

# **Characterization, Genome Organization and Phylogenetic Analysis of Two New Viruses Infecting Legume Crops in Ethiopia**

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# Introduction

- **Legumes particularly cool season food legumes (CSFL) are very important crops (Faba bean, chickpea, fieldpea & lentil)**
- **Major protein sources in some countries of Eastern and Northern Africa and west Asia (WANA)**  
**(Ethiopia, Sudan, Egypt, Morocco & Syria)**
- **Virus diseases are among the main production constraints in the region**
- **Affected by over 50 viruses worldwide**

## In many of these countries, two groups of virus diseases are prevalent

Yellowing, stunting and necrosis caused by

- luteoviruses (Family *Luteoviridae*) and
- nanoviruses (Family *Nanoviridae*)

Mosaic and mottle (less important)

- potyviruses (Family *Potyviridae*) and
- bromoviruses (Family *Bromoviridae*)

## **In Ethiopia:**

- **Ca. 15 viruses identified serologically**
- **Yellowing and stunting are most frequent (luteoviruses and nanoviruses)**
- **None were characterized in detail**

## **Objective:**

**To identify and characterize the virus(es) associated with yellowing and stunting diseases of cool season food legumes at biological, serological and molecular level and develop diagnostic tools**





**Field-grown faba bean infected with a nanovirus at Ambo, Ethiopia**

# Study Approach and Methodology

- **Legume samples obtained from Ethiopia and other WANA countries (Sudan, Egypt, Morocco & Syria)**
- **Serological analyses**  
(DAS-, TAS- and ACP-ELISA procedures)
- **Cloning and sequence analysis including complete genome of new virus isolates (Random-, IC-, and RT-PCR, RACE, RFLP)**
- **Virus purification, Electron Microscopy and CP analysis (SDS-Polyacryl amide gel electrophoresis)**
- **Production of poly- & Monoclonal antibodies**
- **Biological studies (Aphid transmission and host range)**

## Viruses serologically detected from legume samples collected in Ethiopia

Crop	No. of samples	Nanovirus	Luteovirus	Mixed
Faba bean	322	85	57	17
Chickpea	28	0	24	0
Lentil	4	1	2	1
Grasspea	7	1	1	0
Fenugreek	9	0	4	0
<b>Total</b>	<b>370</b>	<b>87</b>	<b>88</b>	<b>18</b>

## TAS-ELISA reaction of luteovirus positive samples with virus-specific luteovirus monoclonal antibodies

Samples	No. of samples tested	Luteo 5G4	BLRV 6G4	BWYV G4C10	BWYV 4D3	BWYV 1-E11	SbDV 2G5
Faba bean	20	+++	-	-	-	-	-
Chickpea	24	+++	-	-	-	-	-
Lentil	2	+++	-	-	-	-	-
Grasspea	2	+++	-	-	-	-	-
Fenugreek	4	+++	-	-	-	-	-

PCR amplification, cloning and sequencing of coat protein gene of an isolate using standard molecular biological techniques followed.

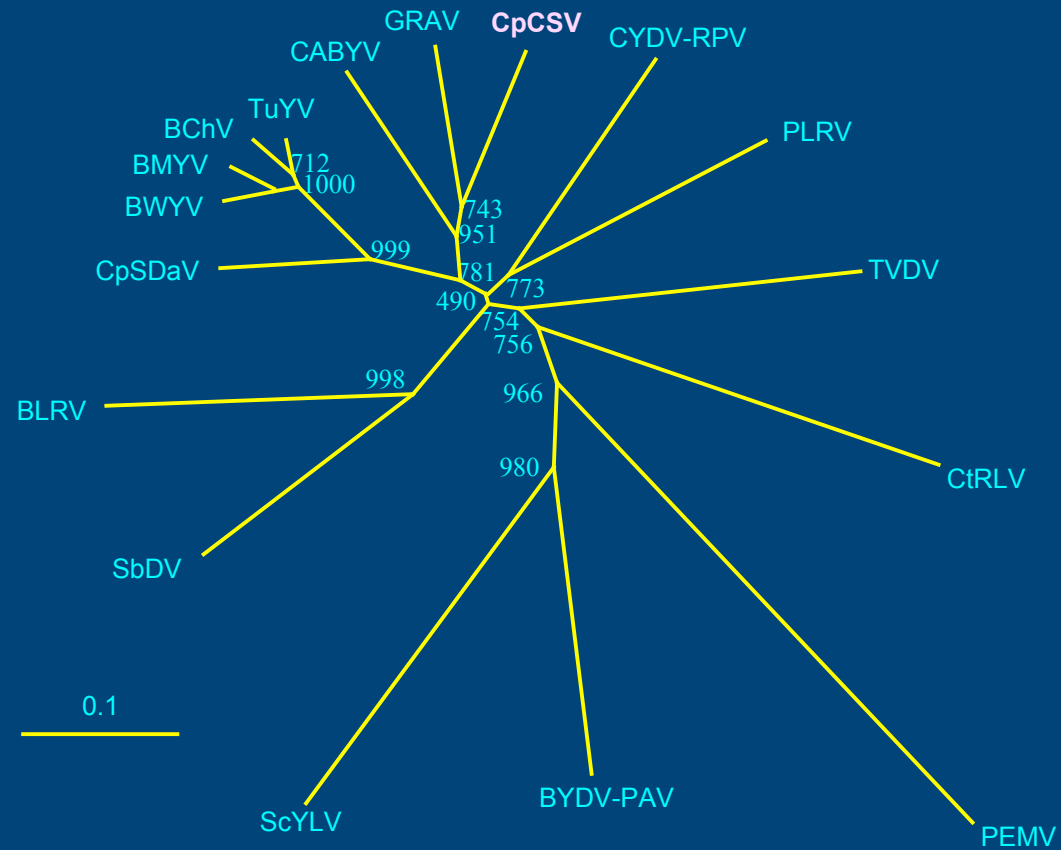


## Percent identities resulting from pairwise comparisons of the deduced coat protein aa sequence of luteo-Eth with that of other luteoviruses

Virus	% identity
<i>Groundnut rosette assistor virus</i>	77.8
<i>Cucurbit aphid-borne yellows virus</i>	71.7
<i>Turnip yellows virus</i>	71.0
<i>Beet western yellows virus</i>	68.5
<i>Chickpea stunt disease-associated virus</i>	66.5
<i>Potato leaf roll virus</i>	65.1
<i>Cereal yellow dwarf virus-RPV</i>	59.8
<i>Bean leaf roll virus</i>	59.0
<i>Soybean dwarf virus</i>	56.8
<i>Barley yellow dwarf virus-PAV</i>	43.7
<i>Sugarcane yellow leaf virus</i>	41.8

**According to the ICTV criteria, distinct luteovirus species should have an identity % of < 90. So luteo-Eth is a new luteovirus named Chickpea chlorotic stunt virus (CpCSV)**

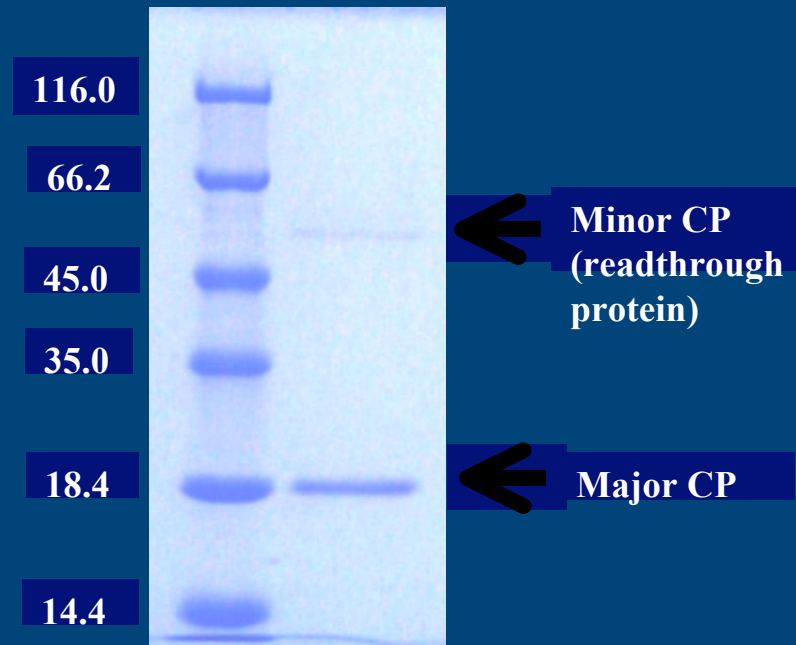
# An unrooted tree showing phylogenetic relationship of CP aa sequence of CpCSV to those of other luteoviruses



## Electron micrograph of purified CpCSV particles



## SDS-PAGE Analysis of CpCSV structural proteins



**A polyclonal and 10 monoclonal antibodies were produced and used in reliable diagnosis of CpCSV**

## Biological studies:

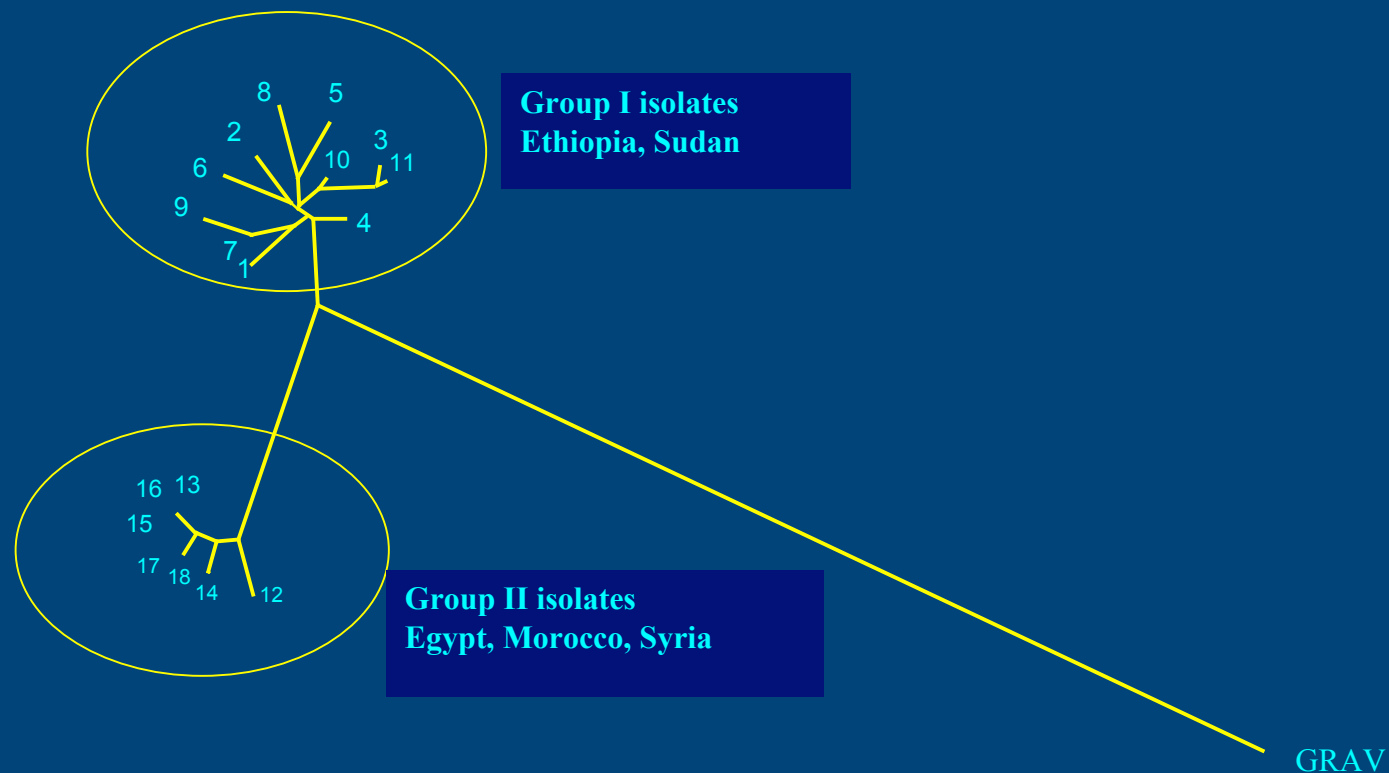
**Transmitted by aphid *Aphis craccivora* but not by *Aphis fabae*, *Myzus persicae* or *Acyrtosiphon pisum***

**Infects only cool season food legumes:**

*Vicia faba*, *Lens culinaris*, *Lathyrus sativus*, *Cicer arietinum*, *Pisum sativum* and *Trigonella foenum-graecum*.

**Several other Legume and non-legumes were not infected.**

# Unrooted tree showing CP phylogenetic relationship among 18 CpCSV isolates from five countries



**Monoclonal antibodies produced for one group do not react with isolates from another group, indicating serological distinctnes of each group**

Variation in plant stunting (upper) and leaf yellowing (lower) caused by two strains of CpCSV



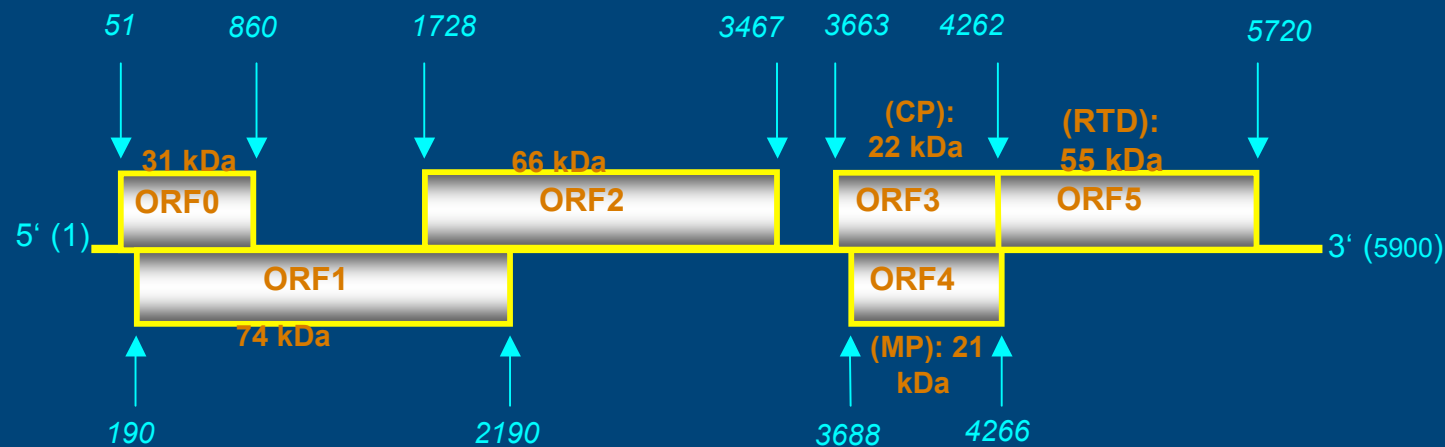
Syria Ethiopia not inoculated



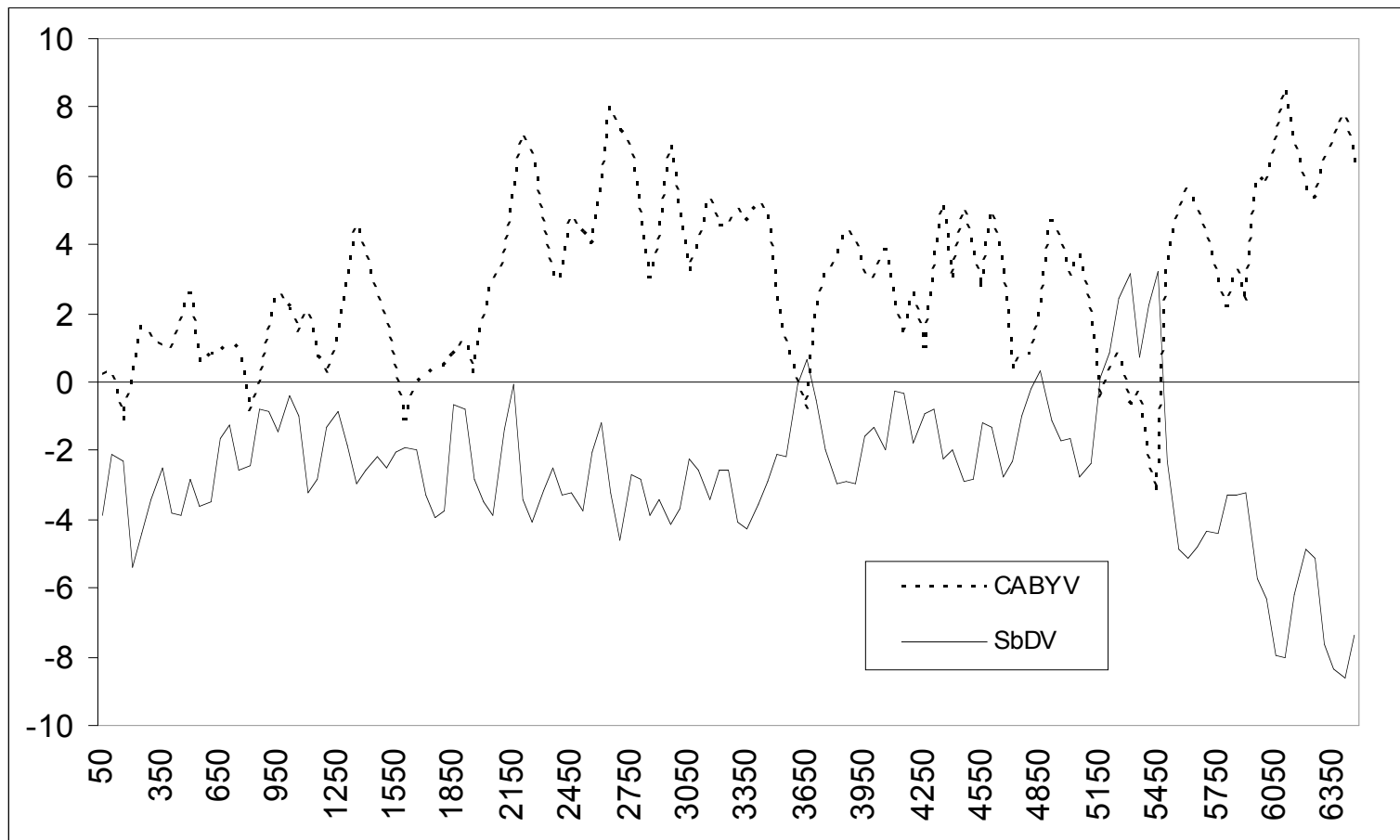
Two Distinct Strains



# Organization of the entire genome of CpCSV, the new luteovirus from Ethiopia



CpCSV belongs Genus *Polerovirus*, Family *Luteoviridae*

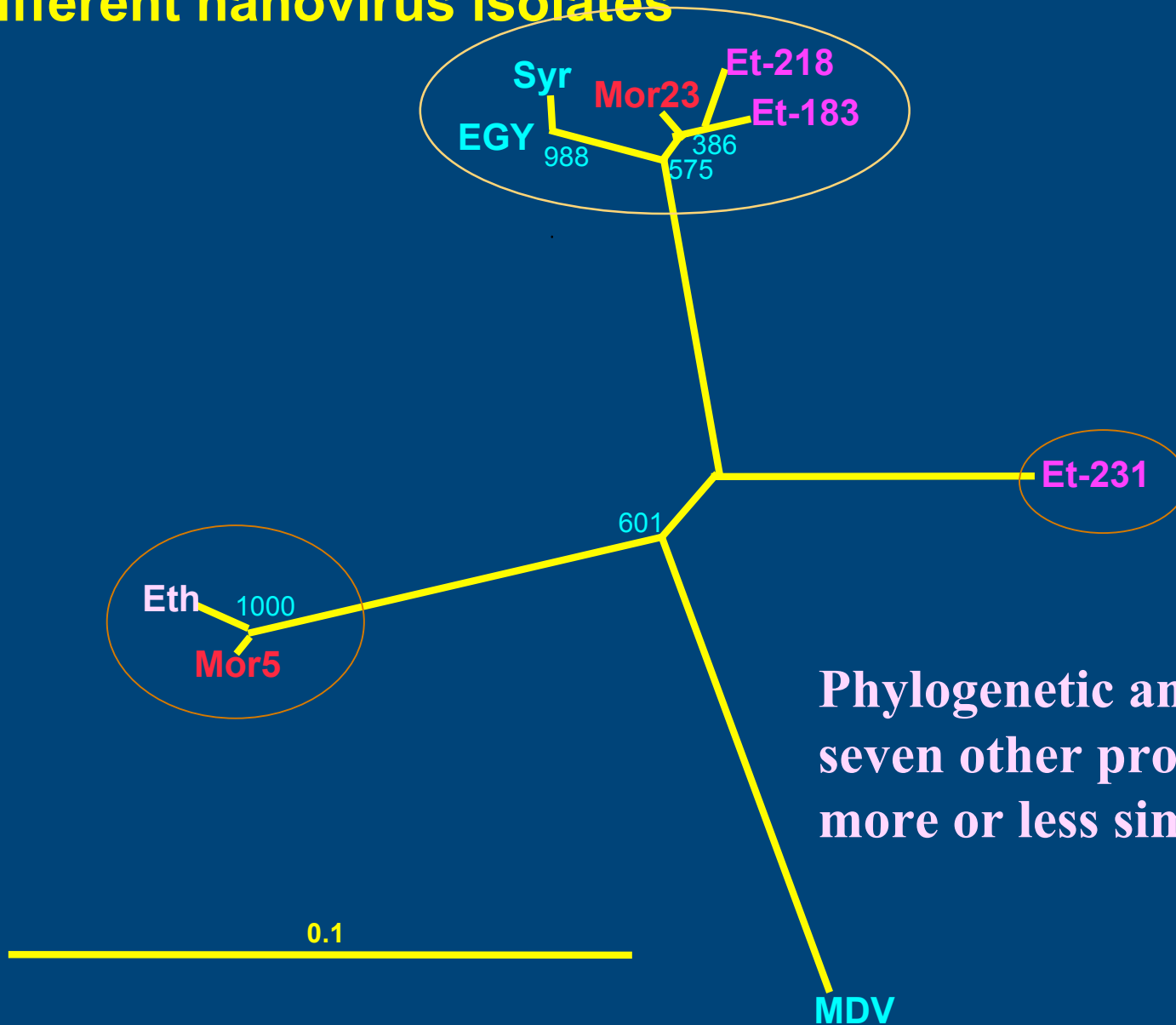


Graph showing the result of the recombination analysis of aligned luteovirus genome sequences using the Sister scanning procedure. The complete nucleotide sequence of CpCSV was compared with those of CABYV (dashed line) and SbDV (solid line). Z score values greater than 3 strongly suggest a close phylogenetic relationship to a certain virus. Note that peaks at nt position 5300 and 5400 have Z score values of greater than 3.

## Serological grouping of nanovirus-positive samples from Ethiopia using with the seven discriminating monoclonal antibodies

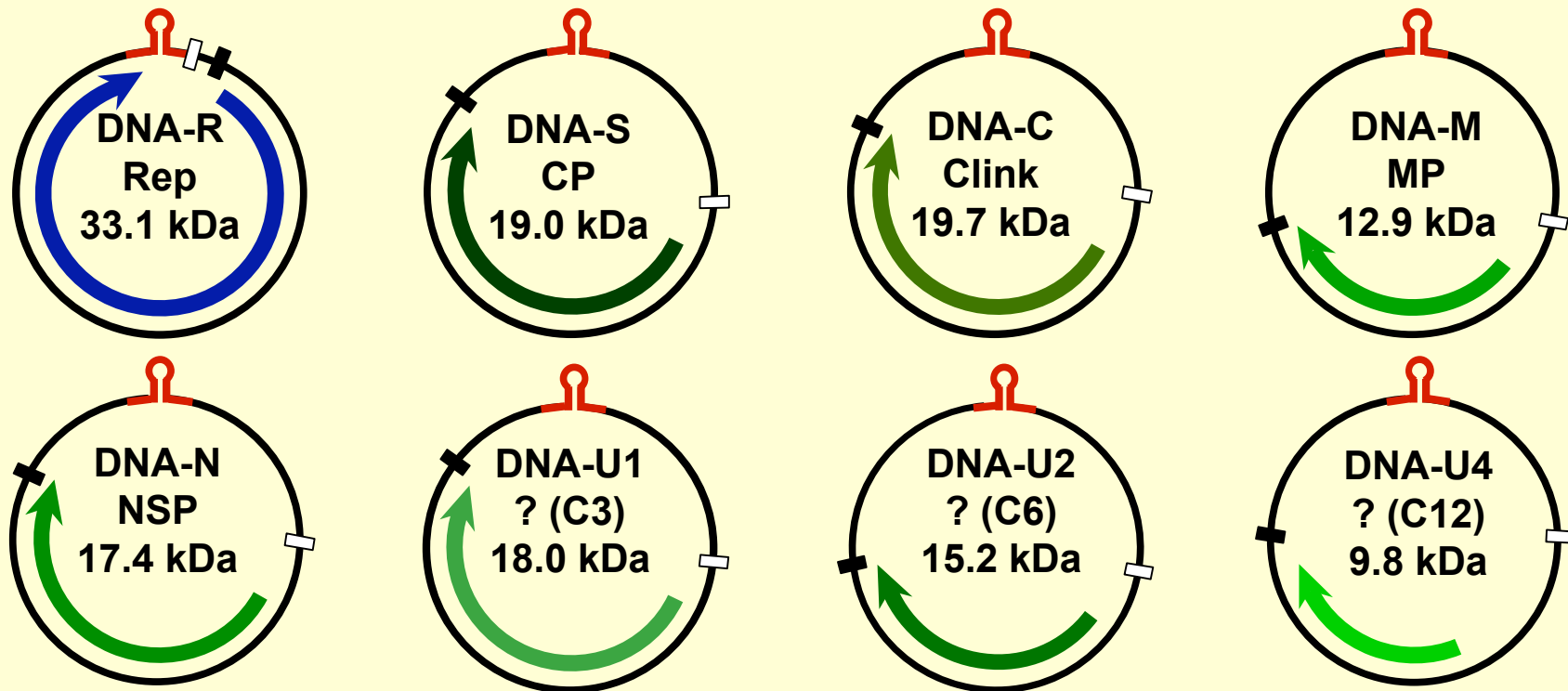
Selected samples	Mab mix	Differentiating FBNYV MAbs raised against							No. of samples giving a similar epitope profile <sup>2</sup>	Sero-group <sup>2</sup>
		FBNSV-Eth				FBNYV-Eg				
		8-6F8	8-4F9	8-2G10	8-3G11	1-3D8	3-4A5	2-3E12-D5		
Eth-2	+++ <sup>1</sup>	+++	+++	+++	+++	-	-	-	62/73	A
Eth-183	+++	+++	+++	+++	+++	+++	+++	-	1/73	mixed, B
Eth-218	+++	-	-	-	+	++	+++	-	4/73	B
Eth-231	+++	-	-	-	-	+++	+	-	4/73	C
Eth-234	+++	-	-	-	-	-	-	-	2/73	others <sup>3</sup>
FBNSV-Eth <sub>4</sub>	+++	+++	+++	+++	+++	-	-	-	-	A
FBNYV-Eth <sub>4</sub>	+++	-	-	-	-	+++	++	+++	-	B

# Unrooted phylogenetic tree showing the relationship between the coat protein amino acid sequences of different nanovirus isolates



Phylogenetic analysis of seven other proteins showed more or less similar topology

## The eight of ssDNA components forming the multipartite genome of Eth-231 isolate (from Gedeo, Ethiopia)



- Each DNA component (~1 kb) is individually encapsidated and encodes a single protein of 10-35 kDa

The overall nucleotide identity of the eight ssDNAs of Eth-231 (serotype C) to other known nanovirus isolates is 70%.

According to current criteria of ICTV criteria, nanoviruses with <75% identity have to be considered as distinct species.

Hence, Serotype C isolates are suggested to represent a new nanovirus named Faba bean yellow leaf virus.



# Conclusions

- A new virus named **Chickpea chlorotic stunt virus (CpCSV)** (*Luteoviridae*) commonly infecting legume crops in Ethiopia is characterized in detail. It also occurs in WANA countries as two geographically distinct strains differing in biological, serological and molecular properties.
- **The genome of an Ethiopian CpCSV isolate is 5900 nucleotides long arranged in six ORFs similar to members of genus *Polerovirus* in Family *Luteoviridae*.**

Diagnostic tools (poly- & monoclonal antibodies as well as oligonucleotide primers) made available in this work for these new viruses will help in further studies and management of these viruses throughout the world.

**Serological properties and complete genome sequence of another new nanovirus from Ethiopia named Faba bean yellow leaf virus has been determined. This virus is related to but distinct from Faba bean necrotic yellows virus isolates found to be more widely distributed in Ethiopia and elsewhere.**

**Finally, serological and molecular studies revealed that viruses causing legume yellowing and stunting in Ethiopia and WANA countries are much more diverse than previously thought. Developing management options such as resistance varieties should take this into consideration.**

*Thank You for Your Attention*