Development of a diagnostic DAS-ELISA for *Soybean mosaic virus* (SMV)- infected Colombian purple passion fruit

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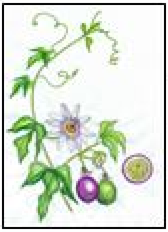
Background



- Colombian purple passion fruit (*Passiflora edulis* Sims.) is increasingly in demand as domestic and international markets recognize its unique taste, antioxidant properties, and potential for industrial processing.
- In Cundinamarca there has been an increase in passion fruit plants exhibiting typical symptoms of viral diseases such as leaf blistering and fruit deformation.
- *Soybean mosaic virus* (SMV) is present worldwide, can cause significant damage in soybean, and has been detected previously in Colombian fields[1].
- SMV has a very narrow host range apart from soybean and is naturally transmitted by aphid species in a non-persistent manner and via infected seeds.

A serological assay for Soybean mosaic virus has been developed for detection in purple passion fruit

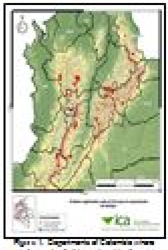
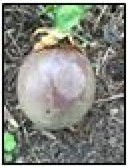


Figure 1. Departments of Colombia where purple passion fruit is produced (red)

Step 1: *Potyvirus* detection by ELISA

Figure 2. Cundinamarca and Boyacá, two departments where purple passion fruit is produced (blue) and the region where the samples were taken (purple).

- Samples of *P. edulis* plant material with virus-suspected symptoms were collected in 2016 and 2017 from 5 different farms in Cundinamarca and Boyacá, Colombia.
- Of 102 samples collected, 9% tested positive using a BIOREBA PTA (Plate-Trapped Antibody) - ELISA and a *Potyvirus*-group specific antibody.



Step 2: SMV detection by NGS

- 1 pooled sample of 4 different plants from 3 different farms (Sutamarchan; Pasca; Sylvania) using NGS (Total RNA, Illumina, RNA-Seq)[2]
- Assembly of several scaffolds and identification of viruses by blastX (NCBI), against taxid 10239 „viruses", rendered a nearly complete genomic sequence of Soybean mosaic virus (SMV) showing 99% aa ID in blastX

Step 3: SMV detection by RT-PCR

- Diagnostic RT-PCR for SMV detection with primers primers based on Botelho, et al. (2016)[3] inside the coat-protein coding region of SMV

✓ SMV-cpF: CAA GCA AAG ATG TAA ATG
✓ SMV cpR: GTC CAT TAG GCA TAT ACG
✓ Fragment Size: 469 bp 1 kb size standard ladder

- RNA isolation from leaf material according to Boom et al. (1990)[4]. cDNA synthesis with pMMLV RTase (fresh material) or Premium RevertAid RTase (frozen material) and random hexamer primers, nad5-PCR for quality control of RNA and cDNA synthesis according to Menzel et al. (2002)[5]

- 4 Samples from one farm appeared positive in a *Potyvirus*-group PTA ELISA and the SMV RT-PCR.

Figure 3. Detection of SMV in 4 passion fruit samples by RT-PCR amplifying 469 bp

Step 4: Validation of SMV DAS-ELISA

- Plant material from positive PCR products was sent to BIOREBA for testing by a SMV DAS-ELISA
- The SMV reagent was made against a recombinant coat protein[6]. For testing leaves, samples are homogenised 1:20 (w/v) in BIOREBA general extraction buffer (Art. No. 110120)
- The product is based on antibodies developed and validated according to Hajimorad (2017)[7]

The SMV DAS-ELISA Kit can detect a strain infecting passion fruit collected in Colombia and will soon be available for purchase from BIOREBA

References

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