

## First report of Phytoplasma associated flattened stem disease on Sapodilla in Rajasthan

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### Abstract

Many tree species growing in tropical and subtropical regions are frequently infected by phytoplasma-associated diseases. Phytoplasma is a pleomorphic and cell wall-less microorganism restricted only to the phloem of plants. About 129 plant species related to vegetables, cereals, grasses, fruit trees, weeds etc. are infect by Phytoplasma in India. The present study is the first report of phytoplasma-associated flattened stem disease on Sapota tree growing in Jodhpur, Rajasthan. *Sapota (Manilkara zapota)* or Sapodilla of the family Sapotaceae is commonly known as Chickoo in India. Disease symptoms such as presence of little leaf, phyllody, proliferation of axillary buds (witches' broom), sterility of flowers, and stem flattening were reported in branches of infected sapota tree. There is a strong possibility that Sapota was infected by locusts which attacked several areas of western Rajasthan during the year 2020-21.

**Key words :** Sapodilla, flattened stem, Phytoplasma, phyllody, witches' broom, Locusts.

**M**ost of fruits and vegetables have suitable circumstances for the growth of various type of microbes. Phytoplasma is such a phytopathogenic bacterium which is a pleomorphic and cell wall-less microorganism and restricted to phloem only. This is primarily spread through insects mainly by the leafhoppers. The plant propagation materials and seeds are the secondary source for transmission of Phytoplasma. Approximately, one hundred twenty-nine plant species related to vegetables, cereals, grasses, fruit trees, weeds etc. are

infected by Phytoplasma in India. Phytoplasma cause many plant diseases like little leaf of brinjal, Sesame phyllody, grassy shoot of sugarcane, sandal spike, including various diseases of ornamental plants. In present study such phytoplasma- associated flattened stem disease is reported in the Sapodilla tree.

*Sapota (Manilkara zapota (L.))* P. Royen also known as sapodilla belongs to family Sapotaceae and is a native plant of southern Mexico. In India, it is commonly known as chikoo, it is a

longlived evergreen fruit tree. This plant is well adapted to the coastal tropics of India therefore considered an indigenous crop to India but it originated from tropical South America<sup>30</sup>. The first seedling plantation of sapota in India was done in Gholwad village of Dahnutaluk, Thane district, Maharashtra in 1898 as per evidence<sup>7</sup>.

Sapodilla is a medium to large tree reaches up to 20-30 metre in height with an average trunk diameter of 1.5 metres. The tree shows a sympodial branching pattern. Branches are horizontal and drooping which makes its canopy pyramidal to round with dense crown. A white, gummy latex exudes from various parts of the tree. The leaves are evergreen, glossy, green, alternate, elliptic to ovate and 7-15 cm in length. Leaves are spirally arranged and clustered on the tip of shoots. The flowers are white, bell-like, with two whorled calyx and a six-lobed corolla. Flowers bloom throughout the year. Sapota fruit is a large round or ellipsoid berry from 4 to 8 cm or more and contains 2-5 seeds. An immature fruit has a firm outer skin with a gummy substance whereas the fully ripe fruit is smooth-skinned and free from latex. The seeds are black with a hook at one end.

Fruits of Sapota are good sources of digestible sugar ranging from 12 to 18 % with significant amounts of fibre, protein, fats, calcium, phosphorus, iron, carotene and vitamins. The white latex of the trunk namely the chicle of the Sapota tree is the main source of the key ingredient of chewing gum.

*Symptoms of Phytoplasma associated disease:*

Phytoplasmas associated plant diseases

display various symptoms which are indicative of instabilities in the normal balance of plant hormones<sup>18</sup>. Phytoplasma- infected plants show virescence which causes green flowers instead of a normal coloured flower, phyllody *i.e* change of floral parts into leaf-like structures, the proliferation of auxiliary shoots subsequently the appearance of “witches’ broom”, phloem necrosis, compact growth at the end of stem, yellowing, and dieback of branches in woody plants.<sup>21</sup>

*Historical background, structure and Transmission:*

In 1967, during the study of Mulberry dwarf disease many large and small (80-800m $\mu$ ) asymmetrical-elliptical mycoplasma-like particles (MLO) were observed within the sections of young leaves and shoots under the electron microscope.<sup>9</sup>, these MLO was named as ‘Phytoplasma’ in the IX<sup>th</sup> International Organization of Mollicutes Conference in 1992.

These pleomorphic, cell wall-less, endocellular prokaryotes parasitized on plants and insects, constitute a large monophyletic group within the class Mollicutes, confirmed by ribosomal rDNA sequencing. Phytoplasma is placed under Mollicutes, as *Candidatus* phytoplasma genus. A total 52 *Candidatus* species, consisting of 34 groups and approximately 100 subgroups are identified on the basis of 16s rRNA gene sequence.<sup>34</sup> Phytoplasmas are cell wall-less prokaryotic microorganism that colonize in plant phloem tissues and salivary glands of insects. Barely few Phytoplasma can cultured in vitro due to its strict culture conditions.<sup>8</sup> The first complete sequencing of the phytoplasma genome of

*Candidatus phytoplasma asteris* was done. It is also the first obligate intracellular bacterium that resides in plants and insects both and whose complete genome is sequenced.<sup>23</sup>

Phytoplasma strains are mainly transmitted by leaf-hoppers, plant-hoppers, and psyllids. Vegetative propagation of the infected host plant parts is another way of transmission of phytoplasma.<sup>32</sup> Indirect biological proof like electron microscopy observation, transmission by insect and dodder<sup>5,13</sup> and elimination of phytoplasma and its symptoms after the treatment of tetracycline<sup>16</sup> proved that phytoplasma is associated with many plant diseases worldwide. Phytoplasma diseases cause huge economic losses to many important crops all over the world, it highly affects the yield and quality of crop plants.

*Host diversity and distribution of Phytoplasma-associated disease :*

In recent years phytoplasma associated diseases have been reported in different countries of Europe<sup>29</sup>, Asia<sup>19,35</sup>, Caribbean and Africa<sup>12</sup>. Phytoplasma-associated plant diseases are widely spread in the Middle East and reported from many plants like almonds, mangoes, Arabic Jasmin, acid lime, date palms *etc.* Phytoplasma-associated diseases in Middle east countries are reported from Iran<sup>14,28</sup>, Oman<sup>33</sup>, Iraq<sup>3</sup>, Lebanon<sup>6</sup>, Isarael<sup>22</sup>, Egypt<sup>10</sup>, Saudi Arab<sup>2</sup> and United Arab Emirates.<sup>11</sup> In India, Phytoplasma-associated diseases reported from seventeen states so far and ten phytoplasma ribosomal groups reported from the south and north parts of India while other parts have a limited number of diseases.<sup>27</sup> Phytoplasma associated flattened stem

disease of Sapota was first reported in India from Uttar Pradesh in July 2019, this was related to 16SrXI-B subgroup.<sup>17</sup>

In Rajasthan, earlier one case of phytoplasma disease was reported on an ornamental plant namely *Jasminum sambac* (L.) in Jaipur in August 2012, during a survey.<sup>20</sup> Recently, In Jodhpur *Candidatus Phytoplasma asteris* associated phyllody disease in Fenugreek is first reported in year 2022, this phytoplasma belongs to 16 SrI-B subgroup is the first time reported of in India also.<sup>31</sup> However, no report of phytoplasma associated disease on any type of fruit tree is documented from any part of Rajasthan.

*First report of Phytoplasma-associated flattened stem disease of Sapota in Rajasthan :*

Phytoplasma-associated disease is mainly reported on ornamental plants but it also affects weeds, crops, fruit, vegetable and other plants. Approximately 172 plant species are confirmed as hosts of ten different groups of phytoplasma, out of these aster yellow group 16SrI is the most predominant group.<sup>26</sup> Present study reported Phytoplasma-associated disease on the Sapota tree for the first time in Rajasthan. Earlier, phytoplasma strains associated with sapota trees have been reported from Cuba which are related to group 16SrII and 16SrV<sup>1,24</sup>. In another case, phytoplasma disease symptoms on Sapota tree like flattened stem, little leaf and internode shortening were reported on two sapodilla trees in the campus of Hormozgan Agricultural and Natural Resources Research and Education Centre, Iran in February<sup>4</sup> 2015.



Figure 1. Infected plant and twigs showing symptoms of Phytoplasma disease

Recently, during a survey of Sapota orchards in India between 2015-2018, phytoplasma-associated disease symptoms like flat stem, phyllody, witches' broom and little leaf were reported in Karnataka, Tripura and Kerala state of India and these symptoms related to three phytoplasma subgroup strains *i.e.* 16SrI-B, 16SrVI-D and 16SrXIV-A.<sup>25</sup> All the phytoplasma strains identified on sapota in India are the first records from the world. In

Rajasthan phytoplasma associated disease on sapota tree is reported for the first time. This disease was reported on *Manilkara zapota* (sapodilla) tree growing in a garden in Jodhpur during April, 2022. An incidence of symptoms of Phytoplasma infection such as little leaf, phyllody, proliferation of auxiliary buds (witches' broom), sterility of flowers, flattening of stem were observed on branches of infected sapota tree.

*Probable source of Phytoplasma infection in Sapodilla tree in Rajasthan:*

Phytoplasma-associated diseases in plants can arise in various probable ways. It can be transmitted by grafting if material is obtained from the infected mother plant or planting the young seedling with latent phytoplasma infection by any insect vector. In India, many common hemipteran insects of different families feed on different herbaceous hosts which cannot be ignored as source of phytoplasma infection<sup>27</sup>. During the month of May, 2020 a huge attack of locusts happened in the north-western part of India. Several other neighbouring countries like Pakistan, Afghanistan, Iran and also some African countries and Arabian Peninsula were also severely affected by this locust attack.<sup>15</sup>

In several areas of western Rajasthan locusts feed on many plant species therefore it is a great possibility that they might carry any type of phytoplasma strains from other countries because locust-swarm travelled across many countries in search of food. Earlier, no case of Phytoplasma disease on the Sapota or any fruit trees was reported in the western or other part of Rajasthan. Therefore, it might be possible locusts may transmit phytoplasma strains from any of their herbaceous hosts to the sapodilla trees; however, the symptoms will appear in the tree after few months later. The observation of the infected branches of the Sapota tree was revealed that the symptoms are very close to earlier study done on two phytoplasma-infected trees of Sapota in Iran which was also the first report of Phytoplasma-associated disease

in Iran in February 2015 and the strain had maximum identity with 16Sr II group<sup>4</sup>. Three phytoplasma subgroup *i.e.* 16SrI-B, 16SrVI-D and 16SrXIV-A was reported in three states of India which is first report of identification of three phytoplasma groups on Sapota in world<sup>25</sup>, similar symptoms were observed in the present study. Further identification on the molecular level of infected plant parts can only confirm the infection causing phytoplasma strain.

Several reviews and reports were published regarding diseases associated with phytoplasmas in different countries. However, no report is available on the phytoplasma diseases in western Rajasthan, which is an important region with semi-arid and arid conditions. This report describes the phytoplasma-associated diseases now entered in this region of India and can spread to other trees of plants in future. Therefore, close monitoring is for the occurrence of Phytoplasma-associated diseases especially after any locust attack near future so that any kind of huge loss for crop plants or fruit trees can be controlled well in time. Extensive and systematic research on the phytoplasma strains regarding their identification and distribution patterns is also necessary to establish effective control measures and any rapid spread of this disease.

References :

1. Acosta, K., B. Piñol, E. Acosta, P. Countín and Y. Arocha (2009). *Plant Pathology*. 58(2): 391-391.
2. Alhudaib, K., Y. Arocha, M. Wilson and P. Jones (2007). *Bulletin of Insectology*. 60(2): 285.

3. Alkuwaiti, N.A.S., T.A. Kareem and L.J. Sabier (2017). Agriculture (Pol'nohos-podárstvo). 63(3): 112-119.
4. Bagheri, A., M. M. Faghihi, H. H. Khankahdani, M.A Seyahoei, N. Ghanbari and S.S Sarbijan (2017). *Australasian Plant Disease Notes*. 12: 1-3.
5. Bertaccini, A. (2007). *Frontiers in Bioscience-Landmark*. 12(2): 673-689.
6. Choueiri, E., F. Jreijiri, S. Issa, E. Verdin, J. Bové and M. Garnier (2001). *Plant disease*. 85(7): 802-802.
7. Chundawat, B. S. (1998). Agrotech Publishing Academy pp. 10-14.
8. Contaldo, N., A. Bertaccini, S. Paltonieri, H.M. Windsor and G.D. Windsor (2012). *Phytopathologia Mediterranea*. 51(3): 607-617.
9. Doi, Y. O. J. I., M. Teranaka, K. Yora and H. Asuyama (1967). *Japanese Journal of Phytopathology*. 33(4): 259-266.
10. El-Banna, O. H. M. and S.H. El-Deeb (2001). *Egyptian Journal of Phytopathology*. 29(1): 101-102.
11. Garnier, M., L. Zreik and J.M. Bové, (1991). *Plant Disease*. 75(6): 546-551.
12. Gurr, G. M., A.C. Johnson, G.J. Ash, B.A Wilson, M.M Ero, C.A Pilotti and M.S. You (2016). *Frontiers in plant science*. 7: 1521.
13. Hogenhout, S.A., K. Oshima, E.D. Ammar, S. Kakizawa, H.N. Kingdom and S. Namba (2008). *Molecular plant pathology*. 9(4): 403-423.
14. Hosseini, S. A. E., M. Salehi, G. Khodakaramian, S.M Mirchenari and A. Bertaccini, (2015). *Phytopathogenic Mollicutes*, 5(1): 9-18.
15. <https://www.bbc.com/news/world-asia-india-52804981>
16. Ishiie, T., Y. Doi, K. Yora and H. Asuyama (1967). *Japanese Journal of Phytopathology*. 33(4): 267-275.
17. Kumar, S., J. Singh, V.K. Baranwal and G.P. Rao (2021). *Journal of Plant Pathology*. 103: 659-660.
18. Lee, I.M. and R.E. Davis (1992). *Mycoplasmas: molecular biology and pathogenesis*. pp. 379-390 American Society of Microbiology, Washington.
19. Li, S., W. Hao, G Lu, J. Huang, C. Liu and G. Zhou (2015). *Plant disease*. 99 (11): 1483-1487.
20. Madhupriya, G.P. Rao and S.M.P. Khurana (2015). *Phytoparasitica*. 43: 77-80.
21. McCoy, R.E., A. Caudwell, C.J. Chang, T.A. Chen, L.N. Chiykowsky, M.T. Cousin, J.L. Dale, G.T. N de Leuw., D.A. Golino, K.J. Hackett, B.C. Kirkpatrick, R. Marwitz, H. Petzold, R.C. Sinha, M. Sugiura, R.F. Whitcomb, I.L. Yang, B.M. Zhu, and E. Seemüller (1989). *The Mycoplasmas*. pp. 545-640 Academic Press.
22. Orenstein, S. I. G. A. L. I. T., T. I. R. T. Z.A Zahavi and P. H. Y. L. L. I. Weintraub, S. (2001). *Vitis*. 40(4): 219-223.
23. Oshima, K., S. Kakizawa, H. Nishigawa, H.Y. Jung, W. Wei, S. Suzuki and S. Namba (2004). *Nature genetics*. 36(1): 27-29.
24. Pérez-López E, M.L. Pantoja, L. Hernández-Rodríguez and I.P. Bárzaga (2013). *New Disease Reports*. 28: 18.
25. Rao G.P., Madhupriya, S. Mitra, J.M. Johnson, P. Debnath, A. Bahadur and S.C Das. (2020). *Physiology and Molecular Biology of Plants*. 26(8): 1685-1693.
26. Rao, G.P. (2021). *Indian phytopathology* 74(2): 371-401.

27. Rao, G. P., V. Thorat, R. Manimekalai, A. K. Tiwari and A. Yadav (2017). *Phytopathogenic Mollicutes*. 7(1): 1-38.
28. Salehi, M. and K. Izadpanah (1992). *Journal of Phytopathology*. 135(1): 37-47.
29. Seemüller, E. and B. Schneider (2004). *International Journal of Systematic and Evolutionary Microbiology*. 54(4): 1217-1226.
30. Shirol, A. M., V.C. Kanamadi, M.P. Duragannavar, N. Thammaiah and S.M. Baragimath (2009). *Asian Journal of Horticulture*. 5(3): 87-90.
31. Singh, K., M. Kumar, K. Rawat, H. Ranebennur, V.S. Meena, N. Shekhawat,... and M. Choudhary (2023). *Plant Disease*. 107(8): 2516.
32. Sugio, A., H.N. Kingdom, A.M. MacLean, V.M. Grieve and S.A. Hogenhout (2011). *Proceedings of the National Academy of Sciences*. 108(48): E1254-E1263.
33. Waller, J. M. and J. Bridge (1978). *Proceedings of the National Academy of Sciences*. 24(3): 313-326.
34. Yang, J., Y. J Liao, J. H. Ning, J. Z. Wang, H. Wang and Z.G. Ren (2020). *Forest pathology*. 50(3): e12592.
35. Zhu S., A. Bertaccini, I. Lee, S. Paltrinieri, and A. Hadidi (2011). *Virus and Virus-Like Diseases of Pome and Stone Fruits*. pp. 255–257. APS Press; St. Paul, MN, USA.