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Phytoplasma infecting weeds in India: A review

Shoeb Ahmad[1*], Akil A Khan[2]
[1, 2] Department of Botany, GF College, Shahjahanpur, Uttar Pradesh, India

Abstract
In 2018 and 2019, suspected phytoplasma symptoms of typical leaf chlorosis, witches' broom and small leaf were recorded on seventeen weed species in and around agricultural fields in various parts of India. Symptomatic weed species were tested for phytoplasma interaction using nested PCR assays using standardised phytoplasma-specific primary pairs, 'P1 / P7 and R16F2n / R16R2'.Ten of the seventeen symptomatic weed species collected, *viz.* Positive to phytoplasma were "*Digitaria ciliaris, Phalaris minor, Eleusine indica, Cynodon dactylon, Parthenium hysterophorus, Dichanthium annulatum, Cannabis sativa, Datura stramonium*, *Digitaria sanguinalis* and *Oplismenus burmanni".* BLAST and phylogenetic study of 16Sr-DNA phytoplasma sequences of ten positive weed species confirmed the relationship of three distinct phytoplasma types. 16Sr-I, 16Sr-II, 16Sr-XIV. Phytoplasma interaction with *D. Ciliaris* (16Sr-XIV), *E. Indica* (16Sr-XIV group) and *P. minor* (16Sr-I group) were new world phytoplasma records. The two weeds, though, *viz*. P. hysterophorus, O. burmanni. The new phytoplasma community (16Sr-II) in India identified burmanni hosts.

Keywords: phytoplasma, o burmanni symptomatic weed species, pathogenic stud**y**

1. Introduction

Phytoplasmas are prokaryotic plant mollicates, phloem-limited bacterial pathogens that cause many significant woody and herbaceous plant diseases worldwide (Bertaccini *et al*., 2014). In addition to several economic crop plants, many weeds are significant phytoplasma reservoirs and play an important function as natural alternative / collateral hosts (Tran-Nguyen *et al*., 2000 [74, 103]; Blanche *et al*, 2003 [8, 84]; Harrison and Oropeza, 2008) [26]. .More than 30 weed species are recorded to be hosting phytoplasmas belonging to four separate classes (16Sr-I, 16Sr-II, 16Sr-VI and 16Sr-XIV) across India and most belonged to 16Sr-I and 16Sr-XIV phytoplasma classes (Baiswar *et al*, 2010 [81]; Tiwari *et al*). Early identification of these weed-related phytoplasmas is very necessary to verify the risk of more transmitting phytoplasma diseases to other crops. Thus, a comprehensive survey of different weed species in and around agricultural fields during the autumn and spring seasons from August 2018 to April 2019 attempted to report some new weed species as host of essential phytoplasmas infecting crops in India. A total of 16 weed species from the families Asteraceae, Poaceae, Cannabinaceae and Solanaceae displaying suspicious phytoplasma symptoms were obtained.

In some weeds, phytoplasmas induce diseases that may serve as alternate natural hosts, encouraging the dissemination of phytoplasmas to other commercially valuable plants and thereby growing agricultural losses. The most unusual signs on weeds include extensive chlorosis, axillary shoots proliferation, witches'-broom, yellowing, and thin leaves. More than 43 weed species have been recorded to have phytoplasma infections worldwide. Nucleotide sequence analyses found that weed-infecting phytoplasmas primarily belong to classes 16Sr-I, 16Sr-IV, 16Sr-VIII, 16Sr-XI and 16Sr-XIV. 16Sr-I and 16Sr-XIV phytoplasmas have a larger incidence in nature worldwide. While weeds known as phytoplasma hosts sometimes develop abundantly around field crops, the possibilities of transmitting phytoplasmas related to important agricultural, economic and horticultural crops from weeds to the crops listed and vice versa cannot be over looked. This may be because phytoplasmas can thrive in several possible economic crops or an insect vector can spread phytoplasmas from other weeds to crops already recognised as phytoplasma hosts. In any scenario, the probability of transmission in the future seems strong, considering the already revealed broad phytoplasma pool, the propensity of emerging new phytoplasma strains. Comprehensive and up-to-date information on the occurrence of phytoplasma, symptomatology, molecular characterization, distribution, taxonomy, genetic diversity and management approaches were explored in this section. Recent research and open molecular techniques for pathogen detection will increase understanding of the variety of phytoplasmas.

Wall less, uncultural Mollicute-class bacteria are grass-pathogenic phytoplasmas contaminated with over 1,000 plant diseases worldwide (Seemuller *et al*., 1998 [67]; Al-Saady and Khan, 2006) [40, 1]. They have a small genome of between 530 and 1350 kilo bases, a G+C content of between 24 and 33 mol each, two rRNA operons, a small number of tRNAs and a limited range of metabolic enzymes. Phytoplasmas are obligatory pathogens that could live and reproduce only in plant hosts and insect vectors. According to phylogenetic research, phytoplasmas are similar to Acholeplasmas than other mollicutes. Acholeplasma and A. Modicum are the nearest recognised phytoplasma relatives. Phytoplasma-induced diseases are characterised by profound disturbances in the equilibrium of the natural growth regulator, flower malformation, growth aberrations, yellowing and/or regression, and are collectively referred to as yellow disease. These diseases were believed to be triggered by viruses before a group of Japanese scientists discovered in 1967 that they were infected with pleomorphic bodies varying from 200 to 800 nm, then called mycoplasma-like species (Doi *et al*., 1967) [20]. DAPI staining and electron microscopy focused on phytoplasma detection for over two decades. However, inside the phytoplasma clade, the developments of DNA-based technologies have rendered it possible to differentiate distinct phylogenetic groups. “To drop the term mycoplasma-like species, the International Research Project for Comparative Mycoplasmology (IRPCM) Phytoplasma Working Party introduced the trival designation 'phytoplasma.' The 'Candidatus Phytoplasma' genus was later suggested and accepted to start systematic classification of these prokaryotes, some of which are correlated with significant or quarantine-subjected plant diseases (IRPCM, 2004) [4].” Koch's up-to - date happiness was not reached, however some indirect facts suggested that they are responsible for multiple plant diseases worldwide. Genetically undistinguishable phytoplasmas have also been shown to be associated with diseases that cause various symptoms and/or affect specific plant species, while genetically distinct phytoplasmas in the same or particular plant host(s) may be associated with associated symptoms.

2. Phytoplasma Disease of Weeds

Several weeds are reservoirs of important phytoplasmas that cause major commercial crops to become serious diseases and play a major role in the spread of phytoplasmas and function as natural alternate hosts (Blanche *et al*., 2003 [8, 84]; Harrison *et al*., 2008) [44, 26]. Phytoplasmas induce diseases in many weeds, resulting as an additional natural host to promote the propagation of phytoplasmas to other economically valuable plants and thereby raise agricultural losses. Early identification of these weed crop-related phytoplasmas is very necessary to verify the risk of further transmission of phytoplasma diseases to other commercial crops.

Many phytoplasma-infected weeds are considered to have a broad variety of symptoms such as white leaf, yellow, witch-broom, and phyllody (Dabek, 1983 [14]; Shiomi *et al*, 1983; Gibb *et al*., 1997 [22]; Tran-Nguyen *et al*, 2000 [103, 84]; Arocha *et al*., 2005 [5]; Marcone, 2008) [44]. Bermuda grass white leaf (BGWL) is a destructive phytoplasmic disease of Bermuda grass, Cynodon dactylon (L.) Pers., first recorded from Taiwan (Chen *et al*., 1972) [92]. Several Asian countries are reported to have the disease (Davis and Dally, 2001 [16]; Jung *et al*., 2003; Rao *et al*., 2007), Sudan, Italy (Marcone *et al*., 1997, 2004), Cuba (Arocha *et al*., 2005) [5] and Australia (Schneider *et al*., 1999; Tran-Nguyen *et al*., 2000 [103]; Blanche *et al*., 2003) [8, 84]. Often named Cynodon White Leaf (CWL). The causative factor, the BGWL phytoplasma, is a part of the phylogenetic BGWL phytoplasma community or 16Sr-XIV community, which includes many other poaceous plant pathogens such as white leaf (Brachiaria distachya), annual blue grass (Poa annua) white leaf (ABGWL), white leaf (Dactyloctenium aegyptium) white leaf (DacWL) and carpet grass (Axonopus compressus) white leaf (CB). BGWL phytoplasma is a distinct taxon at the putative species stage for which the term 'Candidatus Phytoplasma cynodontis' has been suggested (Marcone *et al*., 2004; Arocha *et al*., 2005) [5].

Arocha *et al*. (2005) [5] first reported on the occurrence of phytoplasmas in Bermuda grass in Cuba and also found that BGWL disease is triggered by a 16Sr-XIV community phytoplasma. In Italy, Marcone *et al*. (1997) observed BGWL disease and described the relationship through RFLP study. Sarindu and Clark (1993) [63] recorded Thailand sugarcane white leaf and BGWL diseases. Gibb *et al*. (1997) [22] observed and established white-leaf-related phytoplasmas and stunting disease in Australian turf. Livingston *et al*. (2006) [40] first identified phytoplasma in Polygala mascatense, a plant in Oman. Some plants display stunting, thin leaves, bushy development and symptoms of phyllody. PCR assay reported the existence of infections triggering witches'-broom in Polygala. RFLP findings showed that polygala-infecting phytoplasma is identical to Lucerne phytoplasma community (16Sr-II).

Phytoplasmas were observed in weed E's naturally contaminated plants phloem. Cicutarium collected in Chile's semi-arid coast. The analysis revealed various straight and curved sections of phytoplasma filaments, but no helical bodies were found (Graf *et al*., 1978) [23]. Lee *et al*. (1992) documented phytoplasma Trifolium sp. (Fabaceae) in Canada with standard clover phyllody signs, recognising the causative agent as a 16SrI-C yellow community member. Smrze *et al*. (1981) [72] noticed phytoplasmas sporadically arising in Czechoslovakia. The disease was transferred to healthy plants and the symptoms were mild and inconspicuous relative to those recorded from Australia and USA. In diseased but not healthy phloem tissues, phytoplasma bodies were found. Arocha *et al*. (2006) [5] documented infections of phytoplasma in Ocimum basilicum (Labiatae) rising wildly in Cuba, showing no leaf symptoms.

Brown *et al*. (2008) [85] identified a 16Sr-IV phytoplasma in Emelia fosbergii, Synedrella nodiflora and Vernonia cineria (Asteraceae) in Jamaica with lethal yellowing symptoms. Reeder *et al*. (2008) [62] identified a 16Sr-I community phytoplasma in yellow-sick Senecio jacobaea in UK. Koh *et al*. (2008) [32] observed phytoplasma on Paspalum conjugatum (Poaceae) in Singapore with white-leaf symptoms. Singh *et al*. (1978) revealed phytoplasma bodies in DC phloem cells. Dactylon plants collected near Varanasi, U.P. India and proved the disease-causing function of phytoplasma bodies. Reddy and Jeyarajan (1990) [61] find rice yellow dwarf symptoms in herb, Echinochloa colonum. They have effectively transferred the rice-to-weed phytoplasma. Muniyappa *et al*. (1979, 1982) [47] observed C-phytoplasma. South India's dactylon and yellowing Urocha panicoides. Pleomorphic phytoplasma bodies existed in contaminated plant phloem tissue. They observed that adding oxytetracycline hydrochloride briefly suppressed symptoms. Padmanabhan (1982) [55] recorded South India's Parthenium hysterophorus phyllody disease. Witches'-broom signs describe the disorder. Transmission from diseased to stable plants utilising the insect vector Hishimonus phycitis was also achieved. Rao *et al*. (1990) confirmed phytoplasma-caused Imperata arundinacea white-leaf disease. Also correlated with 16Sr-I community phytoplasmic infections were Cannabis sativa (Cannabinaceae) and Achyranthes aspera (Amaranthaceae) exhibiting witch-broom and yellowing signs in India (Raj *et al*. 2008 [22]; Mall, 2009) [23]. Other recorded weed diseases are Phyllanthus fraternus leaf phyllody, Cenchrus ciliaris grassy and bunchy shoot, Dactyloctenium aegyptium bunchy shoot and Imperata arundianacea white leaf (Mall *et al*., 2007) [30]. A witch-broom disease triggered by phytoplasma on Bupleurum falcatum, Cnidium officinale and Plantago asiatica was observed in Korea (Choi *et al*. , 1985), while a small leaf disease associated with phytoplasma was documented in Jamaica (Dabek, 1983) [14].

3. Detection of Phytoplasmas

A wide range of symptoms is found in phytoplasma-related weeds.

Extensive chlorosis, axillary shoots proliferation, bushy growing habit, thin leaves, decreased stolons and rhizomes, pervasive stunting, virescence / phyllody, witch-broom and all species death are the most characteristic symptoms. In certain weeds infected with phytoplasma, bright green to yellow stripes on the leaves are also present. (Singh *et al*. 1978; Rao *et al*., 2007, 2009; Mall *et al*., 2009). Table lists a different set of symptoms developed by phytoplasmas on different weeds worldwide. Any typical phytoplasma symptoms also occur in Fig. 1.

Table 1: Symptoms of Phytoplasma occurring in weeds

Sl.No.	Weed Species	Disease	Symptoms	Reference
1	Acanthospermum hispidium (Asteraceae)	Little leaf	Little leaf	Raju and Muniyappa (1981) [32]
2	Achyranthes aspera (Amaranthaceae)	Yellowing disease	Yellowing	Raj *et al*. (2008) [46]
3	Asclepias sp. (Asclepiadaceae)	Milkweed yellows (MWY)	Yellowing	Griffiths *et al*. (1994); Gunderseon *et al*. (1994) [45]
4	Axonopus compressus (Poaceae)	Carpet grass white leaf (CGWL)	White leaf	Sdoodee *et al*. (1999) [24]
5	Brachiaria distachya (Poaceae)	Brachiaria white leaf (BraWL)	White leaf	Chen *et al*. (1972)
6	Cannabis sativa (Cannabinaceae)	Wwitches'-broom disease	Wwitches'- broom	Raj *et al*. (2008) [85]
7	Cassia italica (Fabaceae)	Witches'-broom disease	Witches'-broom	Khan *et al*. (2007) [44]
8	Cassia italica (Fabaceae)	Witches'-broom	Witches'-broom	Khan *et al*. (2007) [44]
9	Cenchrus ciliaris (Poaceae)	Grassy and bunchy shoot disease	Grassy and bunchy shoot	Mall *et al*. (2007) [20]
10	Chloris inflate (Poaceae)	Creamy leaf disease	Creamy leaf	Blanche *et al*. (2003) [46]
11	Crotalaria tetragona (Fabaceae)	Wwitches'-broom disease	Wwitches'- broom	Baiswar *et al*., (2009) [61]; Ribeiro *et al*. (2001) [90]
12	Cynodon dactylon (Poaceae)	Bermuda grass white leaf (BGWL)	White leaf	Lee *et al*. (1997) [65]; Marcone *et al*. (1997); Arocha *et al*. (2005) [5]; Marcone *et al*. (1997); Koh *et al*. (2008)
13	Dactyloctenium aegyptium (Poaceae)	Bunchy shoot disease	Bunchy shoot	Mall *et al*. (2007) [20]
14	Dactyloctenium aegyptium (Poaceae)	Grassy shoot disease	Grassy shoot	Blanche *et al*. (2003) [46]
15	Dichanthium annulatum (Poaceae)	White leaf	White leaf	Rao *et al*. (2009) [61]
16	Echinochloa colonum (Poaceae)	Rice yellow dwarf (RYD)	Yellowing	Reddy and Jeyarajan (1990) [5]
17	Emelia fosbergii (Asteraceae)	Lethal yellowing	Yellowing	Brown *et al*. (2008) [15]
18	Erigeron bonariensis (Asteraceae)	Witches'-broom disease	Witches'-broom	Davis *et al*. (1994) [20]
19	Erodium cicutarium (Geraniaceae)	Yellow disease	Yellowing	Graf *et al*. (1978) [65]
20	Imperata arundianacea (Poaceae)	White leaf disease	White leaf	Mall *et al*. (2007) [56]
21	Imperata arundinacea (Poaceae)	White leaf disease	White leaf	Rao *et al*. (1990) [95]
22	Medicago sativa (Fabaceae)	Loofah witches'- broom (LfWB)	Witches'-broom	Lee *et al*. (1993a) [53]
23	Ocimum sp.(Labiatae)	Basil little leaf	Little leaf	Arocha *et al*. (2006) [5]
24	Parthenium hysterophorus (Asteraceae)	Phyllody disease	Phyllody	Padmanabhan (1982) [44]
25	Paspalum conjugatum (Poaceae)	White leaf disease	Bleached leaf	Koh *et al*. (2008) [45]
26	Pennisetum purpureum (Poaceae)	Napier grass stunt disease	Stunting	Nielsen *et al*. (2007 [52]
27	Phyllanthus fraternus (Euphorbiaceae)	Leaf phyllody disease	Leaf phyllody	Mall *et al*. (2007) [20]
28	Poa annua (Poaceae)	Annual blue grass white leaf (ABGWL)	White leaf	Lee *et al*. (1997) [53]
29	Polygala mascatense (Polygalaceae)	Polygala witches'-broom	Witches'-broom	Livingston *et al*. (2006) [95]
30	Rhynchosia minima (Fabaceae)	Rhynchosia little leaf	Little leaf	Dabek *et al*. (1983) [42]
31	Sasa fortunei (Poaceae)	Witches'-broom disease	Wwitches'- broom	Zhang *et al*. (2009) [41]
32	Senecio jacobaea (Asteraceae)	Aster Ragwort yellows	Witches'-broom	Montano *et al*. (2000) [47]
33	Sorghum stipoidem	White leaf disease	White leaf	Blanche *et al*. (2003) [48]
34	Synedrella nodiflora (Asteraceae)	Lethal yellowing	Yellowing	Brown *et al*. (2008) [49]
35	Trifolium sp. (Fabaceae)	Clover Phyllody (Cph)	Phyllody	Lee *et al*. (1992) [66]

36	Trifolium sp. (Fabaceae)	Clover yellow edge (CYE)	Chlorosis, Stunting	Lee *et al*. (1992, 1993) [71]
37	Trifolium sp. (Fabaceae)	Clover proliferation (CP)	Proliferation of axillary shoots	Deng *et al*. (1991); Lee *et al*. (1991) [44]
38	Urochloa panicoides (Poaceae)	Yellowing disease	Yellowing	Muniyappa *et al*. (1982) [75]
39	Vernonia cineria (Asteraceae)	Lethal yellowing	Yellowing	Brown *et al*. (2008) [67
40	Vicia faba (Fabaceae)	Faba been Phyllody (FBP)	Phyllody	Schneider *et al*. (1995) [26]
41	Whiteochloa biciliata (Poaceae)	White leaf disease	White leaf	Blanche *et al*. (2003) [23]
42	Whiteochloa cymbiforms (Poaceae)	White leaf disease	White leaf	Blanche *et al*. (2003) [79]

Fig 1: Symptoms of phytoplasma on weeds: (a) witches'-broom on Cannabis sativa, (b) Bermuda grass with white leaves, (c) yellows streaks on leaves of Oplismenus burmanni, (d) pronounced yellowing of Achyranthes aspera, (e) Dichanthium annulatum showing symptoms of extensive chlorosis (f) Crotalaria tetragonaloba with witches'- brooms along with healthy plant.

4. Genetic Diversity

Weed phytoplasmas display a large distribution (Table 1). More than 43 weed species are confirmed to have phytoplasma infections worldwide. Nucleotide sequence analyses have shown that weed-infecting phytoplasmas belong to five main groups: "16Sr-I, 16Sr-IV, 16Sr-VIII, 16Sr-XI and 16Sr-XIV". 16Sr-I and 16Sr-XIV phytoplasmas have a larger incidence in nature worldwide. In India, 16Sr-XIV group phytoplasmas (= 'Candidatus Phytoplasma cynodontis') are reported as a large group on grass, while aster yellows (16Sr-I) group phytoplasmas are reported in Cannabis sativa, Achyranthes aspera and Parthenium hysterophorus (Rao *et al*., 2007 [63]; 2009; Raj *et al*., 2008 [94]; Mall *et al*., 2009) [20].

5. Management

Unfortunately, no successful majors are required to treat phytoplasma diseases. The effect of these diseases depends on many factors such as the virulence of strains in a given taxon and their ability to mutate, vector existence and dynamics, phytoplasma abundance in both host plants and insect vectors, and environmental and agronomic conditions. Consequently, no single monitoring technique can be implemented. Before interfering, the most important considerations to consider are: seriousness of outbreak, whether or not to rouge contaminated plants, roughing methods, supply of insect vectors, alternate vulnerable plant reservoirs, and outbreak economic effects (Osler and Carraro, 2004) [54]. Using disease-free plant propagating material is the most essential for handling these diseases, since phytoplasmas are mainly transmitted by grafting of infected plant sections. Study was also conducted to examine the efficacy of plant bodies attacking phytoplasmas. Bacteriostatic to phytoplasmas, tetracyclines hinder their development (Singh *et al*, 1978). Singh and Pathak (1982) have confirmed that tiamulin antibiotics suppressed symptoms of Cynodon dactylon-related phytoplasma disease. However, illness signs will reappear without constant antibiotic use. It has long been established that heat therapy can help remove pathogens like virus and likely phytoplasmas from plants and primarily fruit trees (Kunkel, 1936 [33]; Kassanism, 1954[34]; Nyland, 1962) [39]. Laimer and Balla (2003) [83] reported a mixture of heat therapy and meristem culture without tetracycline treatment to remove phytoplasmas. These techniques can refer to cannabis phytoplasmas. The easiest way to treat weed phytoplasmas is to eradicate perennial and biennial weed hosts, rogue and kill symptomatic plants, stop planting prone crops next to crops harbouring phytoplasmas, monitor leafhopper vectors in crops and surrounding weeds early in season (Welliver *et al*., 1999) [77].

6. Conclusion

We infer from above that the described phytoplasmas in weeds mainly belong to "16Sr-I, 16Sr-IV, 16Sr-VIII, 16Sr-XI and 16Sr-XIV classes." 16Sr-I and 16Sr-XIV" phytoplasma classes demonstrated larger occurrence in nature worldwide. This analysis will help explain the variety of phytoplasmas in natural and imported weeds. Discovering new phytoplasma hosts has expanded the diversity of the future disease reservoir. Potential phytoplasma reservoir in weeds may be much greater than recorded. Host plants other than grasses now have evidence that before signs appear once, phytoplasmas can be recognised in plant species, that phytoplasmas are not generally visible in each segment of a plant, and that the location of phytoplasma can vary over time in a plant. Although weeds known as hosts of phytoplasma often grow abundantly around field crops, it is not possible to overlook the possibilities of transmitting phytoplasmas related to major agricultural, economic and horticultural crops from weeds to economic crops, and vice versa. This may be because phytoplasmas can live in several possible economic crops (Harrison *et al*., 2008) [44, 26], or because an insect vector can spread phytoplasmas from other weeds to crops already identified as phytoplasmas (Lee *et al*., 2004; Saady and Khan, 2006 [40, 1]; Harrison *et al*., 2008) [44, 26]. In either case , given the already revealed large phytoplasma reservoir, the likelihood of emerging new phytoplasma strains (Lee *et al*., 2000), and the capacity of several leafhoppers to migrate long distances (Taylor, 1985) and switch to new host plants (Purcell, 1985), the probability of transmission in the future seems high..

7. References

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