Investigations on Citrus tristeza virus (CTV) and its occurrence in citrus orchards in arid and semi arid zones of Sudan

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Introduction

Citrus tristeza virus (CTV) often causes quick decline and death or stem pitting and reduces vigour and longevity in susceptible Citrus varieties. Yields are shortened, hence CTV is 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 by infected budwood and is also spread by several species of aphids. Up to now, there was no serious work in the diagnosis of citrus viruses occurring in the Sudan by other methods than visual inspection.

Results

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 (Fig. 1, 2 and 3) in different areas of the Sudan accompanied by tissue printing on nitrocellulose membranes and RNA extraction of some leaf samples. CTV was detected successfully in thirteen printed samples originating mainly from orange trees but were collected from different orchards (table 1). In two cases also a mandarin and a lime tree respectively reacted positive in this serological assay. Starting from RNA, extracted from fresh leaves, in a nested RT-PCR approach (Olmos *et al.,1999*) from ten samples a specific PCR product was amplified (Fig. 4), 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 specific PCR products proved the presence of CTV in Citrus trees in Sudanese orchards. Nested PCR-products from three samples (S5, S9 and S10) 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 one sample (S6) is identical to the reference sequence (DSMZ PV-0332 from Israel) and showed 99% homology with CTV strains T30 and T36 from Florida (AF260651 and U16304). The two different sequences obtained from Sudan revealed lower nucleotide homology about 97%, indicating at least two different CTV-strains to be present in Citrus orchards in Sudan.

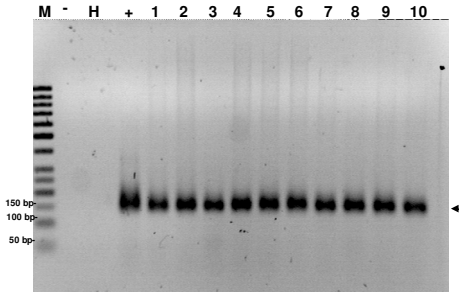

Fig. 4: Detection of Citrus tristeza virus (CTV) in 10 RNA samples from citrus trees using nested RT-PCR assay followed by electrophoretic analysis of PCR-products. Lane M: 50 bp DNA ladder, –: water; +: positive control from CTV infected tree (DSMZ PV-0332), H: healthy control, 1-10: tested samples S1 to S10 as indicated in table 1.

Table 1: CTV detection in different trees examined by Tissue Print, RT-PCR and/or Sequencing

sample	Tree	Location	Source	Test		
				Tissue Print	RT-PCR	Sequencing
S1	Orange 1	Northern State (Algureir)	Abdullah Ahmed Orchard	+	+	n.t.
S2	Orange 2			+	+	n.t.
S3	Orange 4			n.t.	+	n.t.
S4	Orange 6			n.t.	+	n.t.
S5	Orange 1		Aabood Gibril Orchard	n.t.	+	+
S6	Orange 2			n.t.	+	+
S7	Grapefruit 2	Khartoum State (Bahry)	Food Research Centre	n.t.	+	n.t.
S8	Lime 9		University Top Farm	+	+	n.t.
S9	Orange 1		Food Research Centre	n.t.	+	+
S10	Orange 8		University Top Farm	+	+	+
S11	Orange 5	Northern State (Algureir)	Abdullah Ahmed Orchard	+	n.t.	n.t.
S12	Orange		Aabood Gibril Orchard	+	n.t.	n.t.
S13	Orange1	River Nile State (Shendi)	El Hadi Mudthir Orchard	+	n.t.	n.t.
S14	Mandarin 3			+	n.t.	n.t.
S15	Orange 4			+	n.t.	n.t.
S16	Orange 3	Khartoum State (Bahry)	University Top Farm	+	n.t.	n.t.
S17	Orange 5			+	n.t.	n.t.
S18	Orange 10			+	n.t.	n.t.
S19	Orange 11			+	n.t.	n.t.

Samples from Sudan (S1-S19), which were tested positive either by Tissue Print using mAb (3DF1 and 3CA5, Plant Print Diagnostics S.L.), nested RT-PCR or sequencing after cloning of nested PCR products, + = tested positive, n.t. = not testet

Fig. 1: Areas of Sudan, where CTV has been detected by serological and/or molecular methods

Fig. 2: Stunted, CTV-affected orange tree on sour orange showing reduction in growth, yellowing and twig dieback (Shendi, River Nile State)

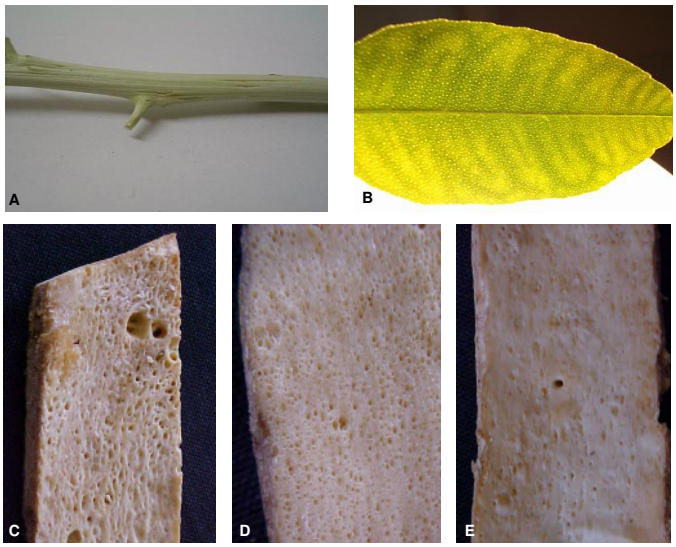

Fig. 3: Typical symptoms of CTV on different affected Citrus trees in Sudan (A) Severe pitting in the peeled stem of a grapefruit (B) Sweet orange leaf showing vein flecking symptom (C) Inner cambial face of piece of bark showing pronounced pinholing on the bark of the sour orange (D) A section of bark cut through the bud-union of a sweet orange on sour orange showing conspicuous pinholing or honeycombing in the bark of the sour orange (E) Inner cambial face of piece of bark showing inverse pitting of a grapefruit on sour orange.

Conclusions

Results indicated that the nested RT-PCR and Tissue Print using specific monoclonal antibodies are sensitive, accurate and efficient methods for CTV detection. The existence of Citrus tristeza virus (CTV) was confirmed in different areas and orchards of the Sudan by other methods than visual inspection and therefore considered as a first report Of CTV in the country.

Reference

Olmos, A., Cambra, M., Esteban,O., Gorris, M.T.,Terrada, E. (1999) New device and method for capture, reverse transcription and nested PCR in a single closed-tube. NAR **27**,15764-15765.

Acknowledgements

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