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CENTRE NATIONAL DE RECHERCHE AGRONOMIQUE

Breeding of cocoa (*Theobroma cacao* L.) genotypes tolerant/resistant to cocoa swollen shoot virus (CSSVs) in cocoa orchards infected by the disease in Côte d'Ivoire

Cocoa program

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PLAN

1. CONTEXT AND JUSTIFICATION

2. MATERIAL AND METHODS

3. RESULTS AND DISCUSSION

4. CONCLUSION AND PERSPECTIVES

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1. CONTEXT AND JUSTIFICATION

CONTEXT AND JUSTIFICATION

Theobroma cacao L. Perennial plant (2n), tropical, native to Central and South America

Cocoa: Chocolate, Food, Cosmetic, pharmacology

Fig 1. Pods and beans of cocoa tree

IMPORTANCE OF COCOA TREE IN CÔTE D'IVOIRE

- **Côte d'Ivoire**: 1st world cocoa producer of cocoa beans with around 2 010 000 tones in 2020
- **Cocoa tree:** 1st exportation crops of Côte d'Ivoire and contribute to 40% of PIB and 44% export revenue

PRODUCTION CONTRAINTS IN CÔTE D'IVOIRE

- Aging of the orchard ;
- Declining of soil fertility ;
- Low mastery of good agricultural pratice by producers
- Poor quality of commercial cocoa.
- High disease and insects pests pressure
  - **COCOA SWOLLEN SHOOT VIRUS DISEASE**

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CONTEXT AND JUSTIFICATION

- **CONSEQUENCES OF CSSVD**

➢ Upsurge in Côte d'Ivoire since 2003

- Losses of productivity of cocoa farms
- Destruction of cocoa orchards

Fig 2. cocoa field infected by CSSVD

PROBLEMATIC

- CSSVD posed a serious threay for Ivoirian cocoa farming

2. Objectives

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OBJECTIVES

General objective

Contribute to improve cocoa tree productivity in Côte d'Ivoire

Specific objective

- Identifed tolerants/resistant cocoa trees to swollen shoot ;

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3. MATERIAL ET METHODS

MATERIAL ET METHODS

PLANT MATERIAL

Departments	Group 1 (APT)	Group 2 (APS)	Total
Agnibilékro	2	0	2
Bangolo	9	0	9
Bouaflé	107	38	145
Duekoue	25	0	25
Issia	7	0	7
Meagui	60	10	70
Sinfra	58	1	59
Soubre	3	0	3
Total	271	49	320

- 320 farms cocoa accessions

✓ Two phénotypics groups :

- Group 1 (APT) : Asymptomatics accessions in field in CSSV infected areas
- Group 2 (APS) : Symptomatics accessions in field

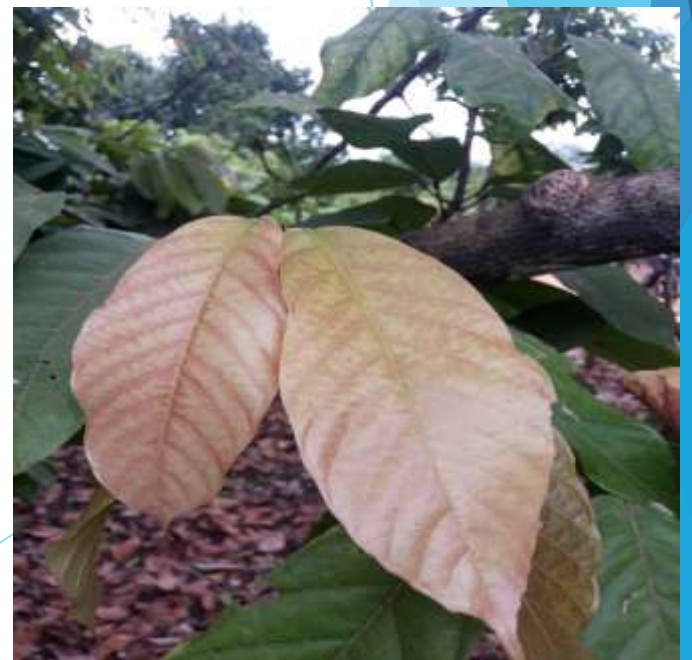

Fig 3. Symptoms of swollen shoot on cocoa tree in field

MATERIAL ET METHODS

METHODS

- **Grafting on Amelonado seedling of 320 farms accessions (10 plants/accession)**

- **Data collect on grafts and rootstook (during 1 year)**

  - Number of plants per accession with CSSV symptoms ;
  - Type of symptom
  - Latency time for the appearance of the first symptom per accession

- **Collect of samples leafs per accession in greenhouse for molecular analysis**

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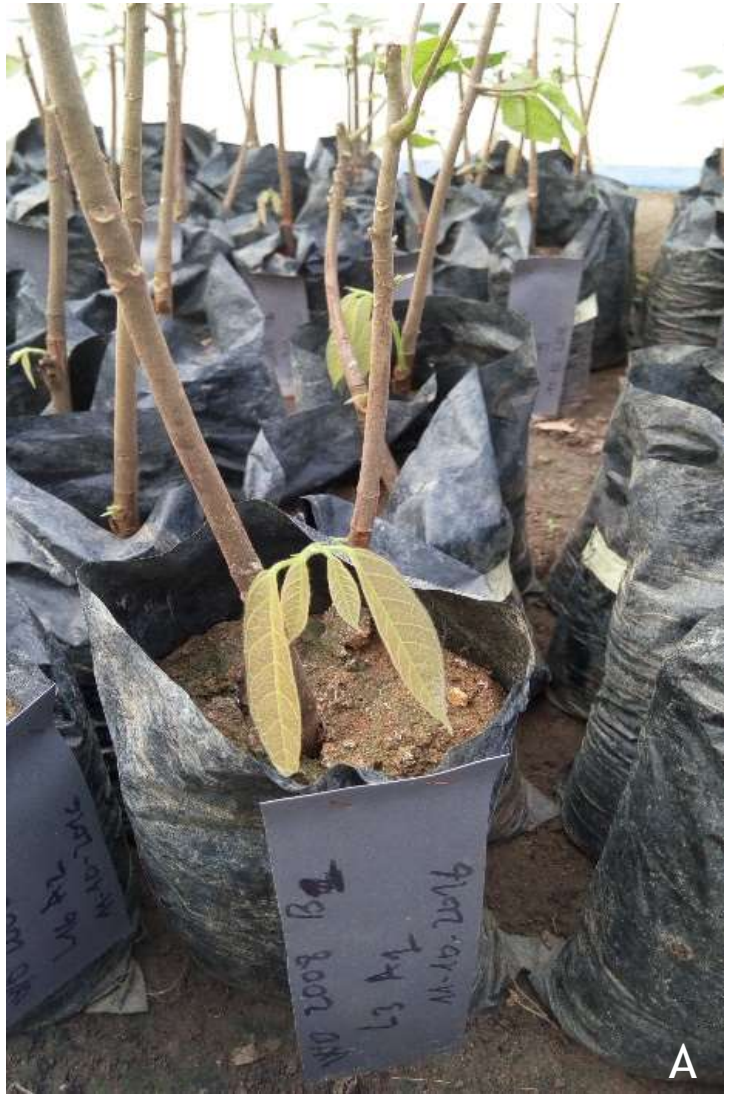

Fig 4. Cloning of symptomatic and symptomatic cocoa trees in nursery (A) & (B)

MATERIAL ET METHODS

METHODS

- **DNA extraction and purification (protocole standard MATAB)**

- **PCR with Badna ¼ CSSV deg 2 ; Tm: 57°C; Size: 626 pb; design in reverse transcritase region of viral genome**

  - Collect and analyse PCR data
  - ✓ Electrophoresis on agarose gel at 1%:
  - ✓ Presence ou absence of bands at size 626 bp

- **qPCR TaqMan (Light Cycler 480)**

  - Primers CSSV Bd2 (mouvemnt protein zone of genome)
  - qPCR data collect:
    - ✓ Sigmoid courbe
    - ✓ Treshold cycle (Ct) inferior to 40
    - ✓ Inflorescence signal red

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4 RESULTS AND DISCUSSION

RESULTS AND DISCUSSIONS

RESULTS

- Three phenotypic groups :
- **Group 1 (APT)** : Asymptomatics accessions in field and greenhouse
- **Group 2 (APS)** : Symptomatics accessions in field and greenhouse
- **Group 3 (APT/APS):** Asymptomatics accessions in field but symptomatic in greenhouse: Presence of CSSV symptom in APT/APS is an indicator of tolerance

RESULTS

Fig 4. Symptom of CSSV on roostoock leaf in greenhouse

RESULTS

Tab 1: Number of cocoa samples positives in **PCR** with Badna ¼ CSSV Deg 2 primers per locality and phenotypic group

Origin of accessions	Number (percentage) of positive samples			
	APT	APS	APT/APS	Total
Agnibilékro	0 (0 %)	0	0	0 (0 %)
Bangolo	6 (66,67 %)	0	0	6 (66,67 %)
Bouaflé	55 (55,56 %)	27 (71,05 %)	5 (62,5 %)	87 (60 %)
Duekoue	9 (36 %)	0	0	9 (36 %)
Issia	0 (0 %)	0 (0 %)	0 (0 %)	0 (0 %)
Meagui	21 (37,5 %)	7 (70 %)	2 (50 %)	30 (42,85 %)
Sinfra	43 (78,18 %)	1 (100 %)	1 (33,33 %)	45 (76,27 %)
Soubre	2 (66,67 %)	0	0	2 (66,67 %)
Positive diagnostic by PCR	**136/255 (53,33 %)**	**35/49 (71,42 %)**	**8/16 (50 %)**	**179/320 (55,93 %)**

53,33 % of asymptomatic accessions are positive in PCR: TOLERANCE of these accessions to CSSV

RESULTS

Tab 2: Number of cocoa samples positives in **qPCR** with Bd2 primers per locality and phenotypic group

Origin of accessions	Number (percentage) of positive samples			
	APT	APS	APT/APS	Total
Agnibilékro	1 (50 %)	0	0	1 (50 %)
Bangolo	4 (44,44 %)	0	0	4 (44,44 %)
Bouaflé	45 (45,45 %)	31 (81,58 %)	7 (87,5 %)	83 (57,24 %)
Duekoue	6 (24 %)	0	0	6 (24 %)
Issia	2 (33,33 %)	0	1 (100 %)	3 (42,86 %)
Meagui	11 (19,64 %)	7 (70 %)	4 (100 %)	22 (31,43 %)
Sinfra	46 (83,64 %)	1(100 %)	3 (100 %)	50 (84,75 %)
Soubre	1 (33,33 %)	0	0	1 (33,33 %)
Diagnostic positif by qPCR	**116/255 (45,49 %)**	**39/49 (79,59 %)**	**15/16 (93,75 %)**	**170/320 (53,13 %)**

The sensitivity of qPCR diagnosis is higher than PCR diagnosis (Poitras & Houde, 2002)

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RESULTS AND DISCUSSIONS

Tab 3: Number of cocoa samples **positives in PCR and qPCR** primers per locality and phenotypic group

Origin of accessions	Number (percentage) of positive samples			
	APT	APS	APT/APS	Total
Agnibilékro	0 (0 %)	0	0	0
Bangolo	3 (33,33 %)	0	0	3 (33,33 %)
Bouaflé	21 (21,21 %)	21 (55,26 %)	5 (62,5 %)	47 (32,41 %)
Duekoue	1 (4 %)	0	0	1 (4 %)
Issia	0 (0 %)	0	0 (0 %)	0 (0 %)
Meagui	2 (3,57 %)	5 (50 %)	2 (50 %)	9 (12,86 %)
Sinfra	21 (38,18 %)	1 (100 %)	1 (33,33 %)	23 (38,98 %)
Soubre	1 (33,33 %)	0	0	1 (33,33 %)
Diagnostic positif PCR & qPCR	**49/255 (19,22 %)**	**27/49 (55,1 %)**	**8/16 (50 %)**	**84/320 (26,25 %)**

- **APT (asymptomatic cocoa accessions) positives to CSSV after PCR and qPCR:**

TOLERANT

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RESULTS AND DISCUSSIONS

Tab 4: Number of cocoa samples **négatives in PCR and qPCR** with Badna ¼ CSSV Deg 2 primers per locality and phenotypic group

Origines des accessions	**Nombre d'échantillons négatifs (%)**			
	APT	**APS**	**APT/APS**	**Total**
Agnibilékro	1 (50 %)	0	0	1 (50 %)
Bangolo	2 (22,22 %)	0	0	2 (22,22 %)
Bouaflé	21 (21,21 %)	4 (10,53 %)	1 (12,5 %)	26 (17,9 %)
Duekoue	10 (40 %)	0	0	10 (4 %)
Issia	4 (66,67 %)	0	0 (0 %)	4 (57,14 %)
Meagui	26 (46,43 %)	1 (10 %)	0 (0 %)	27 (38,57 %)
Sinfra	5 (9,09 %)	0(0 %)	0 (0 %)	5 (8,47 %))
Soubre	1 (33,33 %)	0	0	1 (33,33 %)
Diagnostic négatifs	**70/255 (27,45 %)**	**5/49 (10,20 %)**	**1/16 (6,25 %)**	**76/320 (23,75 %)**

- **27,45% of APT negative to PCR & qPCR: Resistance to CSSV or esquive.**

5 Conclusion et Perspectives

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Conclusion

- 19,22 % of APT are positifs in PCR and qPCR : Tolerance to CSSV
- 27,45 % of APT (negative PCR and qPCR) : Resistance to CSSV or Esquive

Perspective

Tolerant and resistant accessions could be introduce in the multilocal trials in infected areas for evaluate their performances in fields before their introduction in reciprocal reccurent selection of cocoa in Côte d'Ivoire.

5 Akcnowledgments

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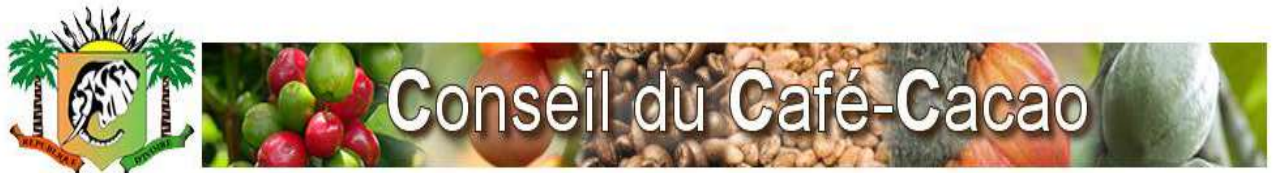
Ackownledgments



Le Conseil de Régulation, de Stabilisation et de Développement de la Filière Café-Cacao

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THANK YOU