How marker-assisted breeding of *Musa balbisiana* genitors devoid of infectious endogenous Banana streak virus sequences contributes to pesticide-free agroecological banana farming systems

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Abstract
Breeding new interspecific banana hybrid varieties with pests and disease resistance characters is instrumental for the development of pesticide-free agroecological banana farming systems. Such breeding relies on the combined use of *Musa acuminata* and *M. balbisiana* parents. Unfortunately, infectious alleles of endogenous Banana streak virus (eBSV) sequences are present in the genome of *M. balbisiana* genitors. Upon activation by biotic and abiotic stresses, these infectious eBSVs lead to spontaneous infections by several species of Banana streak virus (BSV) in interspecific hybrids harbouring both *M. acuminata* and *M. balbisiana* genomes.
In this work, we show that *M. balbisiana* diploid genitors available for breeding host at least one infectious eBSV. We also show how we segregated infectious and non-infectious eBSV alleles in seedy *M. balbisiana* diploids through self-pollination or chromosome doubling of haploid lines. We report on the successful breeding of *M. balbisiana* diploid genitors devoid of all infectious eBSV alleles following self-pollination and on the potential of breeding additional *M. balbisiana* diploid genitors free of infectious eBSVs by crossing parents displaying complementary eBSV patterns. This work paves the way to the safe use of *M. balbisiana* genitors for breeding banana interspecific hybrid varieties with no risk of activation of infectious eBSVs.

Keywords
Musa; Endogenous viral element; Banana streak virus; infectious alleles; marker-assisted breeding

Materials and methods
Plants used in this study originated from the open-field *Musa* collection of the Guadeloupe Biological Resources Center of Tropical Plant (CRB-PT) at Station de Neufchateau, Capesterre Belle-Eau, Guadeloupe, French West Indies. Haploid lines were created from male flower buds of PKW and their ploidy was assessed by flow cytometry [1]. Their homozygosity was assessed by the analysis of simple sequence repeats (SSR). Selfed-progenies were created by self-pollination followed by embryo rescue and their ploidy was assessed by flow cytometry. Interspecific and intraspecific crosses were performed similarly to self-pollination except that male and female flowers originated from separate plants. Progenies were conserved under insect-proof greenhouse conditions and placed under maximized abiotic stress conditions favoring the expression of infectious eBSV alleles when required. eBSV allelic genotyping was assessed for eBSOLV, eBSGFV and eBSIMV by PCR-based screening and Southern blot hybridization [2, 3]. Virus indexing was performed by immunocapture PCR (IC-PCR) [4].

Main results
PCR-based screening and Southern blot showed that all analyzed seedy *M. balbisiana* diploids harbor at least one infectious eBSV: 'Pisang Klutuk', 'Pisang Batu', 'Klue Tani' and model species PKW share the same eBSV allelic patterns, whereas other *M. balbisiana* diploids 'Lal Velchi', 'Singapuri', 'Cameroun', 'Butuhan' and 'Honduras' displayed modified eBSV allelic patterns for either one, two or all three eBSVs (eBSOLV, eBSGFV and eBSIMV).
eBSV modified alleles of 'Cameroun' and 'Honduras' were characterized. We showed that the modified eBSGFV allele of 'Cameroun' is infectious whereas its modified eBSOLV allele is not. Likewise, we showed that the modified eBSGFV allele of 'Honduras' is non-infectious.
For the first time, segregation of eBSOLV and eBSGFV alleles was achieved experimentally by chromosome doubling of haploid lines for model species 'PKW' and by self-pollination for diploids 'Pisang Batu' and 'Klue Tani', leading to improved *M. balbisiana* genitors devoid of some infectious eBSVs. Likewise, improved diploids 'Honduras' genitors devoid of all infectious eBSVs were obtained following self-pollination.

Conclusions

All *M. balbisiana* diploids analyzed harboured infectious eBSVs, confirming previous observations [5] and confirming also the fact no *M. balbisiana* genitor devoid of infectious eBSVs is currently available..

We achieved the first experimental segregation of eBSV alleles in several seedy *M. balbisiana* diploid genitors harbouring both infectious and non-infectious eBSV alleles, following chromosome doubling of haploid lines or self-pollination, leading to the first improved *M. balbisiana* genitors devoid of all infectious eBSV alleles. These results pave the way to the safe use of *M. balbisiana* genitors for breeding much needed improved interspecific banana hybrids with no risk of activating infectious eBSVs. They add to a recently reported complementary approach also resulting in the segregation of eBSVs [6]

The approach described in our work is now being used for creating additional *M. balbisiana* genitors devoid of infectious eBSVs by crossing diploid *M. balbisiana* parents with improved *M. balbisiana* diploid 'Honduras' devoid of all infectious eBSVs [7]. This approach will be extended to more diploid *M. balbisiana* parents with potential for breeding interspecific hybrids.

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