

## RAPID COMMUNICATION

## Occurrence of tea plant necrotic ring blotch virus in Iran

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Vol. 61, No. 2: 200–202, 2021

DOI: 10.24425/jppr.2021.137028

Received: November 17, 2020

Accepted: January 11, 2021

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### Abstract

The tea plant [*Camellia sinensis* (L.) O. Kuntze] is one of the most significant commercial crops in Iran. Symptomatic leaves including chlorina on the edge of the leaf, and multiple necrotic ring blotches on mature leaves from different tea gardens were observed in northern Iran. RT-PCR analysis and transmission electron microscopy observations were applied to characterize the causal agent of tea leaf discoloration. Sequence analyses of the fragments revealed that all the samples were infected with tea plant necrotic ring blotch virus (TPNRBV). To our knowledge this is the first report of TPNRBV in Iran and the second in the world.

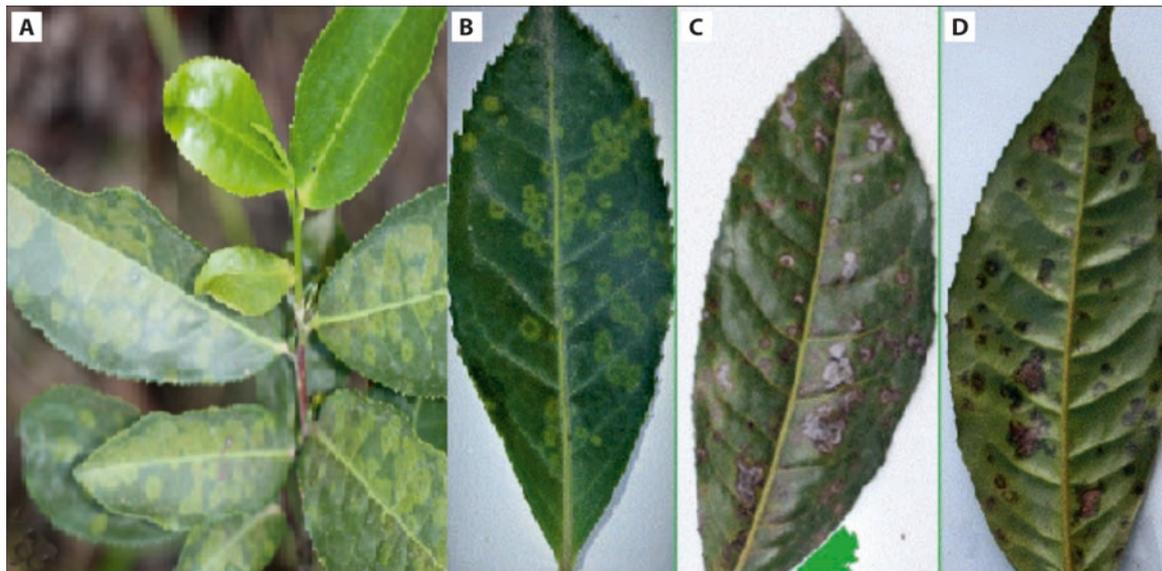
**Keywords:** *Blunervirus*, *Camellia sinensis*, discoloration

The tea plant [*Camellia sinensis* (L.) O. Kuntze] is a perennial woody bush first discovered as a drink and medicine in China approximately 3,000 years ago. The tea plant has been widely cultivated in China, India, Sri Lanka, Kenya, Japan, and other countries (Hao *et al.* 2018). The main area of tea plantations is located in northern Iran. It was thought that the tea plant was a virus-free species because of some polyphenolic compounds such as catechins which have anti-infective activities against a broad spectrum of viruses and other pathogens (Hao *et al.* 2018; Song 2018). Hao *et al.* (2018) discovered new viruses of tea plants namely, tea plant necrotic ring blotch virus (TPNRBV) and tea plant line pattern virus (TPLPV). The most recent threats to tea plantations are fungal and nematode caused diseases (Lehmann-Danzinger 2000), while tea plant necrotic ring blotch virus is a new concern. Leaf discoloration, including albino and chlorina, and necrotic ring blotch are the main symptoms of new viral infections of tea plants. Hao *et al.* (2018) suggested that virus detections in symptomatic and asymptomatic tissues from field plants showing tea plant necrotic ring blotch disease (TPNRBV) had a systemic movement feature. Plants with such symptoms often had

weak growth and even displayed significant symptoms of disease under certain conditions. Despite the economic importance of tea in Iran and the disease distribution in all tea gardens in the Ramsar region, no records and information are available on the incidence of viral diseases and their impact on production. This was the first major survey on the occurrence of tea virus in the main tea producing areas of Iran. The information obtained will be a key element in future research to develop improved control strategies for tea virus diseases in Iran.

In August 2018, leaves from tea plant cultivars (Kashef, Asam) for production of fermented (black) tea, showing discoloration and necrotic ring blotch symptoms were observed and collected from Ramsar (36.9268°N, 50.6431°E) tea gardens (Fig. 1).

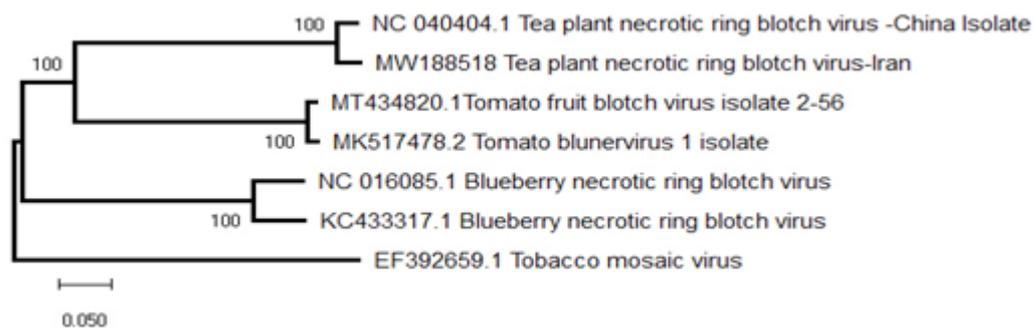
Total RNA from the samples was purified using the TOPAZOL kit (TOPAZGEN, Iran) according to the manufacturer's instructions. RNA quality was verified by 1% gel electrophoresis. The primers (TPNRBV2-F) 5'-(GGGCCGGGGTGTGGAAAACTT)-3' and (TPNRBV2-R) 5'-(TTCTTATCATCCCGGCAAAACA)-3' amplifying a 550 bp fragment of Helicase-RNA polymerase ORF of RNA 2 of TPNRBV, were used for



**Fig. 1.** A – naturally diseased tea, B – early stage of ring blotches formation on mature leaf, C – advanced stage of ring blotches on mature leaf and D – disease symptoms under side of mature leaf

RT-PCR (Hao *et al.* 2018). Reverse transcription (RT) reaction was carried out using Easy cDNA Synthesis kit (Pars Tous biotechnology, Iran) and reverse primer (TPNRBV2-R). PCR reactions were performed on a thermal cycler iCycler (Bio-Rad, USA), using the following pattern: 95°C for 4 min (1 cycle), 95°C for 30 sec, 58°C for 30 sec, and 70°C for 1 min (35 cycles), and 70°C for 7 min (1 cycle). PCR products were monitored by 2% gel electrophoresis. Amplified fragments were purified from gel using DNA Recovery kit (Cinagen, Iran) and directly sequenced (Pishgam Biotechnology, Iran). The obtained sequence was deposited in GenBank with accession number MW188518. Sequences were compared to others existing in the National Center for Biotechnological Information (NCBI) database using Basic Local Alignment Search Tool (BLAST). Phylogenetic analysis was performed using MEGAX software, Clustal W (Thompson *et al.* 1994) was used for multiple alignment and a phylogenetic tree was built with the neighbor-joining method and bootstrap analysis with 1,000 replications. The methods for sample preparation, ultrathin section, and electron microscopic examination were carried out following the descriptions by Wang *et al.* (2014) and Reynolds (1963). Samples showing leaf discoloration and necrotic ring blotch symptoms that were collected from tea gardens located in different regions of Ramsar, Iran, resembled TPNRBV symptoms in China (Hao *et al.* 2018). The annual incidence patterns were blotches and no symptoms were seen before July during the 2 years of study. Symptoms were indicative of a putative viral infection. Electron microscopy did not show any viral particles in symptomatic leaves and attempts to reproduce symptoms on different plants

through mechanical inoculation failed. The use of primers amplifying partial helicase – RNA polymerase ORF of TPNRBV (TPNRBV2-F/ TPNRBV2-R) caused the amplification of the expected bands in the samples, which was in accordance with the classification of the features employed by Hao *et al.* (2018). The amplified sequence fragment of Iranian tea virus isolate had 95.9% identity with tea plant necrotic ring blotch virus RNA2 in BLASTn analysis. According to this identity percentage, Iranian tea virus isolate belongs to tea plant necrotic ring blotch virus species. This sequence had 51.4 and 42.9 in nucleotide level with tomato fruit blotch virus “MK517478.1” and blueberry necrotic ring blotch “YP004901701.1”, respectively (Robinson *et al.* 2016; Ciuffo *et al.* 2020). The phylogenetic tree based on nucleotide sequences of blunervirus isolates revealed a close relationship with TPNRBV (Fig. 2). No polymorphism has been seen between sequences collected from the Ramsar region. Electron microscopy study did not show any virus particles in symptomatic samples. The reported results of electron microscopy about blunerviruses are contradictory. Hao *et al.* (2018) observed multiple virus particles in the cytoplasm of infected tea plant tissue (80–100 nm diameter), whereas Ciuffo *et al.* (2020) did not show any particles in infected tomato tissue. TPNRBV from *Camellia sinensis* was previously reported in China (Hao *et al.* 2018). Considering the current problem of disease in tea plants, diagnosis of the associated pathogen is a key step to developing a successful disease-management program. According to molecular analysis, TPNRBV is the causal agent of viral disease in tea plants in northern Iran. The virus is considered to be one of the most economically important pathogens in tea plants (Hao *et al.* 2018), and it is



**Fig. 2.** Dendrogram constructed by the neighbor-joining method showing the phylogenetic relationship between the Iranian TPNRBV (MW188518), tomato fruit blotch virus "MK517478.1" and blueberry necrotic ring blotch "YP004901701.1", based on the sequences generated using helicase and RNA polymerase ORF

able to act as the primary pathogen. Future studies are required to better characterize this TPNRBV isolate. So far, sanitation and host resistance have been recommended as different methods to manage the disease. Therefore, more studies need to be conducted in order to find a rational solution to this problem.

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