observed in fifteen other markers (2_22541, 2_22099, 2_16708, 2_26364, 2_09924, 2_04951, 2_04048, 2_33548, 2_23395, 2_20447, 2_21262, 2_34044, 2_50243, 2_01068, 2_42732). Polymorphism absence was shown by the bands absence or by the uniformity of bands position in susceptible and resistant varieties (Tableau 5).

2_31831, 2_27367 and 2_02374 markers have shown that the bands linked to the resistance gene against fusarium wilt disease were located in same position in CB46 variety and in TVU410 variety. The association of certain markers to the resistance gen is noted in plant genetic defence system against a pathogen. Thus, results of the present investigation were in accordance with the works of [25] who have demonstrated the dominants microsatellites markers associated to the resistant gene were revealed by the formation of DNA bands in resistant varieties while DNA bands were absent in susceptible varieties. The codominant markers permit to differentiate homozygote genotype form the heterozygote genotype [26].

Table 5 Markers Screening Associated to the Resistant Gene Against *Fot*

Varieties / N° Markers		CB46	TVU410	IT82E-18	IT99-573-2-1
		R	R	S	S
1	**2_0018**	**++**	**+**	**+**	**+**
2	**2_31831**	**+**	**+**	**-**	**-**
3	2_22541	+	-	+	+
4	2_22099	-	-	-	+
5	2_16708	-	-	-	-
6	2_26364	+	+	-	+
7	2_09924	-	-	-	-
8	**2_27367**	**+**	**+**	**-**	**-**
9	2_04951	+	+	+	+
10	2_04048	-	+	+	+
11	2_33548	+	+	+	+
12	2_23395	+	+	+	+
13	2_20447	+	+	+	+
14	2_21262	+	+	+	+
15	2_34044	+	+	+	+
16	**2_51968**	**++**	**++**	**+**	**+**
17	2_50243	+	-	+	+
18	**2_02374**	**+**	**++**	**+**	**+**
19	2_01068	+	+	+	+
20	2_42732	+	+	+	+
21	**2_06275**	**++**	**+**	**+**	**+**

R= Resistant S= susceptible

[27] Reported that, after the screening of SSRs markers, many were not selected because they were not polymorphic. Monomorphism is manifested by the absence of DNA bands in susceptible and resistant varieties or the presence of bands at same level in all varieties [28], [29], [30]. This suggest that, markers should be screened to select the more polymorphic to fulfil the requirement of markers assisted breeding [31]. It is in this regard that [25] have obtained only one polymorphic marker associated to the resistant gene against chickpea fusarium after the screening of 20 microsatellites markers. The

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results of the present study are in accordance with the previous works [11] who have showed the resistance of TVU410 variety against race 3 and 4 of *Fusarium oxysporum* f.sp. *tracheiphilum* pathogen and the resistance of CB6 against race 4 and susceptible to race 4 [11]. This demonstrate that the marker is associated to the resistance gene directed against race 3. In contrary, 2_22099 marker associated to resistant gene in TVU410 is absent in CB46 resistant variety. This result suggests the association of this marker to resistant gene against race 4. It can be explained by the existence of at least two resistant genes in TVU410 variety. Therefore, it is necessary to use 2_22099 markers in addition to others markers to detect if the variety has two or one resistant gene. Nevertheless, in some cowpea varieties, it may exist two resistant genes against cowpea fusarium wilt inherited from two different plants [32]. The absence of DNA bands in 2_16708 and 2_09924 can be explained by the none associability of these oligonucleotides to the specie's genomic. Markers screening has permitted to select those who are polymorphic and therefore discriminate resistant varieties from susceptible varieties.

IV. CONCLUSION

The present study has allowed to determine the pathological status of tested cowpea varieties vis-à-vis the fusarium strain isolated in Sudano-Sahalian Zone of Cameroon. Some varieties were revealed susceptible while a good number of them were confirmed resistant/tolerant. This result offers in perspective the possibility of integrating these varieties in varietal breeding program. A supplementary work is needed for the establishment of genetic map.

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