

A look at the identification, characterization and incidence of cherry leaf roll virus (*Nepovirus avii*) in cherry in South Bulgaria

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INTRODUCTION

Cherry leaf roll virus (CLRV, *Nepovirus avii*, *Secoviridae*) has been known by wide host range including numerous woody and herbaceous plant species. That suggests the existence of a great diversity of variants and isolates of the virus. Among the fruit tree species (CLRV) is an economically important pathogen for sweet cherry and walnut. In Bulgaria, CLRV was studied in walnut, but research on the virus in sweet and sour cherries are limited.

The current study aimed to ascertain the occurrence of CLRV in cherry orchards in the South Central and Southeast regions, and the characteristics of the virus isolates spread in cherry in South Bulgaria.

MATERIAL AND METHODS

Symptoms observation and sampling were carried out in sweet and sour cherry crops located in Plovdiv, Pazardzhik, Stara Zagora, and Burgas provinces. A total of 230 sweet cherry and 23 sour cherry samples collected from symptomatic and asymptomatic trees were tested for the cherry strain of CLRV by Enzyme-linked immunosorbent assay (ELISA). On the base of the symptoms pronounced and ELISA results 180 samples were selected for a subsequent analysis by Reverse transcription – polymerase chain reaction (RT-PCR). Total RNA was extracted by using the Spectrum™ Plant Total RNA Kit (Sigma-Aldrich, USA), according to the protocol of the manufacturer. RT step for synthesis of a complementary DNA (cDNA) was performed with random hexamer primer using a SCRIPT cDNA synthesis kit (Jena Bioscience, Germany). PCR was carried out using two primer pairs corresponding, respectively, to the coat protein (CP) gene (Langer et al., 2016) and 3' end of untranslated region (3'UTR) (Wermer et al., 1997).

Four CLRV-positive accession, representative of the four surveyed provinces were selected as source of inocula for mechanical inoculations on the herbaceous indicators *Chenopodium quinoa*, *C. amaranticolor* and *Nicotiana benthamiana*. Biological tests on woody hosts (peach indicator GF 305, sweet cherry cultivar 'Sam' and cherry rootstock Gisela 5) were conducted by graft inoculation using bud-sticks of two CLRV-infected trees. All artificially inoculated plants were tested for CLRV by RT-PCR.

RESULTS AND DISCUSSION

CLRV was detected in 15.2% of tested sweet cherry samples by ELISA whereas by RT-PCR with the primer pair corresponding to CP genomic region (RNA 2), the virus was identified in 25% of the samples. In the sample of sour cherry ELISA failed to detect CLRV, but the virus was identified by RT-PCR in 8.7% of the tested samples (Table 1).



Fig. 1. Symptoms on the leaves of CLRV-infected tree

Table 1. Results from ELISA and RT-PCR of sweet and sour cherries samples from Plovdiv, Pazardzhik, Stara Zagora and Burgas provinces.

Species	Province	ELISA n/N	RT-PCR n/N	
			CP primers	3'UTR primers
Sweet cherry	Plovdiv	14/93	17/76	16/76
	Pazardzhik	10/73	14/67	12/67
	Stara Zagora	5/30	6/17	6/17
	Burgas	6/34	8/20	8/20
Total		35/230 (15.2 %)	45/180 (25 %)	42/180 (23.3 %)
Sour cherry	Plovdiv	0/12	1/12	1/12
	Pazardzhik	0/11	1/11	1/11
Total		0/23	2/23 (8.7 %)	2/23 (8.7 %)

N – a total number of tested samples; n- number of positive sample

Two CLRV Isolates were successfully transmitted by grafting on the peach indicator GF 305, sweet cherry cultivar 'Sam' and cherry rootstock Gisela 5. The herbaceous species *Chenopodium quinoa*, *C. amaranticolor* reacted with local lesions and systemic symptoms after mechanical inoculation with the four accessions used. Systemic symptoms were observed on the inoculated plants of *Nicotiana benthamiana*. No big differences in the phenotypic reaction of the herbaceous indicators to the used isolates were found.



Fig. 2. Local lesion (left) and systemic symptoms on *C. amaranticolor*, inoculated with CLRV isolate.

CONCLUSIONS

- CLRV occurs at a moderate rate in cherry in surveyed provinces.
- Similar phenotypical reactions of the inoculated herbaceous indicators were observed towards the four CLRV isolates, originated from different provinces.
- RT-PCR based on the detection of CP gene is more reliable analysis for detection of field samples.

Acknowledgements This study is supported by the Bulgarian National Science Fund under Grant No KP-06-N 36/10 from 2019, "Actual phytopathological challenges in fruit tree species in Bulgaria: Unexplored and invasive align phytopathogens with potential risk for biodiversity and biosecurity".