

# Molecular analysis of Citrus tristeza virus (CTV) and *Citrus* Viroids from the Sudan



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## Introduction

In the Sudan the mostly grown *Citrus* species are grapefruit (*Citrus paradisi*, Macfad.) cv. 'Foster pink' and 'march seedless', small fruited acid lime (*C. aurantifolia*, Swingle) cv. 'Baladi' (local), sweet orange (*C. sinensis*, Osbeck), mandarins (*C. reticulata*, Blanco) and lemons (*C. limon*, L.). These are grafted predominantly onto sour orange (*C. aurantium* L.) and also on 'Baladi' lime, which are suitable rootstocks for the arid climate and the high salinity of irrigated soils in the north-eastern region of the Sudan, where Citriculture is most abundant.

Certain viroids as well as *Citrus tristeza virus* (CTV) are long suspected to be responsible for many different graft transmissible diseases of Citrus in the arid and semi arid region of the Sudan creating shortage in food supply and also economic problems. Especially *Citrus* spp. grown on sour orange rootstocks yields a highly susceptible combination for CTV. Infected trees are often showing stem pitting and quick decline or die back. On the other hand, Citrus exocortis viroid (CEVd) and Hop stunt viroid (HSVd) infections of Citrus species are often symptomless on sour orange rootstocks. Exceptions are HSVd infected mandarins, who are chlorotic, stunted and exhibit typical pegs and gumming of the bark (Cachexia).

## Results

Serological and molecular techniques were applied and CTV was detected in oranges, mandarins, grapefruit and lime (fig. 1). Consecutively it was shown by RT-PCR, that some mandarins and orange trees were additionally infected with Citrus isolates of HSVd and CEVd (fig. 2). Full length viroid genomes were cloned and sequences determined. Secondary structure analysis of different Viroid-isolates revealed a characteristic genome-organisation of CEVd belonging to Genus *Pospiviroid* (fig. 3) and established Sudanese HSVd isolates as members of the Genus *Hostuviroid* (fig. 4).

## Detection of Citrus tristeza virus

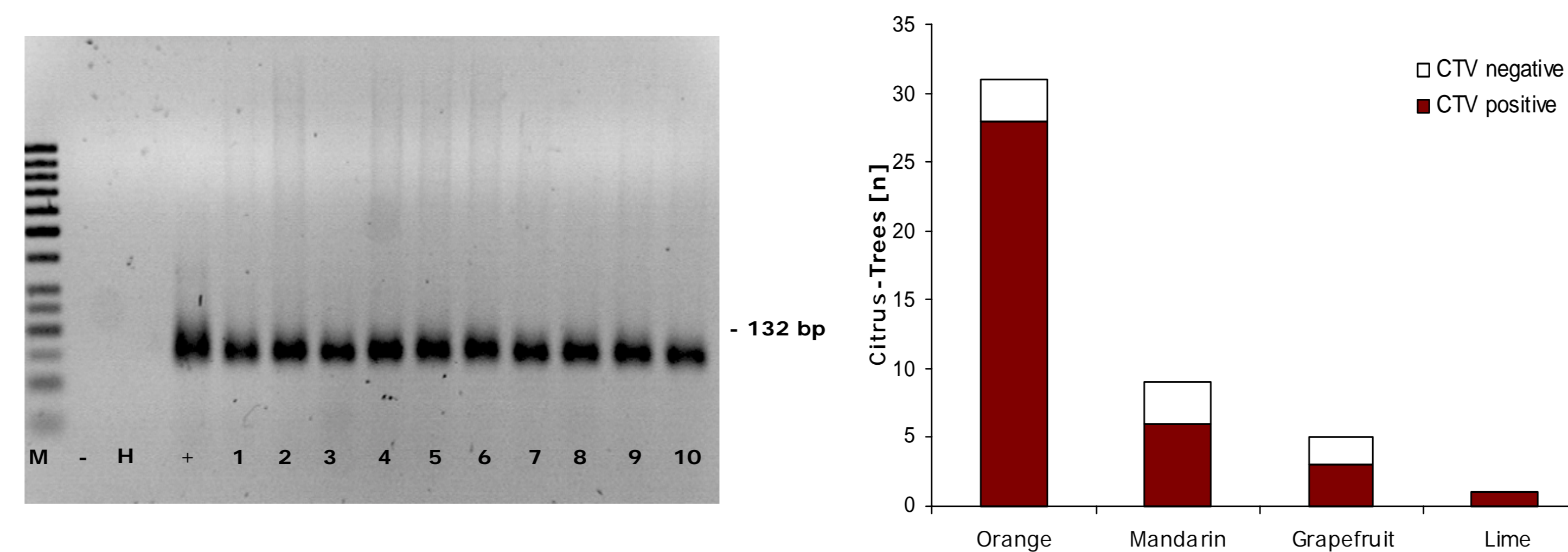


Fig. 1: Detection of CTV by nested RT-PCR; 38 out of 46 samples were tested positive. Citrus-trees originated from eight different orchards located in the provinces Northern State, River Nile and Khartoum of the Sudan (fig. 5).

## Detection of Citrus Viroids

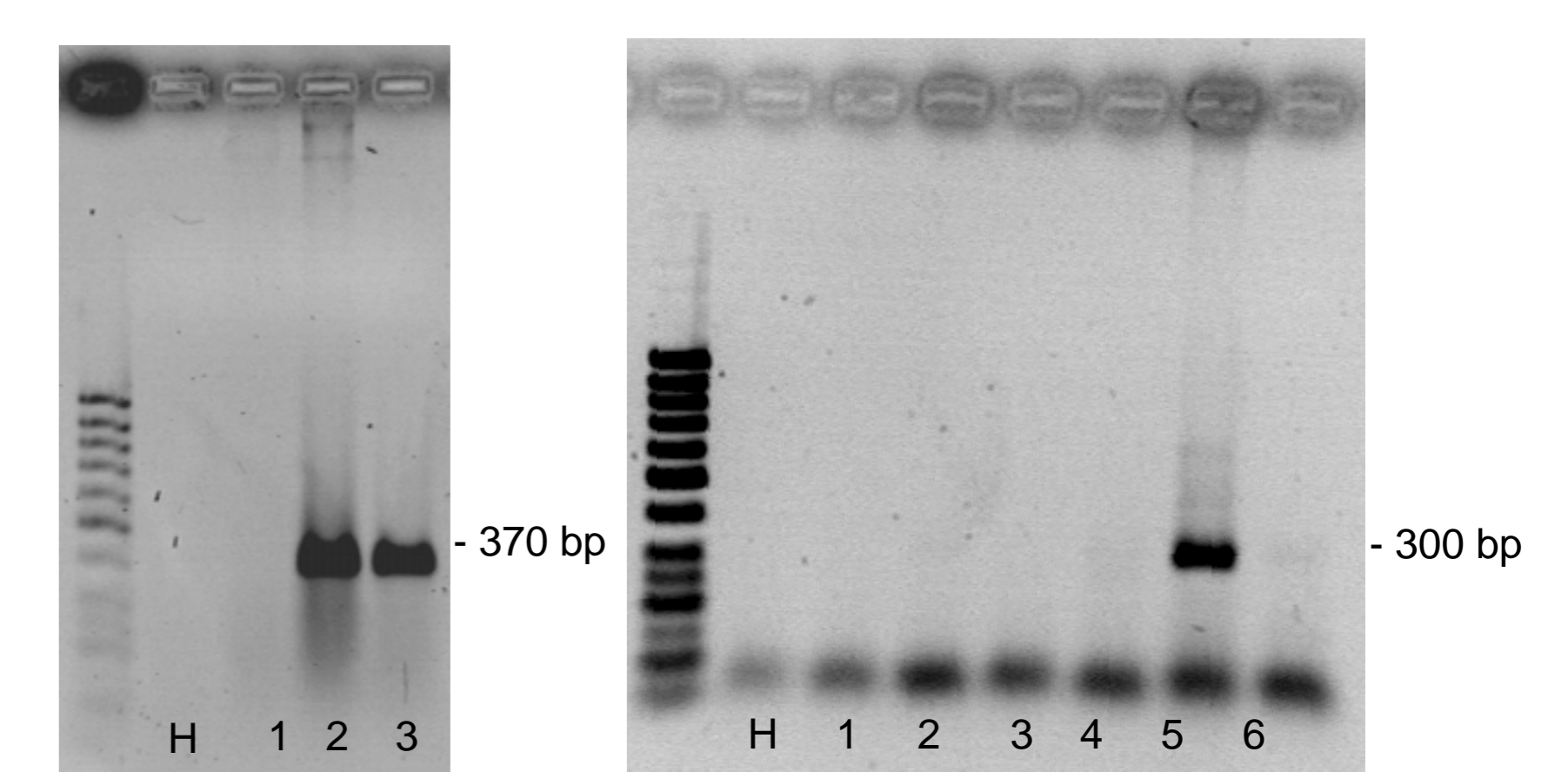


Fig. 2: Detection of Citrus exocortis viroid (left) and Hop stunt viroid (right) in Citrus-trees from the Sudan by RT-PCR using Viroid-specific primerpairs.

## Secondary structure analysis of Citrus Viroids

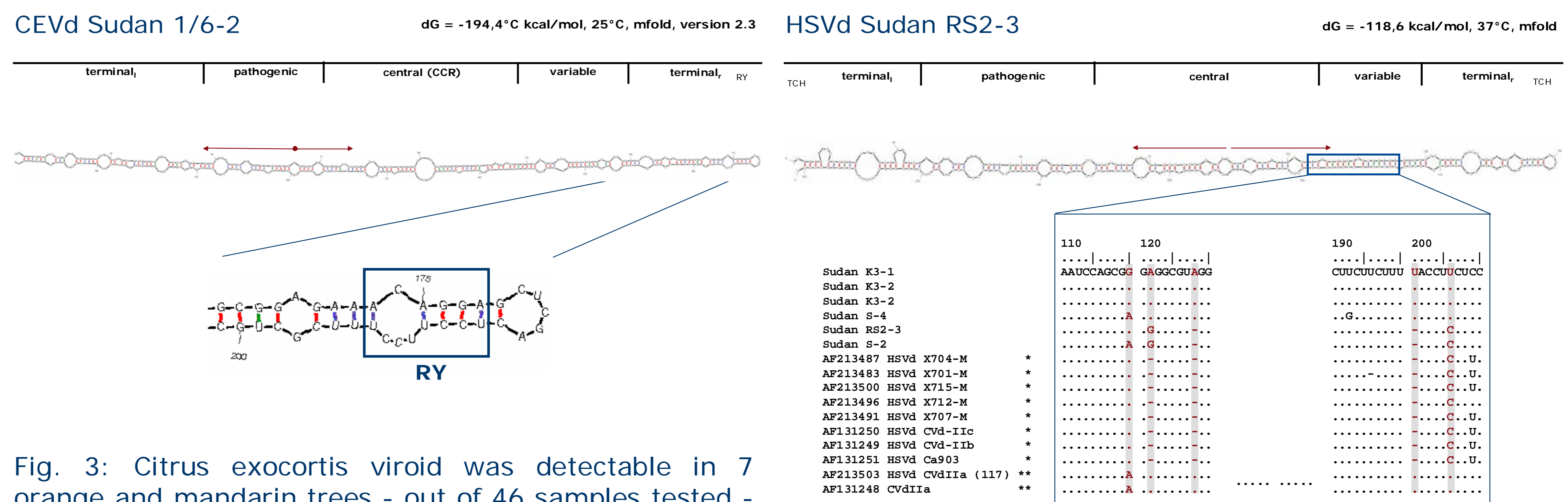


Fig. 3: Citrus exocortis viroid was detectable in 7 orange and mandarin trees - out of 46 samples tested - grafted onto sour orange rootstocks. Infected trees were found in the States River Nile and Khartoum (fig. 5), but exhibited no symptoms. CEVd isolates from the Sudan are 369-371 nucleotides long circular RNAs, with a sequence conservation between 95,9% and 100%. Nucleotide variations were found exclusively in the pathogenic and variable region. Sequence comparison to the CEVd type species (CEVd-Aus) exhibited identities above 92,4 %. One RY-motif proximal to the right Terminus of the Viroid, which is typical of the Genus *Pospiviroid*, is present in all Sudanese CEVd isolates.

Fig. 4: Hop stunt viroid infection was shown in two mandarins exhibiting Cachexia symptoms and in two symptomless orange trees. Sudanese HSVd isolates are between 301-304 nucleotides long, showing typical linear secondary structure, conservation of the central region and terminal conserved helices (TCH). Sudanese isolates RS2-3 and S2 contain the characteristic nucleotide composition (shaded columns) of Cachexia inducing isolates (indicated with a single asteriks \*) in the variable region of the genome. (Non Cachexia inducing isolates are indicated by two asteriks \*\*.)

## Distribution of CTV and Citrus Viroids in the Sudan

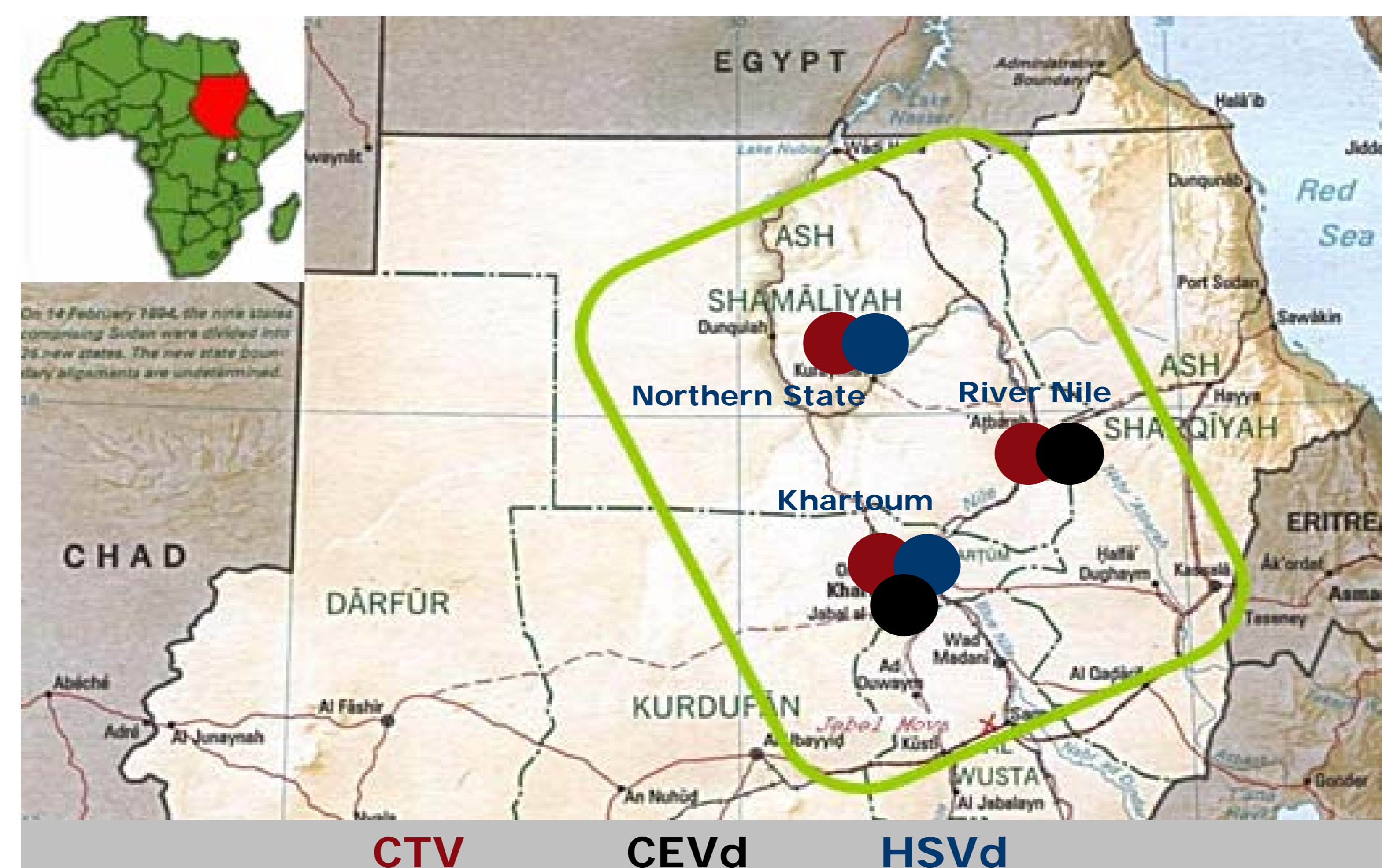


Fig. 5: Occurrence of Citrus tristeza virus (CTV), Citrus exocortis viroid (CEVd) and Hop stunt viroid (HSVd) within the sampled area Northern State, River Nile, Khartoum, Kassala, El Gazira and Blue Nile (green square).

## Conclusions

Citrus tristeza virus is abundant in the Citrus growing area in the Sudan (fig. 5), infecting orange, mandarin, grapefruit and lime. The virus infection is responsible for quick decline and death of Citrus trees, causing severe loss of productivity.

Also, several orange and mandarin trees were found to be infected by Hop stunt viroid or Citrus exocortis viroid, the latter is present as a latent infection.

The presence of Cachexia inducing isolates of HSVd in the Sudan was proved by molecular and secondary structure analysis of the Viroid genome.

## Acknowledgements

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