Strategien zur Einführung eines Zertifizierungsprogramms für virusgetestetes Pflanzenmaterial in Kolumbien

Strategies for the implementation of a certification program for virus-tested plant material in Colombia

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Introduction

Countries in the global south are major exporters of agricultural products, and in certain places no tools for controlling pathogens exist. Plant viruses severely affect Colombian crops, and studies indicate that economic losses caused by phytosanitary problems could be avoided with a standard procedure for preventive management. Three important exports from Colombia have been chosen as model plants for experimentation: ornamental rose (*Rosa sp.*), cape gooseberry (*Physalis peruviana*), and purple passion fruit (*Passiflora edulis* Sims). Selection of farms was carried out based on size and economic importance. 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.

Material and Methods

Biotest experiments, ELISA and Next Generation Sequencing (NGS) were conducted to explore the inventory of known and novel viruses present in the departments of Cundinamarca and Boyacá. Initial visual plant symptom appraisal led collection (Fig. 1) of 67 physalis, 77 purple passion fruit, and 47 ornamental rose samples from14 farms. ELISA was carried out at BIOREBA (CH) using antibodies of known viruses described in RODRIGUEZ et al. (2016). 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, N. tabacum* cv. Samsun*, N. clevelandii* and *Cucumis sativus* cv. Vorgebirgstraube to understand whether the observed blistering symptom was seed and/or mechanically transmissible.

Results and Discussion

ELISA revealed known *Potato virus Y, Prunus necrotic ringspot virus, Tobacco streak virus,* and further viruses belonging to the genus *Potyvirus* in different cultivars (Table 1). Biotest experiments revealed blistering symptoms in germinated passion fruit, mottling in *Nicotiana benthamiana*, leaf deformation in *Nicotiana tabacum* c.v. Samsun 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 current and future samples to discover the distribution of the novel ilarvirus and tymovirus in Colombia.

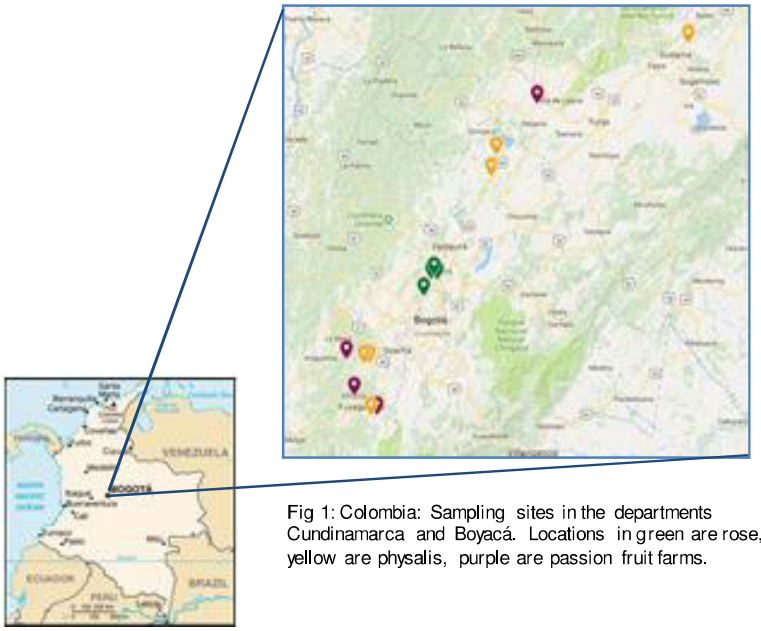

Fig 1: Colombia: Sampling sites in the departments Cundinamarca and Boyacá. Locations in green are rose, yellow are physalis, purple are passion fruit farms.

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

Figure 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.**

Table 1: **ELISA Results 2016**

Table 1 ELISA*	DAS** PVY	DAS TMV	DAS TSWV	PTA*** Poty	
Physalis peruviana	3/61	0/61	0/23	10/61	
Passiflora edulis Sims	0/33	0/33	0/33	4/44	
	DAS PNRSV	DAS TSV	DAS ArMV		DAS ToRSV
Rosa hybrida	17/37	3/37	0/23	1/24	0/14

* numbers indicate positive deterction

** Double Antibody Sandwich ELISA

*** Plate Trapped Antigen ELISA

Conclusion

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 agriculture in international markets depends on the use of healthy plant material and therefore, virus-free certification can improve quantity and quality of yields and contributes to better trade policy decision-making. Rapid expansion in global trade causes distribution of plant pathogens. Small national producers and larger major exporters of horticultural and agricultural products can benefit from tools for controlling pathogens.

Abstract

Plant viruses critically affect Colombian crops. Several studies indicate that economic losses caused by phytosanitary problems could be avoided by having a better understanding of these pathogens and using a standard procedure for preventive management as a guide. Three important exports from Colombia have been chosen as model plants for experimentation: ornamental rose (*Rosa sp*.), cape gooseberry (*Physalis peruviana*), and purple passion fruit (*Passiflora edulis* Sims) -- and the incidence of respective viruses in field tests in 2016-17 is presented. Based on initial findings, recommendations for a program for healthy plant material for nurseries can be provided. The establishment of a national agricultural certification program is being developed in a common project between research institutions, universities, the Colombian Agricultural Institute (ICA), the Colombian Corporation of Agricultural Investigation (CORPOICA), and the International Center for Tropical Agriculture (CIAT).

Literatur

RODRIGUEZ, 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.

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