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Investigations on *Citrus tristeza* Virus (CTV) and its Occurrence in *Citrus* Orchards in Arid and Semi Arid Zones of Sudan

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Abstract

The *Citrus tristeza* closterovirus (CTV) is a filamentous plant virus and has a very narrow host range confined to species of Rutaceae in which it is limited to phloem-associated cells. CTV is a major pathogen of *Citrus* spp., often causes quick decline and death, or stem pitting and reduced vigour, longevity, and yields in susceptible varieties and hence considered as a serious threat to the *Citrus* industry worldwide. In Sudan all *Citrus* trees are grafted mainly on sour orange rootstock and this yields a CTV-susceptible combination with scions of sweet orange, mandarin, grapefruit and others. CTV is a serious problem because it is readily transmitted in infected budwood and is also spread by several species of aphids.

During the trials to detect CTV in the Sudan a survey was initiated in 2003 and 2004. Fresh leaf material was collected from CTV suspected trees in different areas of the Sudan accompanied by tissue printing on nitrocellulose membranes. CTV was detected successfully in thirteen printed samples originating mainly from orange trees but were collected from different orchards. In two cases also a mandarin and a lime tree respectively reacted positively in this serological assay. Starting from RNA, extracted from fresh leaves, in a nested RT-PCR approach from ten samples a specific PCR product was amplified, substantiating the presence of CTV in four trees (three orange, one lime tree), which were presumably tested positive by Tissue Print. Cloning and sequencing of 9 specific PCR products proved the presence of CTV in *Citrus* trees in Sudanese orchards. Nested PCR-products from 5 samples revealed identical sequences. The nested primers enclosed 78 bp sequence showed 99% nucleotide identity to a CTV strain from California causing severe stem pitting symptoms (AF01623) whereas the other 4 samples are identical to the reference sequence (DSMZ PV-0332 from Israel) and showed 99% identity with CTV strains T30 and T36 from Florida (AF260651 and U16304). The two different sequences obtained from Sudan revealed lower nucleotide identity about 97%, indicating at least two different CTV-strains to be present in *Citrus* orchards in Sudan.

Keywords: *Citrus* virus detection, nested RT-PCR, Sequencing, Tissue printing