Development of a certification program for virus-tested plant material in Colombia – a joint initiative

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Background

The goal of this research is to develop a pilot protocol for routine diagnosis that can be applied in **a certification program for virus-tested plant material** for several Colombian horticultural products. Three important exports from Colombia, ***Physalis peruviana*** **(physalis),** ***Passiflora edulis*** **Sims (purple passion fruit),** and ***Rosa hybrida*** **(ornamental rose)** have been selected as model plants for experimentation. A table of viruses affecting the three cultivars was collected for publication in Rodriguez et al. (2016). Biotest experiments, ELISA and Next Generation Sequencing (NGS) according to Massart et al. (2017) were conducted to explore the inventory of known and novel viruses present in the departments of Cundinamarca and Boyacá.

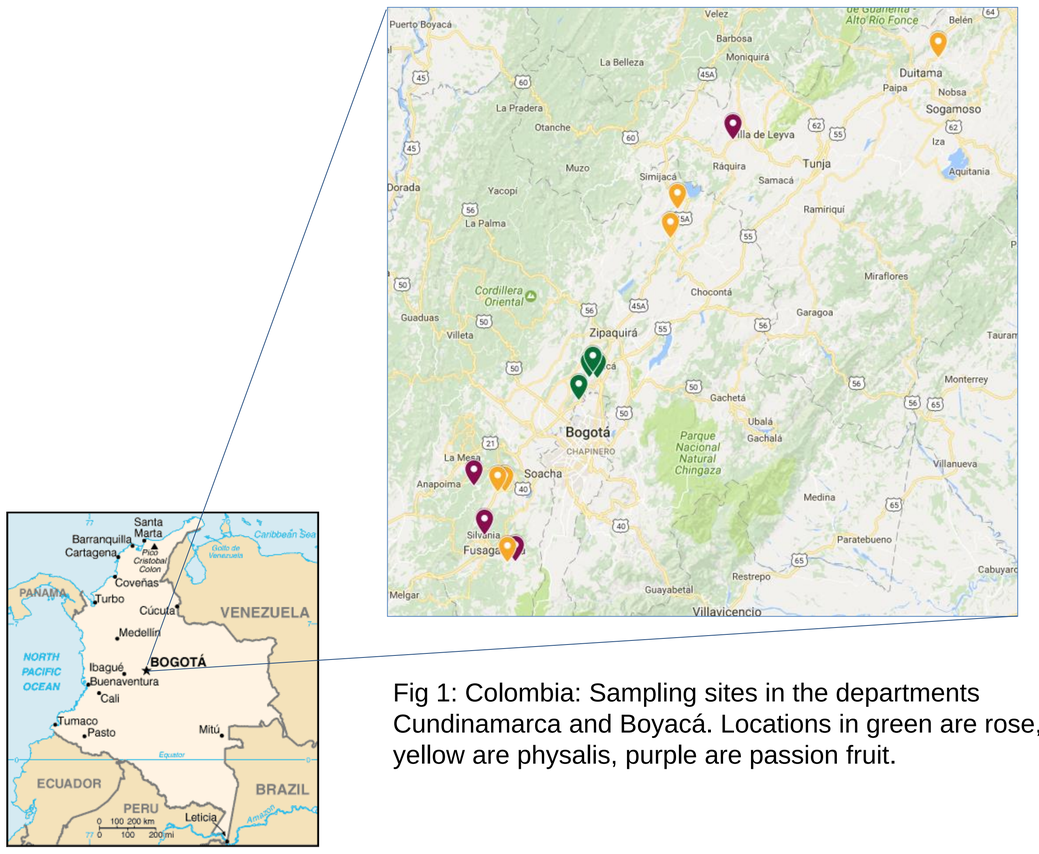

Fig 1: Colombia: Sampling sites in the departments Cundinamarca and Boyacá. Locations in green are rose, yellow are physalis, purple are passion fruit.

Materials and Methods

Fig 2: Virus suspected symptoms: blistering of leaves (a-c), and deformation of passion fruit (b). Local leaf blistering (d), mottling, and yellowing (e,f) of physalis leaves. Oak leaf pattern (g-i) in rose leaves.

Selection of farms was carried out based on size and economic importance. Initial visual plant symptom appraisal (Fig. 1) led collection of 67 physalis, 77 purple passion fruit, and 47 ornamental rose samples from 14 farms. **ELISA** was carried out at BIOREBA (CH) using antibodies of known viruses described in literature. A pooled sample of total RNA of blistered passion fruit (Fig. 2 a,c) and physalis (Fig. 2 d-f) was subjected to **RNA-Seq**. cDNA was synthesized with random hexamers and analysed by BaseClear (NL). *Passiflora edulis* Sims seeds from one farm were germinated under greenhouse conditions and inoculated onto *Chenopodium quinoa, Nicotiana benthamiana, Nicotiana tabacum Samsun,* and *Cucumis sativus cv Vorgebirgstraube* to understand whether the observed blistering symptom was seed and/or mechanically transmissible.

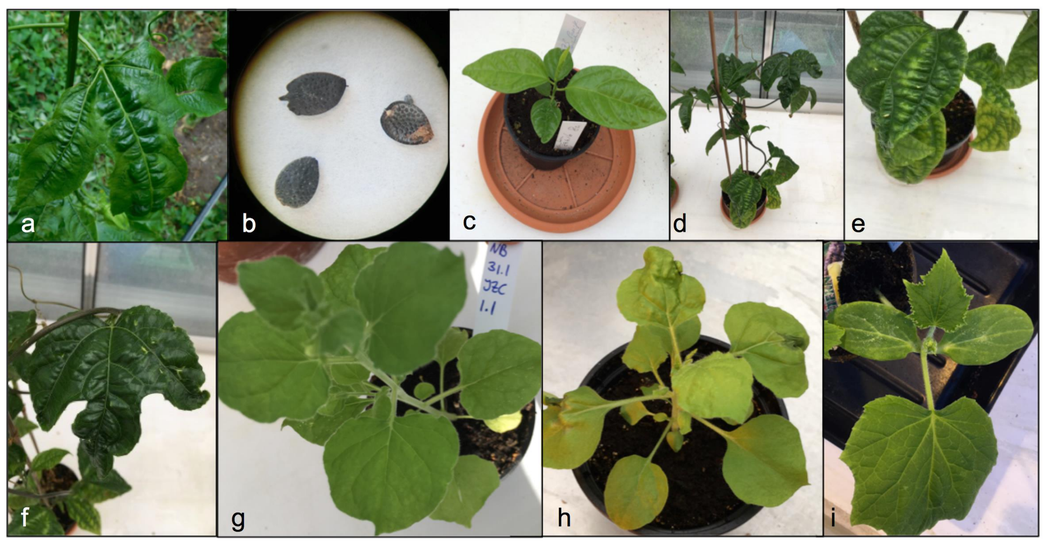

Fig 3: Biotest experiments: Blistering symptoms were observed in passion fruit in the field (a). Seeds were germinated (b). After initial emergence (c), the plant (d) began to express blistering symptoms on leaves (e,f). A first mechanical inoculation of blistered leaves onto *Nicotiana benthamiana* did not express symptoms (g). Leaf deformation was observed upon a second passage of *N. benthamiana* (h). Leaf curling was expressed in a third passage onto *Cucumis sativa* (i).

Results and Conclusion

Table 1 ELISA	DAS PVY	DAS TMV	DAS TSWV	PTA Poty	
Physalis peruviana	3/85	0/61	0/47	11/85	
***Passiflora edulis* Sims**	0/33	0/33	0/33	4/66	
	DAS PNRSV	DAS TSV	DAS ArMV		DAS ToRSV
Rosa hybrida	22/61	3/61	0/47	1/24	0/14

Table 1: Double Antibody Sandwich (DAS) and Plate Trapped Antibody (PTA) Enzyme-linked immunosorbent assay (ELISA) experiments

ELISA revealed known *Potato virus Y* (PVY*)*, *Prunus necrotic ringspot virus* (PNRSV), *Tobacco streak virus* (TSV), *Arabis mosaic virus* (ArMV), and viruses belonging to the genus *Potyvirus* in different cultivars (Table 1). Biotest experiments (Fig. 2) revealed blistering symptoms in germinated passionfruit, leaf deformation in *Nicotiana benthamiana*, and leaf roll in *Cucumis sativus*. NGS analysis of one *Passiflora edulis* Sims sample responded with 57% identity to Lilac ring mottle ilarvirus movement protein and with 65% identity to Tomato necrotic streak ilarvirus replicase. A second *Passiflora edulis* Sims sample from a different farm responded with 45% identity to *Poinsettia mosaic virus* replicase associated protein (genus *Tymovirus)* and with 65% identity to Tomato necrotic streak ilarvirus replicases. Based on this set of contigs, further confirmation by PCR using specific primers will be used on future samples to discover the distribution of the **novel ilarvirus and tymovirus** in Colombia.

Reliable and practical diagnostic tools will be developed for the most important viruses leading to a national agricultural certification program that will be established in a common project between German and Colombian universities, the Colombian Agricultural Institute (ICA), the Colombian Corporation of Agricultural Investigation (CORPOICA), and the International Center for Tropical Agriculture (CIAT). The competitiveness of Colombian products in domestic and international markets depends on the use of healthy plant material and virus-tested certification can improve quantity and quality of yields.

Literature

Rodríguez, MH, et al. (2016). Certificación de material vegetal sano en Colombia: Un análisis crítico de oportunidades y retos para controlar enfermedades ocasionadas por virus. Revista Colombiana de Ciencias Hortícolas 10:164-175

Massart, S. et al. (2017). Framework for the evaluation of biosecurity, commercial, regulatory and scientific impacts of plant viruses and viroids identified by NGS technologies. Front. Microbiol., doi.org/10.3389/fmicb.2017.00045

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