



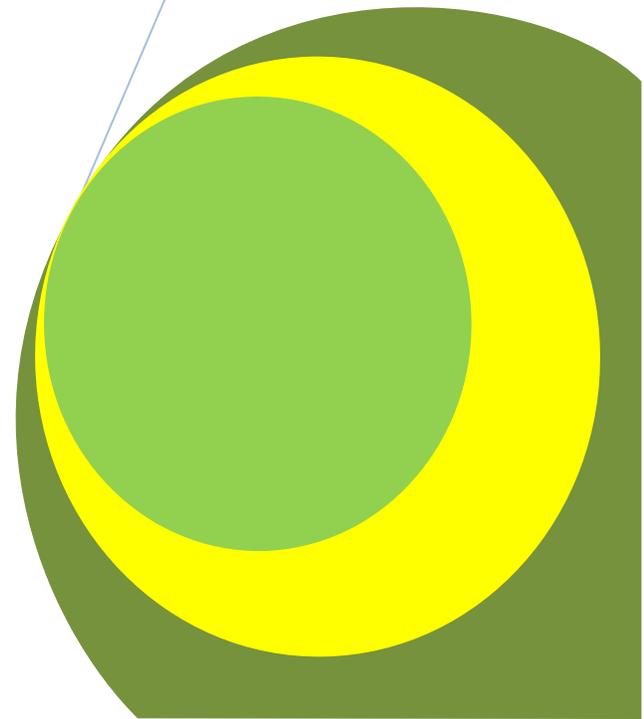
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A Strain of Phytoplasma Related to 16SrVI Group in Datura stramonium in India

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Research Article

A Strain of Phytoplasma Related to 16SrVI Group in *Datura stramonium* in India

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ABSTRACT

During epidemiological survey for phytoplasmas in association with agricultural crops in India, a new species of common weed has been detected to harbor phytoplasmas in India. In 2012, many samples of *D. stramonium* (Family-Solanaceae), commonly known as thorn apple weed were sampled, collected from different adjoining areas of Gorakhpur districts and identification for phytoplasma, Nested polymerase chain reaction analysis using primers specific to the phytoplasma 16SrDNA gene showed samples of *D. stramonium* to be positive. Digestion of amplified 16SrDNA fragments with endonuclease *MseI* identified the same pattern as the one of a reference strain of tomato big bud belonging to the 16SrII ribosomal group. Sequence obtained from the PCR product associated with infected *D. stramonium* was submitted to BLAST analysis which showed a 99% similarity with reference strain of *D. stramonium* witche's' broom from India, belonging to Clover leaf Proliferation (16SrVI) subgroup.

Keywords: *D. stramonium*; 16SrVI; Phytoplasma; PCR; Eastern U.P.

INTRODUCTION

Phytoplasma are specialised bacteria that are obligate plant parasites of plant phloem tissue and transmitting insects (vectors). They were first discovered by scientists in 1967 and were named mycoplasma-like organisms or MLOs (Doi et al., 1967). The natural occurrence of little leaf and witches'-broom was noticed on about 5-10%. *D. stramonium* growing widely along the road sides at Gorakhpur districts Uttar Pradesh, in India during winter session January 2012. The infected plants showed excessive green, tiny narrow leaves, shortening of internodes, little leaf and witches'-broom like symptoms. Earlier, natural occurrence of stolbur phytoplasma (16SrXII group) in *D. stramonium* from Hungary and Greece (Lotos et al., 2012; Viczián et al., 1998) has been reported to be associated with chlorosis and yellows disease. Moreover, *D. stramonium* is also described as experimental alternate host of tomato stolbur phytoplasma in Turkey and of lime witche's' broom phytoplasma in Iran (Ozdemir, 2009; Salehi et al., 2000).

However, another species of *Datura*, i.e. *D. innoxia* was reported as natural reservoir of clover proliferation phytoplasma in India (Raj et al., 2009.)

D. stramonium is used as medicine that treated asthma, and gastrointestinal problems, also aches, abscesses, arthritis, boils, headaches, hemorrhoids, rattlesnake bites, sprains, swellings, and tumors (Sandoval, 1998). It acts as a sedative in large doses and as a stimulant and deliriant in high ones. *Datura* is an anodyne, antibiotic, antispasmodic and narcotic. Relieving the pains of rheumatism and sciatica when applied as an ointment, and easing spasms of Parkinsons disease are unproven accounts of the effects of Jimson weed. Most of the part of plant is used for medicinal reasons. Eating the seeds rapidly gets the plant to the nervous system, but also increases the risk of lethal overdose. The leaves can be dried and smoked to relax the bronchiole muscles of the throat, and leaves are used also to line beds of those with insomnia. Annette Sandoval in Homegrown Healing recommends using the fresh leaves, flowers, or seeds. In an infusion, fresh leaves take to hot water, or a poultice using any variation of the recommended parts. *D. stramonium* contains active compounds such as hyoscyne, as well as atropine, hyoscyamine, apohyoscyne, and meteloidine. Thus it is poisonous and hallucinogenic as well as acting as a pain killer (Duke 1985: 161-162). *D. stramonium* has been found to rapidly clear 2,4,6-trinitrotoluene (TNT) from munition waste sites, and to transform it via nitroreductio *D. stramonium* might very well have similar properties as remediators of explosives(L.M.H. et al., 1999.)

Phytoplasmas belonging to the Clover leaf Proliferation group (16SrVI) have been recorded from weeds and cultivated plants worldwide, causing in significant losses in lime, '*Vaccinium myrtillus*' phytoplasma, Brinjal little leaf

phytoplasma, Candidatus *Phytoplasma castaneae*, Potato witch's broom phytoplasma. Phytoplasmas of the 16SrVI group have been found. In India, phytoplasmas of the 16SrVI group have so far been detected in several weed species, and also cultivated plants (Davino et al., 2007; Parrella et al., 2008; Tolu et al., 2006).

MATERIALS AND METHODS

(A) Survey: During surveys of weeds at different locations of Gorakhpur, Eastern Uttar Pradesh, India in January 2012 for the incidence of phytoplasma associated diseases, *D. stramonium* plants showing suspected phytoplasma symptoms like little leaf and witch's broom were observed. The symptomatic 10 samples were collected together with 5 samples from asymptomatic plants towards identification and characterization of associated phytoplasma.

(B) DNA Isolation: Total DNA was extracted from approximately 100mg of leaf tissue employing phytoplasma enrichment procedure (Ahrens and Seemüller, 1992). In DNA isolation I followed CTAB methods. (2% CTAB, 1.4M NaCl, 20mM EDTA, pH 8.0, 100mM Tris HCl, 0.2 % mercaptoethanol and 0.5% sodium sulphite) was added (Dellaporta et al., 1993).

(C) Primers and PCR Amplification: For amplification of phytoplasmal ribosomal DNA (r DNA) by PCR assays, the universal phytoplasma primer pairs P1/P7 (Deng and Hiruki, 1991; Schneider et al., 1995). Further nested PCR was done with primer pair R16F2n/R16R2 (Gundersen and Lee, 1996).

(D) Sequencing of PCR Product: Nested PCR product (1.2 kb amplicon) was purified using the Wizard[®] SV Gel and PCR Clean-up System (Promega). The purified amplified product was sequenced directly in both directions using the same primers as for the nested PCR amplification.

(E) Sequence Comparison and Phylogenetic Analysis: From sequencing and alignment of R16F2/R2 amplicon a 1081bp DNA sequence of *D. stramonium* phytoplasmas, designed as *Datura* little leaf phytoplasma (DLL) was obtained and submitted in GenBank with Accession No. KC468279. The partial 16Sr DNA sequence for the *Datura* phytoplasma isolate was presented.

The sequences were aligned using CLUSTAL W method of Bio-Edit software (Bio-edit Sequence Align Editor). The consensus sequence was submitted to GenBank and used in Blast search. The sequence generated from the present study and reference phytoplasma strains sequence retrieved from GenBank were used to construct phylogeny through MEGA 4.0 version software (Tamura et al., 1997).

Phylogenetic tree (generated by MEGA 4.0 tool with 100 bootstrap values) showing close Phylogenetic relationship of the *Datura* little leaf phytoplasma (DLL) isolate from India (highlighted) with isolates of brinjal little leaf phytoplasmas from India, clover proliferation phytoplasma group.

RESULTS AND DISCUSSION

A disease characterized by little leaf and witch's broom symptoms was observed on *D. stramonium* plants growing widely along the road sides at Gorakhpur, Uttar Pradesh, India during January 2012. The symptomatic plants were further processed for phytoplasma detection and characterization. Universal primer pair of P1/P7 amplified the ~1.8 kb DNA fragment of phytoplasma 16S-23S rRNA from nucleic acid extracted from symptomatic *D. stramonium* plant. In nested PCR assay with R16F2n/R16R2, a DNA fragment of ~1.2 kb was obtained. Neither by direct nor by nested PCR amplification was present from any non-symptomatic plant indicated the phytoplasma association with the little leaf and witch's broom disease of *D. stramonium*. BLAST analysis of the 16S rRNA partial sequence of the phytoplasma identified in *D. stramonium* revealed its highest identity (99%) with that of Brinjal little leaf phytoplasma (EF186820), Periwinkle little leaf phytoplasma (AF228053), *Ca* phytoplasma trifolii (AY390261), Lucerne virescence phytoplasma (EF186821), Potato witches'-broom phytoplasma (HQ609491), a member of *Ca Phytoplasma trifolii* 16SrVI group (Clover proliferation group). The association of 16SrVI (Clover proliferation group) is the first report from India on *D. stramonium*.

D. stramonium found in nature in 2-6 plants patches irregular manner not found in regular. In the season November-July found phytoplasma infection on *D. stramonium* this sp. is very common in Eastern U.P. I conclude that every 2-6 plants one or two plants are healthy and left all are severally infected to phytoplasma, causes loss of productivity of *D. stramonium*.

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Figure -1: (A) Healthy plants of *D.stramonium*, (B –C) Infected plants of *D.stramonium*. Showing little leaf and witches' broom.

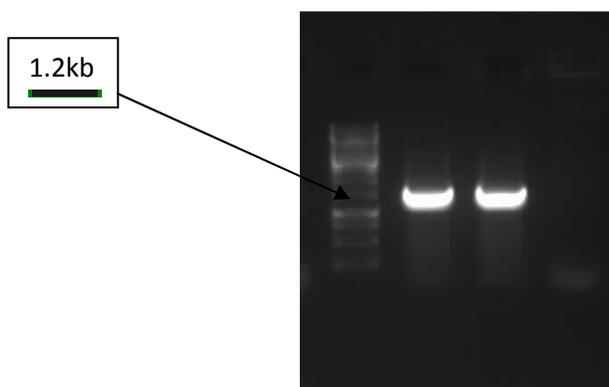


Figure-2: Nested PCR results using primers R16F2n/R16R2 on nucleic acid extracted from *Datura stramonium* samples.

Lane 1: 1 kb DNA ladder (G biosciences); lane 2: toria phyllody phytoplasmas (16SrIX, Pigeon pea witches' broom), lane 3: *Datura* symptomatic sample; Lane 4: *Datura* asymptomatic sample.

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1 aagttgac ctggctcagg attaacgctg gcggcgtgcc taatacatgc aagtcgaacg
61 ggaacctcta aaaggaggtt ttagtgccg aacgggtgag taacacgtaa gtaacctgcc
121 ttaagacga ggataacaac cgaagggtta ctaagactgg ataggaaaca aaaaggcatc
181 ttttgttt taaaagacct tctacaagg tatgctaaa gaggggcttg cgccacatta
241 gtagttgt agagtaaaag cctaccaaga cgatgatgtg tagctggact gagaggtga
301 acagccacat tgggactgag acacggccca aactcctacg ggaggcagca gtagggaatt
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481 tgaataagcc cgggctaact atgtgccagc agccgcggtg agacataggg ggcgagcgtt
541 atccggaatt attggcgta aagggtgctg aggctgttag ataagtctat aatttaatt
601 cagtgctaa cgctgcttg ttatagaaac tctctgact agagtgat agaggcaagc
661 ggaattccat gtgtagcggg aaaatgtgta aatatatgga ggaacaccag aagcgtaggc
721 ggcttgctgg gtcctactg acgctgaggc acgaaagcgt gggtagcaaa caggattaga
781 taccctgta gtccacgccg taacgatga gtaactaagt tggggtaaa actcggctact
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901 aattgacggg actccgcaca agcgggtgat catgtgtt aattcgaaga tacacgaaaa
961 atctaccag gtctgacat actctgcaaa gctatagaaa tatagtggag atcagggata
1021 caggtggtgc atggtgtcg tcagttcgtg tcgtgagatg ttaggttaag tcctaaaacg
1081 aacgcaacc ttgtcgttaa ttgccagcac ataatggtgg ggactt

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Figure-3: *D. stramonium* little leaf and witches' broom phytoplasma partial 16Sr RNA gene sequences, Accession No. KC468279 (Gorakhpur District bases 1 to 1081 bp).

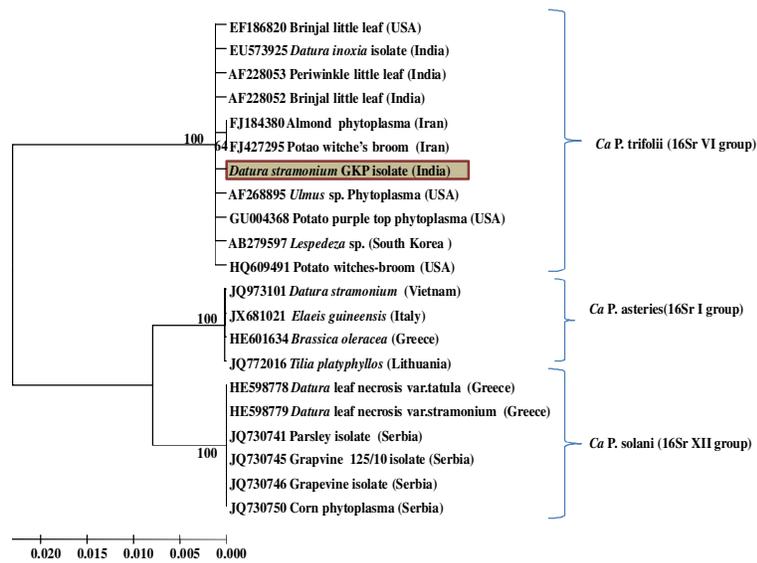


Figure-4: Phylogenetic tree.