RISK ASSESSMENT OF SPREADING *BANANA STREAK VIRUS* (BSV) THROUGH *IN VITRO* CULTURE

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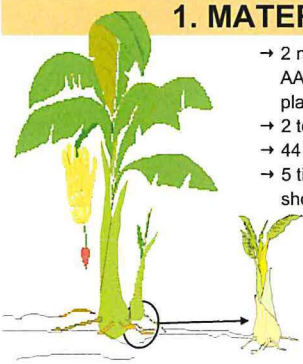
BACKGROUND

***In vitro* multiplication** is one of the main **abiotic stresses** triggering the production of episomal infectious particles of *Banana streak virus* (BSV) in inter-specific banana hybrids, through the activation of BSV **endogenous pararetrovirus** (EPRV) sequences integrated into the genome of *Musa balbisiana* (noted B). Nevertheless, mass production of vitroplantlets remains the most widely used method for diffusing wild *Musa* cultivars or new improved hybrid species. Therefore, there is a need to evaluate the effects of *in vitro* culture on the activation of BSV EPRVs and to **assess the risk of spreading BSV through the diffusion of micropropagated banana vitroplants**. Our work aims at:

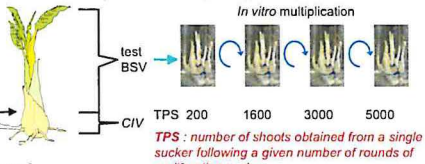
Identifying which steps of *in vitro* culture are involved in the activation of BSV EPRVs, for natural and created banana interspecific hybrid cultivars,

1. Checking whether all the hybrid cultivars studied here go through similar activation patterns during *in vitro* culture,
2. Checking whether distinct BSV EPRVs corresponding to distinct BSV episomal strains display similar activation patterns,
3. Checking whether distinct genotypes of banana hybrid cultivars behave differently during *in vitro* culture.

1. MATERIAL



→ 2 natural (**Kelong Mekintu & Penkelon**, genotype AAB) et 1 hybrid (**CRBP39**, genotype AAAB), plantain cultivars, all **healthy** (not infected by BSV)
→ 2 to 3 lines propagated *in vitro* for each cv,
→ 44 shoots analyzed at each time point,
→ 5 time points corresponding to TPS (total produced shoots) values of 0, 200, 1600, 3000 and 5000.



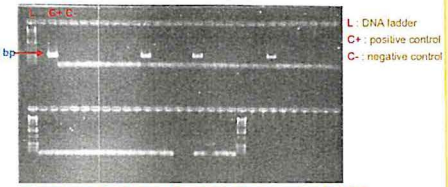
***TPS** : number of shoots obtained from a single sucker following a given number of rounds of proliferation cycles.*

Healthy mother plant and sucker

In vitro multiplication of plant material was performed by VITROPIC., a subsidiary of CIRAD specialized in mass propagation and commercialisation of banana and pineapple vitroplants.
Vitropic SA, ZAE des Avants, F-34270 St Mathieu de Tréviers, France - http://www.vitropic.fr/

2. METHODS

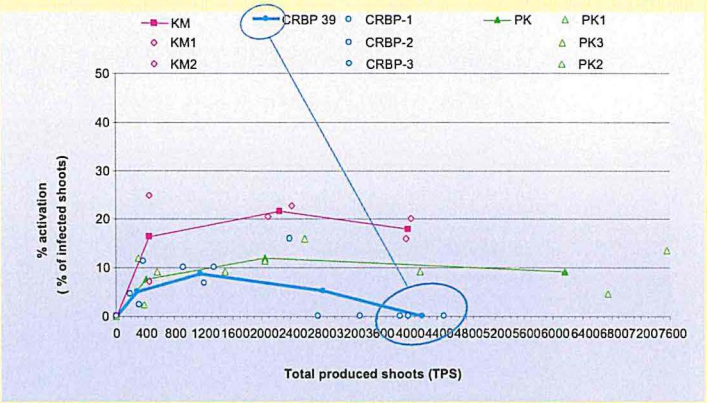
→ Detection of BSV strains Obino l'Ewaï (BSV-OI) and Goldfinger (BSV-Gf) by immunocapture PCR.



Detection of BSV-OI by IC-PCR in Penkelon line N°3317

3. RESULTS

% of plantlets infected by BSV-OI following increasing numbers of proliferation stages

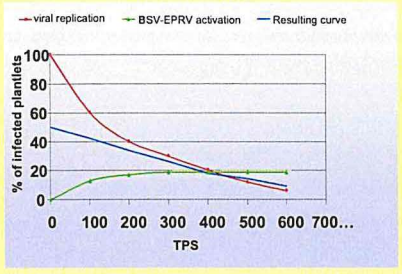


1. **The percentage of infected plantlets increases steadily at the beginning of the *in vitro* proliferation stage.**
2. **A percentage of infected plants comprised between 10% (Penkelon and CRBP 39) and 20% (Kelong Mekintu) is reached for TPS values comprised between 800 and 2000, depending on lines and cultivars. This percentage reaches a plateau then decreases. For hybrid CRBP39, 0% activation is reached at TPS = 2200.**
3. **There are differences in activation patterns depending on BSV strains. No BSV-Gf strain could be detected in the KM nor PK cultivars, although the *M. balbisiana* genome harbours activatable BSV-Gf EPRVs.**
4. **Shapes of the activation curves are similar for the three cultivars studied. Only TPS values registered at given times differ between cultivars.**

CONCLUSIONS & PROSPECTS

The pattern of infected plants observed during *in vitro* culture is the same for all the cultivars and BSV strains studied. It could result from concomitant **viral replication** and **BSV EPRV activation**.

Theoritical pattern of infected plants observed during *in vitro* culture



1. BSV-EPRV activation by *in vitro* culture.
2. **Viral replication**, with a dilution effect resulting from cellular multiplication being faster than viral replication during *in vitro* culture.
3. Addition of both phenomena could result in BSV-free plantlets being regenerated despite the presence and activation of BSV-EPRVs.

Have healthy (BSV-) vitroplants obtained at high TPS values lost their ability to express pathogenic BSV EPRVs?

Further experiments are in progress to check whether BSV- vitroplants obtained at high TPS values retain their healthy status. These experiments aim at unraveling EPRVs activation processes during *in vitro* culture, in order to mass produce safe banana germplasm by micropropagation

Does the activatable state of BSV EPRVs persist in healthy plantlets obtained at high TPS values?

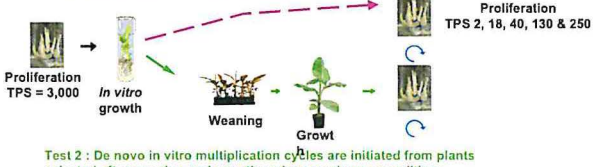
What is the level of transmission of BSV to the progeny from infected shoots following *in vitro* mass propagation?

Testing EPRV BSV reactivation by *in vitro* culture

(on 15 shoots from CRBP39 line 4791, certified BSV- at TPS = 2800)

This test aims at checking whether certified BSV- vitroplants obtained at high TPS values can give rise to BSV episomal particles when used as starting material for further *in vitro* mass propagation.

Test 1 : De novo *in vitro* multiplication cycles are initiated from vitroplants selected at the end of their growing phase (*in vitro*)



Test 2 : De novo in vitro multiplication cycles are initiated from plants selected after weaning and growth under greenhouse conditions

Testing the virus dilution hypothesis

BSV- OI infected CRBP39 shoots are used as starting material for de novo in vitro mass propagation.