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**

<table>
<thead>
<tr>
<th>Methods tested</th>
<th>Green banana</th>
<th>Mature banana</th>
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<tbody>
<tr>
<td>1</td>
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<tr>
<td>2</td>
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<td>X</td>
<td>X</td>
</tr>
<tr>
<td>7</td>
<td>X</td>
<td>X</td>
</tr>
</tbody>
</table>

*Trizol® Reagent (Invitrogen); 2:SV Total RNA Isolation System (Promega); 3: Liu et al., 1999; 4: Mbéguié-A-Mbéguié (Personnal communication); 5: Asif M.H. et al., 2000; 6: Gehler et al., 2000 (GITC); 7: Gehler et al., 2000 (CDAFC).*

**Total RNA, PolyA+ mRNA synthesis, mRNA, cDNA-AFLP**

### 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 allowed to obtain an interpretable number of bands of cDNA. Currently, a screening is carried out to select others combinations of selective 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 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.

### ACKNOWLEDGMENT and REFERENCES

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**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.