Risk assessment of spreading *Banana streak virus* (BSV) through *in vitro* culture

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In vitro multiplication is one of the main abiotic stresses triggering the production of episomal infectious particles of *Banana streak virus* (BSV) in banana hybrid species harbouring some or all of the B (*Musa balbisiana*) genome, through the activation of BSV endogenous pararetrovirus (EPRV) sequences integrated into the B genome [1, 2]. Nevertheless, mass production of vitroplantlets remains the most widely used method for the diffusion of either *Musa* germplasm or new improved hybrid species. Therefore, there is a need to evaluate the effects of *in vitro* culture on the activation of infectious BSV EPRVs, in order to assess the risk of spreading BSV through the diffusion of micropropagated *Musa* germplasm. In this regard, it is of particular relevance to check (i) whether BSV EPRV activation occurs through *in vitro* culture in all inter-specific hybrid species as well as in « natural » plantain species and (ii) whether a correlation exists between the duration of *in vitro* subculture steps and the percentage of plantlets exhibiting BSV episomal particles. Experiments were carried out in order to answer these questions.

Virus-free suckers from two natural triploid plantains (AAB), Kelong Mekintu (KM) and Black Penkelon (PK), and the tetraploid hybrid (AAAB) CRBP 39 were selected and mass propagated using standard *in vitro* budding methods. During the successive multiplication (proliferation) subcultures, at least 40 shoots were randomly picked and screened for the presence of episomal BSV particles, using immuno-capture PCR based detection methods [3].

BSV episomal particles were detected during *in vitro* culture in both natural plantains and CRBP39 hybrid, with BSOLV being the predominantly detected BSV species. Both natural plantains and CRBP39 displayed similar patterns of activation. Percentages of plantlets indexed positive for BSOLV rapidly increased after the first subculture cycles. Depending on cultivars, maximum percentages of BSOLV positive plantlets ranged between 10 % (cv. Penkelon and CRBP 39) and 20 % (cv. Kelong Mekintu) and were reached for TPS (total produced shoots) values comprised between 800 and 2000. Following this increase step, a steady state phase was observed. Then the percentage of BSOLV positive plantlets decreased for the three cultivars studied when increasing the number of subcultures. This was especially striking for CRBP 39 hybrid, for which values of zero (i.e below the sensitivity threshold of detection tests) were reached from TPS values of 4000 onwards, although the decrease was less pronounced for the two other cultivars studied. These results will be presented and their impact on *in vitro* mass propagation and diffusion of *Musa* germplasm will be discussed.

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