

## Molecular identification of Jujube witches'-broom phytoplasma (16SrV) associated with witches'-broom disease of *Ziziphus oenopia* in India

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Phytoplasmas are intracellular obligate prokaryotes which lack cell wall, have small genome and are mainly transmitted by hemipteran insect vector of the families Cicadellidea (leafhoppers) and Fulgoridea (planthopper). Phytoplasma are associated with typical phyllody, virescence, yellowing, proliferation of axillary buds, witches' broom, stunting of whole plant and die back symptoms on number of plant species worldwide. Phytoplasma are also associated with severe yield losses in a variety of plant species of horticultural, agricultural and ornamental importance in India.

*Ziziphus oenopia* (L.) Mill. (Family Rhamnaceae) commonly well known as makai in hindi and Jackal Jujube in english, is a straggling shrub distributed all over the hotter regions of Pakistan, Sri Lanka, India, Malaysia, and Tropical Asia. The fruits are edible, and it is widely used in Ayurveda for the treatment of various diseases, such as ulcer, stomachache, obesity, asthma, digestive, antiseptic, hepatoprotective, wound healing and diuretic property.

Phytoplasmas associated with witches'-broom disease in *Ziziphus jujube* and *Z. nummularia* in Bahraich district, in India, are considered isolates of 'Ca. Phytoplasma ziziphi'. Presently only one report has been published based on symptomatology on *Ziziphus oenopia* expressed witches'-broom appearance by proliferation of axillary buds from Dakshin Dinajpur district of West Bengal, India West Bangal.

The *Z. oenopia* plants were found to phytoplasma like symptoms rosetting, proliferation of axillary shoots, exhibiting witches'- broom, excessive branching accompanied with little leaf symptoms with 40-45% disease incidence during the survey in February 2019 in Barkatullah University campus, Bhopal. The two positive nested PCR

amplicons (~1.2 kb) were purified by Wizard SV gel extraction kit (Promega Pvt., Ltd., USA) and sequenced from both the direction (Bioinnovations Pvt. Ltd., Mumbai, India).

For molecular detection of phytoplasma total DNA was isolated from symptomatic and asymptomatic plant leave samples (100 mg) using phytoplasma enrichment protocol and quantity of DNA preparation as checked by taking its O.D at 260/280 nm is 1.8 and concentration is also checked by the 1% agarose gel electrophoresis. The status of phytoplasma strain under study (MK975463 and MK975462) were also verified by in silico RFLP analysis with isolates of Jujube witches'-broom phytoplasma (MH972556) and 'Ca. Phytoplasma balanitae' (HG937644) from India of Elm yellows group (16SrV) using 06 restriction enzymes. In silico RFLP analysis of 16S rRNA sequences of phytoplasmas strain under study were generated using pDRAW32 program. Each 16S rRNA sequences were digested In silico with restriction enzymes: AluI, BamHI, EcoRI, HaeIII, KpnI, and TaqI and a virtual gel electrophoresis image were generated. The analysis In silico RFLP revealed a silently difference between the Elm yellows group (16SrV) taken for study. RFLP analysis with BamHI and KpnI showed no sites in phytoplasma strain both the under-study isolates (MK975463 and MK975462) as well as any of the Jujube witches'-broom phytoplasma (MH972556) and 'Ca. P. balanitae' (HG937644) 16SrVI group representative

Phylogenetic analyses were perused using Molecular Evolutionary Genetics Analysis (MEGA version 7.1) program with 100 replicates bootstrapping and phylogram were generated with Neighbour-joining method. Dendrograms were viewed by the NJplot program. In silico RFLP analysis of 16S rRNA sequences of phytoplasmas strain under study

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Note: 19th World Conference on Environmental Toxicology and Pharmacology

August 19-20, 2019 | Tokyo, Japan

Volume 1, Issue 3

were generated using pDRAW32 program. The natural occurrence of witches'- broom with little leaf disease of *Z. oenoplia* was detected by nested PCR using phytoplasma specific primers and 1.2 kb sequence data were analysed by NCBI BLASTn.

There are limited reports available in the literature worldwide related to phytoplasma study in *Ziziphus* species such as 'Ca. *Phytoplasma ziziphi*' in China, Japan, Korea and India [7, 8]. The *Z. oenoplia* is totally different in their nature to other *Ziziphus* plant species. It is basically a creeper plant and spreading, sometimes climbing, however the possibility of phytoplasma infection is more prominent to spread the phytoplasma disease to one healthy plant species to another healthy plant species. Therefore detection and identification of phytoplasma species is more essential for proper management of phytoplasma disease

On the basis of sequence analysis, closest phylogenetic relationships and In silico RFLP of the under study both the phytoplasma isolates associated with witches'-broom disease of *Ziziphus oenoplia* identified as a strain of Jujube witches'-broom phytoplasma as a member of Elm yellows group (16SrV ) from Barkatullah University, Bhopal, Madhya Pradesh, India. To the best of our knowledge, this is the first report on the association of Jujube witches'-broom phytoplasma species of Elm yellows group (16SrV) with witches'-broom disease of *Z. oenoplia* in India.

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