COMPARISON OF RNA EXTRACTION METHOD ON BANANA AND APPLICATION OF cDNA-AFLP TECHNIQUE TO IDENTIFY BANANA'S GENES ASSOCIATED WITH SENSITIVITY TO *Colletotrichum musae*

Ludivine Lassois and M. Haïssam Jijakli.
Gembloux Agricultural University, Plant Pathology Unit. Passage des Déportés 2, B-5030 Gembloux, Belgium. lassois.l@fsagx.ac.be

INTRODUCTION

Crown rot and anthracnose constitute two major post-harvest diseases of exported bananas. Anthracnose results from the single action of *C. musae* while crown rot is caused by a parasitic complex which can contain *C. musae*. Geographical and seasonal variations in the symptoms expression were observed for both diseases. The symptoms expression is governed by two components; first a parasitic one which appears as the level of banana's contamination and the second one is a physiological component which determines the susceptibility of the fruit and the response of the fruit to the inoculum pressure..The biological answers of the fruit and thus its sensitivity to *C. musae* are controlled by the expression of certain genes. One of the approaches to understand mechanisms and reactions implicated in the variation of sensitivity in relation to the physiological state consists in identifying genes implicated in these processes and studying their expression. cDNA-AFLP is a non-bias molecular technique allowing analysis of the gene differentially expressed between several populations of cells. The first step of the cDNA-AFLP is the selection of a total RNA extraction method.

OBJECTIVES

1. To identify an efficient RNA extraction method on peel and pulp of green and mature bananas.
2. To develop and test the cDNA-AFLP strategy on banana model.

MATERIALS AND METHOD

Sample preparation

Peel and pulp tissues of green and mature bananas

***Musa acuminata* (AAA)**

Total RNA extraction method

Methods tested	Green banana	Mature banana
1	X	X
2	X	X
3	X	X
4	X	X
5	X	X
6		X
7		X

1:Trizol® Reagent (Invitrogen); 2:SV Total RNA Isolation System (Promega). 3: Liu *et al.*, 1998; 4: Mbéguié-A-Mbéguié (Personnal communication); 5:Asif M.H. *et al.*, 2000; 6:Gehrig *et al.*, 2000 (GITC); 7:Gehrig *et al.*, 2000 (GHCL)

Comparison of the RNA quantity and quality

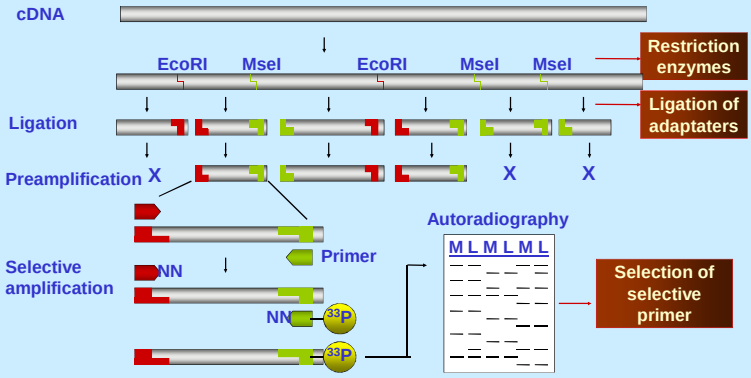
- RNA quantity: A_{260}
- RNA quality: A_{260}/A_{280}
- RNA integrity: running on agarose gel

Selection of the most efficient method on peel and pulp of green and mature banana

Total RNA → PolyA⁺ mRNA synthesis → mRNA

cDNA-AFLP

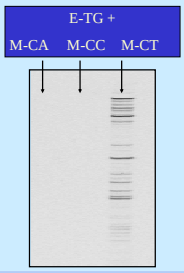
Reverse transcription (sscDNA)



RESULTS AND DISCUSSION

1. Selection of the most efficient RNA extraction method

After comparison, it arises that the technique 4 of Mbéguié-A-Mbéguié (personnal communication) is the best. Average yields were 70 µg/g and 48 µg/g of fresh green pulp and peel of bananas respectively, 63 µg/g and 44 µg/g of fresh pulp and peel of mature bananas respectively. A260/A280 was always ranged between 1,8 and 2,2 and rRNA bands were systematically observable after running on an agarose gel 1%.



2. Application of the cDNA-AFLP

Bands of cDNA were observable on autoradiography with the use of EcoRI and MseI as restriction enzymes.
In this case, the couple of selective primer E+TG/M+CT allows to obtain an interpretable number of bands of cDNA.
Currently, a screening is carried out to select others combinations of selectives primers potential E+2 and M+2 among 256 combinations.

CONCLUSION

Among the 7 RNA extraction methods tested, Mbéguié-A-Mbéguié method was selected. This technique is reproducible and makes it possible to obtain RNA of quality and in sufficient quantity for the realization of the cDNA-AFLP. Furthermore, the cDNA-AFLP technique was successfully applied on banana and is ready to be use to compare gene expression of banana sensitive and less sensitive on *C. musae*. It will be allow a further characterization of the cellular mechanisms and the genetic determinants which underlie the reactions of sensitivity of bananas to the post-harvest diseases caused by *C. musae*.

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