Characterization of a novel strain of Candidatus Phytoplasma aurantifolia infecting cowpea (Vigna unguiculata) based on 16S rDNA sequence analysis

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Keywords: Cowpea, phytoplasma, bud proliferation

Posted Date: January 21st, 2022

DOI: https://doi.org/10.21203/rs.3.rs-1278395/v1

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Abstract

An attempt was made to characterize the Phytoplasma in suspected cowpea (Vigna unguiculata L.) samples showing symptoms consisting of bud proliferation and stunting at Experimental Farm, ICAR-NBPGR, New Delhi during Kharif 2020. Associated phytoplasma was characterized based on sequence analysis of target 16S rDNA fragments of 1247 bp amplified from symptomatic samples of cowpea by nested-polymerase chain reaction assay using universal phytoplasma specific primers pairs (P1/P7 and R16F2n/ R16R2). The 16S rDNA sequences understudy shared 98.6% similarity with that of the Candidatus Phytoplasma aurantifolia (GenBank accession: U15442). In phylogenetic analysis, the target 16S rDNA fragment from two samples (Cow-1 and Cow-2) clustered with strains of the 16SrII group of Ca. Phytoplasma aurantifolia. The virtual RFLP pattern showed the highest similarity with the reference pattern of the 16SrII-D subgroup (GenBank accession: Y10097) with a coefficient of 0.97. Since obtained coefficient value is equal to the threshold (0.97) for designating a different strain. Therefore, this phytoplasma infecting cowpea is proposed to be a new strain of Ca. P. aurantifolia as 16SrII-Y subgroup.

Introduction

Cowpea (Vigna unguiculata L.) is one of the most important legumes across the semi-arid tropics valued for its pods and dried seeds (Kumar et al. 2012). Pests and diseases are the major biotic stresses that decrease yield, raise production costs, and limit the storability and marketability of food and feed. Among biotic stresses, phytoplasma diseases have been reported to infect a large number of plants that includes ornamentals, fruits trees, vegetables, cereals, legumes and grapevines worldwide. Phytoplasma is prokaryote without cell-wall restricted to sieve tubes in plants and transmitted by phloem-feeding insects. They have also been reported to infect many leguminous crops such as greengram (Hameed et al. 2017), chickpea (Latha et al. 2021), French bean (Arocha et al. 2008), cowpea, pigeonpea and lentil (Rao et al. 2017a).

Association of phytoplasma caused by Candidatus Phytoplasma asteris, a 16SrI-B subgroup in cowpea showing bud proliferation on the main shoot and stunting was first reported from India by Kumar et al (2012). Subsequently, Thorat et al (2016) and Rao et al (2017b) reported the association of respectively, Ca. P. aurantifolia, 16SrI-D subgroup and Ca. P. cynodontis, 16SrXIV-A subgroup from cowpea plants showing little leaf, witches’ brooms and flat stem symptoms. Phytoplasma 16SrVI-A subgroups have also been reported infecting cowpea from Iran (Kardani and Jamshidi 2018) and 16SrXIV- A subgroup from Iraq (Al-Kuwaiti et al. 2019). Besides these reports, some sequences of phytoplasma obtained from cowpea have also been submitted at NCBI database indicating the presence of phytoplasma16SrI peanut WB group from China (KC953009 to KC953019) and Taiwan (KU170534 and KU170535).

Symptoms of bud proliferation and stunting suspected to be caused by phytoplasma were noticed in cowpea (accession number IC16966) growing at Experimental Farm, New Area, ICAR-National Bureau of Plant Genetic Resources (ICAR-NBPGR), New Delhi. Since there is meagre information available on the association of phytoplasma in cowpea from India, the present investigation was carried out to identify
Materials And Methods

This study was undertaken jointly at ICAR-NBPGR, New Delhi and ICAR-IIPR, Kanpur, India during the year 2020-21.

Collection of samples and DNA isolation: Five samples (Cow-1, Cow-2, Cow-3, Cow-4 and Cow-5) of cowpea genotype (accession no. IC016966) showing bud proliferation and stunting along with two healthy samples (Cow-6 and Cow-7) collected from Experimental Farm, New Area, ICAR-NBPGR, New Delhi (Fig. 1a & b) were brought to the laboratory. Total DNA was extracted from 100 mg of symptomatic as well as healthy plant parts using DNeasy Plant Mini Kit (QIAGEN GmbH, Hilton) following the manufacturer's instructions. The DNA extracted from the phytoplasma affected chickpea sample (Akram et al. 2016) was used as a positive control.

Amplication and cloning of 16Sr DNA fragments: Phytoplasma-specific primers P1 (5’ AAGAGTTTGATCCTGGCTCAGGATT 3’) and P7 (5’ CGTCCTTCATCGGCTCTT 3’) (Deng and Hiruki, 1991; Smart et al., 1996) amplifying ~1,800 bp fragment of 16Sr DNA were used to detect phytoplasma in first-round PCR. Another primer pair (R16F2n-5’GAAACGACTGCTAAGACTGG3'/ R16R2-5’TGACGGGCGGTGTGTACAAAACCCC3’) which amplify a DNA fragment of ~1,250 bp from a portion of the 16S rDNA was used in nested-PCR (Lee et al. 1998). The first round-PCR and nested-PCR were conducted simultaneously in a total volume of 50 μl PCR reaction mix prepared using Dream Taq Green Master Mix 2X (Fermentas) containing 25 μl 2x master mix, 25 pmol each primer, 2 μl template DNA and nuclease-free H₂O. The PCR conditions involved an initial denaturation at 94°C for 2 min, 35 cycles of denaturation at 94°C for 2 min, annealing at 55°C for 2 min for P1/P7 and 56°C for 1 min for R16F2n/R16R2, primer extension at 72°C for 2 min and final extension at 72°C for 10 min. PCR products were separated by electrophoresis in 1% (w/v) agarose gel prepared in a 1xTAE buffer. The DNA was stained with ethidium bromide added to the gel. The DNA bands were visualized on a UV trans-illuminator and photographed using a mobile digital camera. The DNA extracted from two healthy plants and double-distilled water (negative control) was used as an experimental control. The DNA extracted from the phytoplasma affected chickpea plant (Akram et al. 2016) was used as a positive control.

The amplicons of the expected size ~1250bp were excised from the gel and purified using Nucleopore Gel/ PCR clean up kit. The concentration of purified fragments was measured using Nanodrop 2000 spectrophotometer (Thermo Scientific) and the 50 ng DNA was ligated into CloneJET/1.2 blunt vector and transformed in E. coli (DH5α) cells using CloneJET and Bacterial Transformed Aid Kits (Fermentas) following the manufacturer's instructions. Initially, the transformed bacterial cells were confirmed by colony-PCR using primers (pJETF/pJETR) and finally by using restriction digestion of the plasmids. The cloned 16S rDNA fragments were sequenced from both sides through the sequence service provider (Genotypic Technology). For each sample, two clones were sequenced.
Sequence analysis: The sequence data obtained were blasted and trimmed using Bioedit (v.7.2) to remove the vector sequences. Since the sequences obtained from five samples were identical, only two representative 16S rDNA sequences were submitted to GenBank under accession no. MW827058 and MW827059. These sequences were subjected to the program iPhyClassier online tool (http://plantpathology.ba.ars.usda.gov/cgi-bin/resource/iphyclassifier.cgi) for further analysis. The computer-simulated restriction analysis of the subjected sequences (MW827058 and MW827059) generated the restriction profile with 17 restriction enzymes (Alul, BamHI, BfaI, BstUI, DraI, EcoRI, HaeIII, HhaI, HinfI, HpaI, HpaII, KpnI, Sau3AI, MseI, RsaI, SspI and TaqI) which gave output in the form of a virtual gel. The 16Sr DNA sequences obtained from the present study and phytoplasma sequences consisting of one representative sequence of each known phytoplasma group, subgroups of 16SrII and those infecting cowpea (Table 1) retrieved from NCBI database were used to generate phylogeny using MEGA X (Kumar et al. 2018).

Results

Field observations: During Kharif 2020, symptoms of bud proliferation and stunting were observed in cowpea genotype (IC016966) (Fig. 1a & b) with 4% incidence. The symptoms like bud proliferation on the main shoot and stunting (Kumar et al. 2012) and little leaf, witches' broom, leaf yellowing and stem fasciations (Thorat et al. 2016; Rao et al. 2017b, Al-Kuwaiti et al. 2019) have been reported to be caused by phytoplasma in cowpea. Similarly, symptoms of little leaf and thickened leaves, phyllody, the proliferation of shoot, wrinkled and malformed leaves, stem fasciations and stunting associated with phytoplasma infection in cowpea have also been reported from Iran (Kardani and Jamshidi 2018).

Molecular characterization: In the present study, the nested-PCR using universal primer pairs, P1/P7 and R16F2n/R16R2 gave positive results (Fig. 1d) with all the five cowpea samples (Cow-1 to Cow-5) showing symptoms of bud proliferation and stunting symptoms typically associated with phytoplasma infection in plants, whereas negative results were obtained with the healthy samples (Cow-6 and Cow-7). The presence of the DNA fragments of expected size ~1247 bp in the PCR products of all the five samples (amplified by R16F2n/R16R2 primers pair) in the gel confirmed the association of the phytoplasma in symptomatic cowpea plants. These amplicons were successfully purified from the gel and cloned into pJET/1.2 cloning vector. The positive clones identified by colony-PCR were used to isolate plasmids. The plasmid DNA was subjected to restriction digestion released desired DNA fragments confirming them to be the correct clones (Fig. 1e). Two such clones of each sample were sequenced. Both the sequences obtained were found 100% similar. Only two sequences were, however, submitted at NCBI database under the accession numbers MW827058 and MW827059 and analyzed by iPhyClassier (Zhao et al. 2009).

Sequence analysis: The sequence analysis of the sample Cow-1 and Cow-2 confirmed the presence of phytoplasma infection in cowpea samples tested as these sequences have a phytoplasma-specific partial rRNA operon and a partial 16S–23S rRNA intergenic spacer. Sequences of 16S rRNA gene of these isolates subjected to iPhyClassier for species/subspecies identification. Both of the sequences obtained in this study showed 98.6% identity with that of the reference strain of Ca. P. aurantifolia (GenBank
accession: U15442). This indicated that the phytoplasma understudy is closely related to *Ca. Phytoplasma aurantifolia*.

The virtual RFLP pattern derived from the query 16S rDNA F2nR2 fragment (MW827058) indicated most similarity with a reference pattern of the 16Sr group II, subgroup D (GenBank accession: Y10097) with a similarity coefficient of 0.97, a threshold to designate/consider a different strain (Zhao et al. 2009). Among 17 restriction enzymes used to generate virtual RFLP, *Hae* I restriction enzyme gave different RFLP pattern from reference strain (Y10097-16SrII-D-Australia-Papaya). The enzyme (*Hae* I) released four DNA fragments in reference strain (Y10097), whereas in understudy (MW827058) phytoplasma, it produced five DNA fragments (Fig. 1c & f). Thus, the phytoplasma found associated with cowpea samples in the present study is a new subgroup of *Ca. Phytoplasma aurantifolia* under the 16SrII group.

**Discussion**

In this study while compiling this manuscript, 24 subgroups designated after English alphabets A to X (Yang et al. 2017; Al-Subhi et al. 2017; Omar et al. 2020) have been reported. The 16S rDNA sequence generated in the present study and 61 sequences of different phytoplasma sequences representative of each known groups and 16SrII subgroups retrieved from GenBank were used to construct phylogeny by neighbour-joining method with MEGA X (Kumar et al. 2018). Phylogenetic analysis revealed that all the strains of phytoplasma belong to 16SrII group formed a major cluster. Further this cluster (16SrII group) was subdivided into clad for each subgroup. The present strain of *Ca. Phytoplasma aurantifolia* formed separate clad (Fig. 2). It is therefore proposed to name the phytoplasma associated with cowpea disease with symptoms of bud proliferation and stunting at Delhi as *Ca. Phytoplasma aurantifolia*, 16SrII-Y subgroup.

*Phytoplasma* 16SrI-B (*Ca. Phytoplasma asteris*) and 16SrXIV-A (*Ca. P. cynodontis*) from India (Kumar et al. 2012; Rao et al. 2017a & b; Rao 2021), 16SrVI-A subgroup (Clover Proliferation Group) with similarity coefficient 1.00 from Iran (Kardani and Jamshidi, 2018) and 16SrXIV-A subgroup (Bermuda White Leaf Group) from Iraq (Al-Kuwaiti et al. 2019) infecting cowpea have been reported. The 16SrXII-B strain of ‘*Ca. Phytoplasma australiensis*’ associated with witches’broom and small leaves of *V. unguiculata* var. *sesquipedalis* (snake bean) has been reported from Australia and shown to be infecting cowpea upon transmission (Saqib et al. 2006). Based on the sequences available at NCBI database (Table 1), 16SrII (peanut WB group) has been reported to infect cowpea from China (KC953009 to KC953019) and Taiwan (KU170534 and KU170535).

*Phytoplasmas* have been found associated with several diseases in plant species including crops and causes significant economic losses. Cowpea is one of the most important legumes used as pods, dried seeds and fodder in many places of the world. Various phytoplasma groups and subgroups are reported infecting cowpea crops from Iraq, Iran, India, China, Taiwan and Australia. However, in the present study, we reported that the phytoplasma strain is different from all the previously established 16SrII subgroups, which appears to represent a new subgroup within the 16SrII from India. Reports of association of
phytoplasma with different plant species including agri-horticultural crops including legume crops are increasing. It is therefore imperative to conduct a countrywide survey to understand the current status of phytoplasma diseases per se and to decipher the diversity of this pathogen in the changing scenario of climatic conditions.

**Declarations**

**Funding:** Not applicable

**Conflicts of interest/Competing interests:** The authors declare that they have no conflict of interests.

**Availability of the data and material (data transparency):** Not applicable

**Code availability (software application or custom code):** Not applicable

**Ethics approval:** The research does not involve any human participation and/or animals. The materials in the article have not been published in whole or in part elsewhere and not currently being considered for publication in another journal

**Consent to participate** (include appropriate statement): Yes

**Consent for publication** (include appropriate statement): Yes

**Acknowledgements**

The authors wish to express sincere thanks to ICAR, New Delhi, Director, ICAR-NBPGR, New Delhi and Director, ICAR-Indian Institute of Pulses Research, Kanpur for providing laboratory facilities.

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Table

Table 1. Details of the phytoplasma sequences used and some selected sequences retrieved from NCBI database.
| Host         | Associated Disease               | Group                      | Sub Group | Accession number | Country | Reference     |
|--------------|---------------------------------|----------------------------|-----------|------------------|---------|---------------|
| Cowpea       | Cowpea bud proliferation         | *Indian cowpea bud proliferation* | 16SrI     | HM449952         | India   | Kumar et al. 2012 |
| Cowpea       | Cowpea bud proliferation         | *Vigna unguiculata bud proliferation phytoplasma* | 16SrI     | OK586501         | India   | Unpublished   |
| Cowpea       | Cowpea bud proliferation         | *Vigna unguiculata bud proliferation phytoplasma* | 16SrI     | OK586500         | India   |               |
| Soybean      |                                  | *Ca. P. costaricanum*      | 16SrII    | HQ225630         | Costa Rica | Lee et al. 2011 |
| Cowpea       | Peanut Witches broom             | *Ca. P. aurantifolia*      | 16SrII    | KP677497         | India   | Unpublished   |
| Cowpea       | Peanut Witches broom             | *Vigna unguiculata subsp. Sesquipedalis phytoplasma* | 16SrII    | KU170535         | Taiwan  | Unpublished   |
| Cowpea       | Peanut Witches broom             | *Vigna unguiculata subsp. Sesquipedalis phytoplasma* | 16SrII    | KU170534         | Taiwan  |               |
| Cowpea       | Peanut Witches broom             | *Vigna unguiculata subsp. Sesquipedalis phytoplasma* | 16SrII    | KU170533         | Taiwan  |               |
| Cowpea       | Peanut Witches broom             | *Cowpea phyllody phytoplasma* | 16SrII-A  | KC953001         | China   | Unpublished   |
| Medagaskar   | Peanut                           |                            | 16SrII-A  | L33765           | USA     | Gundersen     |
| Plant Family   | Plant Species | Disease | Genus and Species | 16SrII-RFLP | Accession | Location | Reference          |
|---------------|---------------|---------|-------------------|-------------|-----------|----------|-------------------|
| periwinkle    | Witches       | broom   | phytoplasma       |             |           |          | et al. 1994       |
| Key Lime     | Peanut        | Witches | broom             | Ca. *P. aurantifolia* | 16SrII-B  | U15442   | France           | Zreik et al. 1995 |
| Faba bean    | Fababeans     | phyllody|                  |             |           |          |                   |                   |
| Papaya       | papaya        | yellow  | crinkle disease   | *Ca. P. australasia* | 16SrII-D  | Y10097   | Australia         | White et al. 1998  |
| Bristy oxtongue | Picris        | echiodes| phyllody          |           |           |          | Italy            | Seemueller et al. 1998 |
| Cactus       | Peanut        | Witches | broom group       | *Cactus Witches broom-YN11* | 16SrII-F  | EU099556 | USA              | Cai et al. 2008    |
| Cactus       | Peanut        | Witches | broom group       | *Cactus Witches broom-YN23* | 16SrII-G  | EU099568 | USA              |                     |
| Cactus       | Peanut        | Witches | broom group       | *Cactus Witches broom-YN24* | 16SrII-H  | EU099569 | USA              |                     |
| Cactus       | Peanut        | Witches | broom group       | *Cactus Witches broom-YN06* | 16SrII-I  | EU099551 | USA              |                     |
| Plant Type          | Plant Type          | Phytoplasma Species                      | Accession Code | Country | Reference          |
|--------------------|--------------------|------------------------------------------|----------------|---------|--------------------|
| Cactus             | Peanut             | *Cactus Witches broom*                   | EU099552       | USA     |                    |
|                    | Witches broom group|                                          |                |         |                    |
|                    | Cactus             | *Cactus Witches broom*                   | EU099572       | USA     |                    |
|                    | Peanut             |                                          |                |         |                    |
|                    | Witches broom group|                                          |                |         |                    |
|                    | Cactus             | *Cactus Witches broom*                   | EU099546       | USA     |                    |
|                    | Peanut             |                                          |                |         |                    |
|                    | Witches broom group|                                          |                |         |                    |
| Tephrosia purpurea | Tephrosia purpurea | *Tephrosia purpurea*                     | HG792252       | India   | Yadav et al. 2014 |
|                    | Witches broom      |                                          |                |         |                    |
| Papaya             | Bunchy top symptom | *Papaya bunchy top phytoplasma*          | JF781309       | Cuba    | Lopez et al. 2016 |
|                    | Phytoplasma        |                                          |                |         |                    |
| Tabebuia pentaphylla| Tabebuia pentaphylla| *Tabebuia pentaphylla phytoplasma*       | EF647744       | UK      | Mafia et al. 2007 |
|                    | Witches broom phytoplasma |                                 |                |         |                    |
| Papaya             | Cuban papaya phytoplasma |                                 | DQ286948       | Cuba    | Arocha et al. 2006 |
| Papaya             | Papaya bunchy top phytoplasma |                                 | JF781310       | Cuba    | Lopez et al. 2015 |
| Easter lily cactus | Echinopsis sp. yellow |                                   | DQ535900       | Mexico  |                    |
| Plant                | Species/Species Group | Phytoplasma Name | 16SrII Group | Accession | Location | Notes |
|---------------------|-----------------------|------------------|--------------|-----------|----------|-------|
| Amaranthus          | hypochondriacus       | Amaranthus       | 16SrII-S     | FJ357164  | Mexico   | -     |
| Tomato              |                       |                  | 16SrII-T     | EU125185  | Mexico   | -     |
| Mountain Papaya     | Peanut                | Vasconcellea     | 16SrII-U     | KP057205  | China    | -     |
| Cowpea              | Peanut                | Ca. P.           | 16SrII-V     | MZ831316  | China    | -     |
| Praxelis clematidea | Peanut                | Praxelis         | 16SrII-V     | KY568717  | China    | -     |
| Crotalaria          | aegyptiaca            | Crotalaria       | 16SrII-W     | KY872734  | Oman     | Al-Subhi et al. 2017 |
| Potato              | Peanut                | Ca. P. aurantifolia | 16SrII-X     | MH423498  | Soudi Arabia |     |
| Cowpea              | Peanut                | Ca. P. aurantifolia | 16SrII-Y     | MW827058  | India    | This study |
| Cowpea              | Peanut                | Ca. P. aurantifolia | 16SrII-Y     | MW827059  | India    |       |
| Plant Name     | Disease Group                              | Host Name            | Phytoplasma Name                  | Genbank ID | Country  | Reference                             |
|---------------|--------------------------------------------|----------------------|-----------------------------------|------------|----------|---------------------------------------|
| Soybean       | X disease group                            | *Soybean veinal necrosis phytoplasma* | 16SrIII | AF177383 | Lithuania | -                                     |
| Veitchia merillii | Coconut lethal yellows group              | *Coconut lethal yellowing phytoplasma* | 16SrIV | U18747   | USA      | -                                     |
| Elmyellow     | Elm yellows group                          | *Ca. P. ulmi*        | 16SrV    | AY197655 | USA      | Lee et al. 2004                       |
| Alsikeclover  | Clover proliferation group                 | *Ca. P. trifolii*    | 16SrVI   | AY390261 | Canada   | Hiruki and Wang 2004                  |
| Cowpea        | Clover proliferation group                 | *Vigna unguiculata virescence phytoplasma* | 16SrVI | MK088178 | Iran     | -                                     |
| Cowpea        | Clover proliferation group                 | *Vigna unguiculata virescence phytoplasma* | 16SrVI | KC633094 | Iran     |                                        |
| Cowpea        | Clover proliferation group                 | *Vigna unguiculata virescence phytoplasma* | 16SrVI | KC633093 | Iran     |                                        |
| Cowpea        | Clover proliferation group                 | *Vigna unguiculata virescence phytoplasma* | 16SrVI | KC633092 | Iran     |                                        |
| Chestnut      | Clover proliferation group                 | *Ca. P. castaneae*    | 16SrVI   | AB054986 | South Korea | Jung et al. 2002                   |
| Medagaskar periwinkle | Ash yellows group                          | *Ca. P. fraxini*     | 16SrVII  | AF092209 | USA      | -                                     |
| StraWitches   |                                            | *Phytoplasma sp.*    | 16SrVIII | U96614   | USA      | Jomantiene                           |
| breamerry | Stra Witches broom1 | B | et al. 1998 |
|---|---|---|---|
| StraWitches broomerry | Ash yellows group | Argentinean straWitches broomerry phyllody phytosplasma | 16SrVIII-C | JN368423 | Argentina |
| Cowpea | Pigeonpea witches broom group | Ca. P. phoenicium | 16SrIX | MH547072 | India |
| Peach | European stone fruit yellows group | Ca. P. prumorum | 16Sr-X | AJ542544 | Germany | Seemuller et al. 2004 |
| Rice | Rice yellow dwarf group | Ca. P. oryzae | 16SrXI | AB052873 | Thailand | Jung et al. 2003 |
| Potato | Potato purple top ru group | Russia potato purple top phytosplasma | 16Sr-XII | EU344890 | Russia federation | Girsova et al. 2008 |
| Brocolli | Brocolli stunt group | Pytoplasma asteris related strain | 16Sr-XIII | JX626328 | Brazil |
| Cowpea | Bermud white leaf group | Ca. P. cynodontis | 16SrXIV | MK367419 | Iraq |
| Cowpea | Bermud white leaf group | Ca. P. cynodontis | 16SrXIV | MK367418 | Iraq |
| Cowpea | Bermud white leaf group | Ca. P. cynodontis | 16SrXIV | MK367417 | Iraq |
| Plant          | Disease Group                      | Phytoplasm Type                      | 16SrX-ID | Accession    | Country  | Reference   |
|---------------|-----------------------------------|--------------------------------------|----------|--------------|----------|-------------|
| Cowpea        | Bermuda white leaf group           | Ca. P. cynodontis                    | 16SrXIV  | MK367416     | Iraq     |             |
| Cowpea        | Peanut Witches broom group         | Vigna unguiculata' flat stem         | 16SrXIV-A| KY439870     | India    |             |
| Hemp          | Hemp little leaf group             | Ca. P. cynodontis                    | 16SrXIV-A| KM220612     | India    |             |
| Sunhemp       | Sunhem yellowing group             | Phytoplasma brasiliense related strain| 16SrXV-A| KF878382     | Brazil   | Bianco et al.2014 |
| Bermuda grass | Cynodon white fly group            | Ca. P. graminis                      | 16SrXVI-A| AY742327     | Cuba     | Arocha et al.2005 |
| Potato        | Texas potato top wilt             | Ca. P. americanum                    | 16SrXVIII-A| DQ174118     | USA      | Lee et al.2006 |
| Stervia       | Stevia rebaudiana phytoplasma      | Stevia rebaudiana phytoplasma        | 16SrXXIV-A| JF970603     | India    | Samad et al.2011 |

**Figures**
Figure 1

Symptoms of bud proliferation and stunting on cowpea plants (a, b); PCR amplification of 16SrII DNA fragments using P1/P7 and R16nF/R16nR primers (M=1kb DNA marker, Lane 1 and 2=Healthy cowpea samples, Lane 3-7=phytoplasma infected samples, Lane 8=negative control and Lane 9=Positive control (d); restriction digestion of positive clones plasmids (M=1kb DNA marker, Lane 1-4=positive clones releasing the desired fragments ~1250bp (e); virtual RFLP pattern of cowpea phytoplasma strain understudy (MW827058) and reference phytoplasma strain (c and f). Among 17 restriction enzymes, HaeI RE give different RFLP pattern from reference strain (Y10097-16SrII-D-Australia-Papaya).
Figure 2

Phylogenetic relationship between cowpea phytoplasma understudy (16SrII-Y) with other selected phytoplasma groups and subgroups constructed from 16S rDNA sequences. The evolutionary history was inferred using the Neighbor-Joining method. The optimal tree is shown. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) are shown next to the branches. The tree is drawn to scale, with branch lengths in the same units as those of the
evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the Maximum Composite Likelihood method and are in the units of the number of base substitutions per site. This analysis involved 63 nucleotide sequences. All ambiguous positions were removed for each sequence pair (pairwise deletion option). There were a total of 1877 positions in the final dataset. Evolutionary analyses were conducted in MEGA X. WB= Witches broom group; BWL= Bermuda white leaf group; CP= Clover proliferation group; BP= Bud proliferation group; AY= Ash yellows group; EY= Elm yellows group; YD= Yellow dwarf group; LY= Lethal yellows group; XD= X disease group; YCD= Yellow crinkle disease; NA= Not available