Universität Basel

Research Project

ISCB - Genetic diversity and RNAi-based control of cassava mosaic geminiviruses in India.

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Principal Investigator(s) Hohn, Thomas ;
Organisation / Research unit
Departement Umweltwissenschaften / Molekulare Pflanzenvirologie (Hohn)
Department
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ISCB - Genetic Diversity and RNAi-based control of Cassava mosaic geminiviruses in India

Cassava is a crop used by subsistence farmers in tropical and subtropical regions. It is extensively cultivated in certain regions of India (e.g. Kerala, Andhra Pradesh, Maharashtra, Tamil Nadu) and also in other Asian and African countries, and its cultivation is expected to further increase due to climate changes and increased water shortage, since cassava is drought-adaptive. Viruses, especially the begomoviruses *Indian cassava mosaic-, ăSri Lankan cassava mosaic-* and *African Cassava mosaic viruses* (ICMV,SLCNV,ACMV here commonly I/SL/ACMV) cause dramatic losses in cassava cultivation. A consortium of Swiss and Indian scientists T Hohn and K Veluthambi, experts in begomo-virology, transgenesis and RNA-interference was formed to gain knowledge of the genetic diversity of I/SL/ACMV and their satellites in Southern India, as well as of the host plants affected and about their interaction. On this basis a durable resistance strategy shall be developed.

We aim to make use of a natural response of plants to viruses: “RNA interference (RNAi)”. The plant recognizes viral RNA in the form of double-strand (ds)RNA, a by-product of virus replication. Long dsRNA is chopped by dicer enzymes into small interfering (si)RNAs, 21 toă 24 base pair duplexes, consisting of a guide and a passenger-strand. The guide-strand forms together with the Slicer protein AGO a “RISC”-complex which recognizes and cleaves cognate, i.e. in our case viral RNA. A remarkable feature of RNAi is that, once started, it initiates a positive feedback loop, whereby the sliced RNA fragments are converted to more dsRNA by host RNA-dependent RNA polymerase, which give rise to more siRNAs. Furthermore, siRNAs or other components of the silencing pathway invade the plant systemically and protect from virus proliferation (reviewed by Ding and Voinnet, 2007; Hohn et al., 2007).

Viruses would not be successful, if they would not have found a countermeasure: silencing suppression. Viral silencing suppressors interfere with one or another step in the silencing pathway, by inhibiting e.g. dicing or slicing. Consequently, an equilibrium is established in the infected plant responsible for phenomena of full infection (the virus wins), recovery (the plant wins), meristem exclusion (silencing is most active in new growth), cross protection (silencing initiated by one virus is directed against a related virus) and synergism (the suppressors of two different viruses cooperate).

Our antiviral strategy is to shift this equilibrium in favour of the plant by providing dsRNA cognate to the virus sequences. As a result, more siRNAs are produced, more virus RNA is destroyed and more systemic silencing signal reaches the growing tissue. Finally plants recover fast from initial infection or even become immune to incoming virus.

Our project involves isolation, sequencing and cloning of the I/SL/ACV virus populations and their satellites occurring in the states of Andhra Pradesh, Maharashtra, Kerala, and Tamil Nadu. From these se-

quences a series of constructs will be derived that express hairpins, i.e. dsRNA with a loop between the RNA arms. We will construct variants that target all these viruses, including those that mimic natural micro (mi)RNAs or transacting (ta)siRNAs down-regulating host genes, and those provided with constitutive or virus-inducible promoters. We will optimize these constructs in transient assays to select those most efficient in initiating siRNA production. Selected constructs will then be cloned in Agrobacterium Ti-plasmid vectors and used for transformation of host plants. Although virus-resistant cassava is our goal, preliminary tests of the constructs could also be performed with the easily transformable *Nicotiana benthamiana* in one of our labs, as has been done by the applicants for African cassava mosaic begomovirus or with a more easily transformable model cassava cultivar with the help of Dr. Peng Zhang, Chinese academy of Science, Shanghai, thus extending a former collaboration of the Basel group with him.

When promising constructs are at hand we will approach proper industrial partners for producing appropriate transgenic elite Cassava cultivars and perform green house and netted field tests.ă Alternatively we will evaluate whether the transient approach (plant vaccination) will be economically feasible.

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Specify cooperation partners

ID	Kreditinhaber	Kooperationspartner	Institution	Laufzeit - von	Laufzeit - bis
270572	Hohn, Thomas	Veluthyambi Karrupanan	Madurai Agricultural University	01.01.2002	31.10.2012