Title: Detection and molecular characterization of a phytoplasma associated with Cucumber (*Cucumis sativus*) and its first report from India

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Abstract

Cucumber (*Cucumis sativus*) plants exhibiting typical phyllody symptoms were collected from farmers field of Chintamani, Chickballapur districts of Karnataka (India). Disease incidence of phyllody was 2-3%. The etiology of the cucumber phyllody phytoplasma (CuPP) was confirmed by amplifying 16S rRNA gene from symptomatic plants using PCR followed by nested PCR using universal primers pairs P1/P7 and R16F2n/R16R2. After general detection the non-ribosomal *Sec*Y and *rp* (ribosomal protein) genes was amplified using specific primers. The PCR amplified products 16SrRNA (1.2 kb), SecY (1.6 kb) rp gene (1.2 kb) was cloned and sequenced. Sequence and phylogenetic analyses of 16S rRNA, secY and *rp* genes revealed that the detected phytoplasma is a member of the 16SrI group (*Candidatus* Phytoplasma asteris). Further on the basis of computer-simulated RFLP (= *in silico* RFLP) analysis of amplified F2n/R2 region of 16S rRNA gene indicates that, the detected phytoplasma was belongs to the subgroup X (16SrII-X). This is the first report on phytoplasma associated with phyllody disease of cucumber in India.

Key words: cucumber, phyllody, 16SrI phytoplasma, characterization, India

Introduction

Cucumber (*Cucumis sativus* L.) is an important vegetable crop belongs to family *Cucurbitaceae* grown widely in warm regions of the world. It is the world's ancient vegetable crop originated in India (Yamaguchi 1983) and popularly known as *'khira'* and gherkins. The crop is extensively cultivated for its edible tender fruit, preferred as salad ingredient, pickles and also as cooked vegetable (Navitha et al. 2019). In India cucumber is cultivated with in an area of 78,000 ha with the production of 1,142,000 MT (Anonymous, 2018 NHM data base). The cucumber is mainly gown during spring-summer and rainy season in commercial scale as well as kitchen gardens.

55 The cultivation of cucumber is threaten by number insects and diseases from germination till
56 harvest and causes heavy economic losses to the growers (Venkataravanappa et al. 2019).

57 Cucumber is reported to be attacked by plethora of viruses, bacteria and fungi in India,
58 but not yet by phytoplasmas. All over the world, phytoplasmas cause a multitude of diseases in
59 several hundred crop species including fruits, vegetables, cereals and trees (McCoy et al. 1989;
60 Lee et al. 2000). Many cucurbits belongs to the *Cucurbitaceous* family have been infected with
61 different phytoplasma has been well documented in different parts of the world that includes
62 witches' broom of *Cucurbita pepo* in India (Rao et al. 2017), cucumber phyllody in Iran
63 (Hosseini et al. 2015) and cucumber yellows (Valiunas et al. 2017). Yellows of *C. maxima*,
64 (McCoy et al., 1989), *C. moschata* (Montano et al. 2006), *Luffa cylindrica* L. (Lee et al. 1993;
65 Gundersen et al. 1994), *Momordica charantia* L. (Alves et al. 2017), *Sechium edule* (Jacquin)
66 Swartz (Villalobos et al. 2002), *Sicana odorifera* (Montano et al. 2007), *Trichosanthes*
67 *cucumerina* (Weng et al. 2021) and *C. moschata* L., (Xu et al. 2021).The phytoplasma is one of
68 the emerging pathogens in many vegetable crops and causing huge loss to various crops. Based
69 on the 16Sr RNA gene analysis, 16 different ribosomal groups and subgroups phytoplasmas
70 infecting different vegetable crops have been well documented across the world (Kumari et al.
71 2019). Considering nutritional benefits of cucumbers in human diet, the crop is protected against
72 biotic stress. With this backdrop, the present was attempted to identify and characterize strain of
73 the phytoplasma associated phyllody disease of cucumber based on PCR assays and 16S rRNA,
74 *Sec*Y and rp gene sequence comparison analysis.

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Materials and Methods

Collection of disease samples

During April 2021, typical phytoplasma like symptoms were observed in cucumber in different farmers fields in Chintamani (13.40°N 78.06°E.), Chickballapur (Dist.) Karnataka (India). The infected cucumber plants displayed typical symptoms of phytoplasma infection such as witches' broom, flower viresence, small leaves, and dense clusters of highly proliferating apical shoot. Disease incidence was calculated as the percentage of symptomatic plants over total number of plants observed in an area of 20 X 20 meter plot. Due to creeping growth habit of cucumber, care was taken not to sample the same plant more than once, total five symptomatic and one symptomatic leaf samples were collected from same location. A part of the collected leaf samples were used for DNA isolation and the remaining sample was stored at -80°C for further use.

DNA extraction and PCR amplification of 16SrRNA, *SecY* gene, and ribosomal protein (rp) gene

Total genomic DNA of symptomatic and asymptomatic cucumber plants was extracted using cetyl-trimethyl ammonium bromide (CTAB) method. (Dolye and Doyle 1990). The DNA extracted from the phytoplasma infected bitter gourd (16SrI, KX179474) and bottle gourd (16SrI, MT386510) was included as positive control in PCR assays (Venkataravanappa et al. 2017; Ashwathappa et al. 2020). Using universal 16Sr RNA gene specific primers pairs (P1/P7, (R16mF2/R16mR2), the phytoplasma infection in cucumber was confirm in both direct and nested PCR assays (Deng and Hiruki 1991; Schneider 1995); (Gundersen and Lee 1996).The 16S rRNA gene sequence is highly conserved and insufficient for finer level differentiation of phytoplasma strains, therefore housekeeping genes such as translocase subunit *sec*Y and

101 ribosomal protein (*rp*) was amplified by PCR using primer pairs (SecYF1/SecYR1) (Lee et al.
102 2010) and (*rp*(II)F1A/*rp*(II)R1A) (Martini et al., 2013). PCR reactions were carried out in a
103 thermal cycler (Master cycler, Profelex, Hamsburg, Germany) and the cycling protocol used for
104 PCR assay was followed as earlier described (Venkataravanappa et al. 2017).Then five
105 microliters of PCR products were analysed in 0.8% (W/V) ethidium bromide stained agarose gel,
106 and visualized with a UV transilluminator. The amplified PCR products of 16SrRNA, *Sec*Y and
107 *rp* genes were purified from agarose gel using Qiaquick gel extraction kit (Qiagen, Hilder, USA)
108 and cloned into pTZ57R/T cloning vector according to manufacturer's directions (MBI
109 Fermentas, USA). The transformation was performed using *Escherichia coli* (DH5α) cells. The
110 recombinant plasmids were purified and confirmed with restriction digestion (*Pst*1 and *Sac*1
111 enzymes). The confirmed clones were sequenced in both directions at Eruofins Genomics Pvt.
112 Ltd., Bengaluru, India.

113 **Sequence analysis**

114 The full-length sequences of 16S rRNA, *secY* gene and *rp* genes obtained in the present study
115 were subjected to BLAST search for finding the similar sequences in the database. The
116 sequences showing maximum homology with 16SrRNA (Table 1), *secY* gene (Table 2) and *rp*
117 gene (Table 3) of cucumber phyllody phytoplasma were retrieved from NCBI database and
118 aligned using BioEdit (Hall 1999) and ClustalW (Thompson et al. 1997) program. MEGA X
119 software was used for constructed phylogeny using Neighbour-Joining method with 1000
120 bootstrapped replications (Kumar et al. 2016). Sequence of *Acholeplasma laidlawii* (Accession
121 number NR076550) for 16S rRNA*, Leptospira interrogans* (Accession number MH376290) for
122 *secY* gene sequences and *Acholeplasma laidlawii* (Accession number M81465) were used as out
123 groups in the phylogenetic tree construction.

124 **Virtual RFLP analysis**

125 Virtual RFLP (Restriction fragment length polymorphism) analysis was carried out for F2n/R2
126 region of the 16Sr RNA gene derived from cucumber phyllody phytoplasma using
127 *iPhy*Classifier online tool (https://plantpathology.ba.ars.usda.gov/cgi-bin/resource
128 /iphyclassifier.cgi) (Zhao et al. 2009) to identify the phytoplasma to group and subgroup level.
129 The virtual RFLP patterns were compared, and the similarity coefficient was calculated (Wei et
130 al. 2008). Restriction pattern obtained for the phytoplasmas associated with cucumber phyllody
131 was compared with already published RFLP patterns of phytoplasma representatives of available
132 ribosomal groups and 16SrI subgroups (Lee et al. 1998).

133 **Results**

134 The predominant symptoms observed on phytoplasma infected cucumber plants are little leaf,
135 excessive proliferation along the stem, flower virescence and phyllody, internode shortening
136 phyllody and witches' broom (Fig1a-d). These symptoms are similar to those described for
137 cucumber phyllody disease reported to occur in other countries (Salehi et al. 2015). The
138 cucumber phyllody disease incidence was ranged from 2-5%.

139 The resulting PCR amplicon of 1.2 kb corresponding to the phytoplasma 16Sr RNA gene,
140 *secY* gene (1.6 kb) and *rp* gene (1.2 kb) were amplified in all four cucumber samples displaying
141 phyllody disease symptoms and positive control samples. However, no such amplification was
142 observed in asymptomatic plants with each of primer pair employed. Amplified PCR fragments
143 of each gene was cloned separately and sequenced in both the direction. The consensus
144 sequences of 16S rRNA (accession number MZ401356), *secY* gene (accession number
145 MZ463022) and *rp* gene (accession number MZ463023) were deposited in GenBank.

146 BLASTn searches for homologous gene sequences of 16S rRNA gene sequence of
147 cucumber phyllody isolate obtained in the study was more homologous to those of phytoplasmas
148 classified in the, 16SrI group ('*Candidatus* Phytoplasma asteris') having the maximum
149 nucleotide(nt) sequence identity of 99.13% with cucumber viresence from Taiwan (GU361755).
150 The result was well supported in phylogenetic analysis of 16S rRNA gene with selected
151 reference strains indicated that the cucumber phyllody phytoplasma is clustered together with
152 several strains of phytoplasma belongs to the 16SrI subgroup (Fig. 2a).

153 The computer-simulated RFLP (*in silico* RFLP) analysis of F2n/R2 region derived from
154 16S rRNA gene sequence of cucumber phyllody phytoplasma was identical (similarity
155 coefficient 0.98) to the reference pattern of 16Sr group I, subgroup X (GenBank accession
156 number JF781308). Therefore, cucumber phyllody phytoplasma having similarity coefficient
157 more than the threshold level (Wei et al. 2008) and it may be considered as a variant of 16SrI-X
158 subgroup under "*Candidatus* Phytoplasma asteris" 16Sr I group.

159 The *secY* sequence of cucumber phyllody phytoplasma showed the maximum nt
160 similarity of 98.75% to that of *Trema levigatum'* witches'-broom from China (GenBank
161 accession number MW032211). Similarly, *rp* gene of cucumber phyllody phytoplasma shared
162 maximum nt similarity of 99.34% to that rapeseed phyllody phytoplasma from Poland (accession
163 number CP055264). This result was also well supported by the phylogenetic analysis, in which
164 *secY* (MZ463022) and *rp* (MZ463023) gene sequences of the cucumber phyllody phytoplasma is
165 closely cluster with several phytoplasmas belongs to *Candidatus* Phytoplasma asteris" 16Sr-I
166 group (Fig. 2b and 2c).

167 Attempt to detect phytoplasma in the cucumber samples by LAMP assay using different
168 LAMP primers specific to 16S RNA gene showed that CuP was successfully detected in the

169 infected cucumber samples (Fig. 7a). No amplification was observed from the DNA of negative
170 sample and water control. The LAMP amplicons (a ladderlike pattern) were observed on 1.5%
171 agarose gel from positive samples, but not from the negative sample and water control. The
172 positive reactions can be visualized directly by the naked eye with samples showing sky blue
173 color pattern, whereas a violet color change is observed in negative and water control (Fig. 7b).

174 Phytoplasma diseases cause significant economic losses in numerous *Cucurbitaceae*-
175 related vegetable crops, including *C. maxima* (Giant pumpkin), *C. mixta* (winter Squash), *C.*
176 *pepo (summer squash)* (Rao et al. 2017), *Lagenaria leucantha* (Bottle gourd) (Ashwathappa et
177 al. 2020; Tripathi et al. 2017), *C. moschata* (Butternut Pumpkin) (Montano et al. 2006; Xu et al.
178 2021), *Momordica charantia* (Bitter gourd) (Montano et al. 2007; Venkataravanappa et al.
179 2017). However, there is no information is available for phytoplasma infection in cucumber in
180 India. Literature survey showed that cucumber acts as natural host for different phytoplasma
181 groups, that includes 16SrII-M from Iran (Dehghan et al. 2015; Ghayeb Zamharir and Azimi
182 2018), 16SrVI-A (Salehi et al. 2015) from Iran. In the present study the cucumber phyllody
183 phytoplasma resulted from association of 16SrI group phytoplasma was confirmed based 16Sr
184 RNA gene characterization including *secY* and *rp* genes. This study represents the first recorded
185 of phytoplasma association with cucumber in Karnataka (India) is related to '*Ca*. P. asteris'-
186 related strain belonging to 16Sr I-X subgroup. This disease become major threat for cultivation
187 of cucumber as it causes 100% yield losses due to shoot and floral malformation and sterility.
188 Moreover, the crop is a regular and widely cultivated throughout India in large farming
189 community, therefore, further studies on epidemiology along with possible natural spread
190 sources and management is also a foremost need.

191

192 **Contributions:**

193 MM, HDV and MN performed the biological experiment, SH, VV and KSS involved in the
194 bioinformatics analysis CRJB and CNL involved in experimental design, CNL conceptualized
195 work and provided the overall direction, all authors are involved in literature mining and
196 manuscript preparation. All authors read and approved the final manuscript

197 **Ethics declarations**

198 This article does not contain any studies requiring ethical approval.

199 **Conflict of interests**

200 The authors declare that they have no competing interests**.**

201 **Human and animal rights**

202 This article does not contain any studies with human or animal subjects performed by any of the
203 authors.

204 **Informed consent**

205 Informed consent was obtained from all individual participants included in the study.

206

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290 **Table:1 16Sr RNA gene sequences of phytoplasmas employed in analysis**

Phytoplasma disease / country	**16S rDNA group/subgroup**	**GenBank accession number**	**Identity with Cucumber phytoplasma**
Cucumber phyllody phytoplasma/ Iran	16SrII-D	KR822804	67.30
Cucumis sativus' phyllody/Iran	16SrVI	KR633068	61.90
Cucumber phyllody phytoplasma/Iran	16SrII	JN574839	72.20
'*Candidatus* Phytoplasma trifolii'/Iran	16SrVI	KT807467	62.20
Cucumber viresence /Taiwan	16SrI	GU361755	99.10
Aster yellows/USA	16SrI-B	M30790	68.60
Potato purple top/Russia	16SrI-B	EU333396	68.60
Potato purple top/Russia	16SrI-P	EU333397	68.60
Apricot aster yellows/Spain	16SrI-F	AY265211	68.30
Aconitum proliferation/ Lithuania	16SrI-A	AF510323	68.40
Aster yellows-AV2192/Germany	16SrI-L	AY180957	68.60
Aster yellows-AVUT/Germany	16SrI-M	AY265209	68.70
Rhododendron x hybridum/ Czech	16SrI-C	KC009838	68.40

Republic			
Aster yellows-BB/USA	16SrI-A	AY180955	68.40
Blueberry stunt-BBS3/USA	16SrI-E	AY265213	68.40
Aster yellows Btsv2CarD3/USA	16SrI-B	AY180945	68.60
Cherry little leaf/ Lithuania	16SrI-Q	AY034089	68.50
Japanese maple witches' broom/	16SrI-B	JQ015183	68.70
Clover phyllody/Germany	16SrI-C	AY265217	68.40
Valeriana yellows/ Lithuania	16SrI-M	AY102274	68.10
Aster yellows / USA	16SrI-O	AF268405	68.70
Cucumis sativus' stunt virescence/ Lithuania	16SrI-A	KX298483	68.40
Cucumis. sativus' phytoplasma /Iran	16SrVI-A	MH004460	62.50
Cucumis sativus' phytoplasma /Iran	16SrVI-A	MH004459	39.40
Western X-disease/USA	16SrIII-A	L04682	61.90
Coconut lethal yellowing/USA	16SrIV-A	U18747	62.20
Elm yellows/USA	16SrV-A	AY197655	62.20
Jujube witches' broom/Korea	16SrV-B	AB052876	61.30
"Flavescence dorée/France	16SrV-C	AF176319	62.20
Clover proliferation/Canada	16SrVI-A	AY390261	62.20
Ash yellows/USA	16SrVII-A	AF092209	62.40
Apple proliferation/ Germany	16SrX-A	AJ542541	63.40
Spartium witches' broom/Italy	16SrX-B	X92869	63.50
Pear decline/ Germany	16SrX-C	AJ542543	63.60
European stone fruit yellows/ Germany	16SrX-F	AJ542544	63.50
Rice yellow dwarf/Germany	16SrXI-A	AB052873	60.80
Cucumis sativus phytoplasma /Iran	16SrVI-A	MH004458	39.40
Squash phyllody phytoplasma/Iran	16SrII-D	KR822805	67.40
Strawberry yellows / Lithuania	16SrXII-E	DQ086423	66.80
Mexican periwinkle virescence/Mexico	16SrXIII-A	AF248960	66.40
Bermudagrass white leaf/Italy	16SrXIV-A	AJ550984	62.00
Chestnut witches' broom/South Korea	16SrXIX-A	AB054986	61.40

Hibiscus witches' broom/Brazil	16SrXV-A	AF147708	61.50
Sugarcane yellow leaf/Cuba	16SrXVI-A	AY725228	64.80
American potato purple top wilt/USA	16SrXVIII-A	DQ174122	66.10
Buckthorn witches' broom/USA	16SrXX-A	X76431	63.10
Pine shoot proliferation/Europe	16SrXXI-A	AJ632155	62.00
Awka wilt/Nigeria	16SrXXII-A	Y14175	61.80
Buckland Valley grapevine yellows/ Australia	16SrXXIII-A	AY083605	66.70
Sorghum bunchy shoot/USA	16SrXXIV-A	AF509322	59.20
Cassia italica witches' broom/Oman	16SrXXIX-A	EF666051	61.40
Weeping tea witches' broom/Australia	16SrXXV-A	AF521672	61.20
Sugarcane yellows D3T1/ Mauritius	16SrXXVI-A	AJ539179	64.40
Salt cedar witches' broom/China	16SrXXX-A	FJ432664	63.80

291

292 **Table:2 *SecY* gene sequences of phytoplasmas employed in the analyses.**

Phytoplasma Species /Country	Sub-group	GenBank accession No.	Identity with Cucumber phytoplasma
Tomato big bud phytoplasma/USA	16SrI-A	AY803178	83.40
Chrysanthemum yellows phytoplasma/ Germany	16SrI-A	AY803170	83.20
Hydrangea phyllody phytoplasma/Belgium	16SrI-A	AY803181	83.20
Chrysanthemum yellows phytoplasma/Italy	16SrI-B	DQ787851	87.80
Trema levigatum' witches'-broom/China	16SI-B	MW032211	98.75
Primrose virescence phytoplasma/Germany	16SrI-B	AY803176	86.80
Clover phyllody phytoplasma/Germany	16SrI-C	AY803183	83.80
Paulownia witches-broom phytoplasma/Taiwan	16SrI-D	AY803184	86.50
Blueberry stunt phytoplasma/ Michigan, USA	16SrI-E	AY803169	84.20
Apricot chlorotic leaf roll phytoplasma/Spain	16SrI-F	AY803166	83.50
Strawberry multiplier phytoplasma/Florida, USA	16SrI-K	AY803180	83.50
Aster yellows phytoplasma/USA	16SrI-M	AY803168	86.60
Ipomoea witches-broom phytoplasma/Taiwan	16SrI-N	AY803182	98.58
Peanut witches-broom phytoplasma/Taiwan	16SrII-A	GU004331	26.80
Sesame phyllody phytoplasma/ Thailand	16SrII	GU004322	28.40
Sesame phyllody phytoplasma/ Thailand	16SrII	GU004362	37.50
Soybean phyllody phytoplasma/ Thailand	16SrII-C	GU004324	28.50
Picris echioides phytoplasma/Italy	16SrII-E	GU004348	37.80
Australian tomato big bud phytoplasma/ Australia	16SrII-D	GU004347	37.70

Sesame phyllody phytoplasma/ Myanmar	16SrII	AB703253	37.80
Milkweed yellows phytoplasma/ New York, USA	16SrIII-F	GU004340	30.00
Potato purple top phytoplasma/ Montana, USA	16SrIII-M	GU004333	29.90
Clover yellow edge phytoplasma/ Lithuania	16SrIII-B	GU004332	30.30
Spirea stunt phytoplasma/ New York, USA	16SrIII-E	GU004326	28.20
Poinsettia branch-inducing phytoplasma/USA	16SrIII-H	GU004328	30.00
Peach X-disease phytoplasma/Canada	16SrIII-A	GU004327	28.10
Walnut witches-broom phytoplasma/ Georgia, USA	16SrIII-G	GU004325	30.00
Elm yellows phytoplasma/ New York, USA	16SrV-A	AY197690	41.00
Cherry lethal yellows phytoplasma/China	16SrV-B	AY197693	41.30
Alder yellows phytoplasma/Germany	16SrV-C	AY197692	41.20
Rubus stunt phytoplasma/Italy	16SrV-E	AY197696	41.50
Flavescence doree phytoplasma/USA	16SrV-D	AY197685	41.40
Dry bean phyllody phytoplasma/ Washington, USA	16SrVI-A	GU004353	40.30
Ash yellows phytoplasma/ NY California, USA	16SrVI-A	GU004329	28.60
Brinjal little leaf phytoplasma/India	16SrVI-D	GU004356	30.30
Potato witches'-broom phytoplasma/Canada	16SrVI-A	GU004316	29.00
Clover phyllody phytoplasma/Canada	16SrVI-A	GU004315	29.10
Potato purple top phytoplasma / Alaska, USA	16SrVI-A	GU004343	29.20
Lucerne virescence phytoplasma/Frans	16SrVI-A	GU004318	30.00
Vinca virescence phytoplasma/ California, USA	16SrVI-A	GU004317	28.70
Potato purple top phytoplasma/ Alaska, USA	16SrVI-A	GU004344	37.30
Potato purple top phytoplasma/ Alaska, USA	16SrVI-A	GU004342	38.20
Potato purple top phytoplasma/ Alaska, USA	16SrVI-A	GU004351	38.70
Dry bean phyllody phytoplasma/USA	16SrVI-A	GU004352	40.00
Coconut lethal yellows phytoplasma/USA	16SrIV-A	GU004320	29.60
Candidatus Phytoplasma fraxini/USA	16SrVII-A	GU004329	28.60
Apple proliferation phytoplasma/Italy	16SrX-A	GU004335	29.60
Pear decline phytoplasma/Italy	16SrX-C	GU004363	36.60
Mexican periwinkle virescence /Meixco	16SrXIII-A	GU004336	29.10
American potato purple top wilt phytoplasma/USA	16SrXVIII-B	GU004338	29.50
Stolbur-It phytoplasma/Italy	16SrXII-A	GU004355	47.50
'*Candidatus* Phytoplasma americanum'/USA	16SrXVIII-B	MN227134	38.00

Table.3 Rep gene sequences of phytoplasmas employed in analysis

Phytoplasma Species/ Country	**Sub-group**	**GenBank accession No.**	**Identity with Cucumber phytoplasma**
Gynostemma pentaphyllum' witches'-broom/China	16SrI-B	MN543070	99.00
Aster yellows phytoplasma/Hungary	16SrI-B	MN526023	99.10

Rapeseed phyllody/Taiwan	16SrI	CP055264	99.30
Plum witches'-broom phytoplasma/Poland	16SrI-B	MH061366	96.10
Oilseed rape phytoplasma/China	16Sr1-A	KX551965	99.10
Melochia corchorifolia' phyllody/China	16Sr1-A	KX158198	99.10
Tilia platyphyllos' phytoplasma/ Lithuania	16SrI-B	JX184926	97.90
Salix tetradenia' witches'-broom/China	16SrI-B	KC117314	99.10
Periwinkle phyllody/Korea	16SrI-B	AB741642	98.20
Sesame phyllody/Korea	16SrI-L	AB741638	97.70
Larch phytoplasma strain / Lithuania	16SrI-B	JF767009	98.60
Periwinkle phyllody/China	16SrI-B	HQ285145	99.20
Iranian tomato phytoplasma /Iran	16SrI-B	HQ286478	99.10
Onion proliferation phytoplasma/ Lithuania	16SrI-A	GU228514	96.90
Aster yellows/USA	-	AY183710	96.90
Paulownia witches'-broom/Germany	-	AY264857	96.80
Aster yellows phytoplasma/Germany	-	AY183708	96.10
Green gram phyllody/ Myanmar	16SrII	AB703244	96.70
Black gram witches' broom/Myanmar	16SrII	AB703243	57.10
Parthenium weed witches' broom/ Yunnan	16SrII	KU958723	57.10
Sword bean witches' broom/ Yunnan	16SrII	KC953009	57.70
Peanut witches' broom/ Yunnan	16SrII	JX871469	57.60
Cucumber phyllody/Iran	16SrII	KY365523	57.50
Sesame phyllody/ Myanmar	16SrII	AB703247	56.80
Peach X-disease/USA	16SrIII-A	EF186813	55.60
Goldenrod yellows/USA	16SrIII-D	EF186810	57.80
Vaccinium witches' broom/ Germany	16SrIII-F	EF186809	57.70
Poinsettia branch inducing/ USA	16SrIII-H	EF186811	58.30
Gliricidia little leaf/ Honduras	16SrIX	EF186800	57.80
Knautia arvensis phyllody/ Italy	16SrIX	EF186801	60.40
Pigeon pea witches' broom/USA	16SrIX-A	EF183495	60.20
Almond witches' broom/ Lebanon	16SrIX-B	EF186803	59.70
Picris echioides phyllody/Italy	16SrIX-C	EF186802	59.90
Elm yellows/USA	16SrV-A	AY197675	59.40
Alder yellows/Italy	16SrV-C	AY197666	59.00
Spartium witches' broom/Italy	16SrV-C	AY197672	58.60
Hemp dogbane yellows/USA	16SrV-C	AY197674	58.20
"Flavescence dorée"/Italy	16SrV-D	AY197664	58.80
Catharanthus phyllody/Sudan	16SrVI	EF183494	58.60
Ash yellows/USA	16SrVII-A	EF183492	58.50
Apple proliferation/Italy	16SrX-A	EF193366	58.60
Pear decline/Germany	16SrX-C	EF193370	67.60
Mexican periwinkle virescence/Mexico	16SrXIII-A	EF193365	67.70
American potato purple top wilt/USA	16SrXVIII-A	EF193362	77.90
Tomato "stolbur"/Italy	16SrXII-A	EF193364	78.00
Palm lethal yellowing/Florida	16SrIV-A	DQ297677	80.10
Coconut lethal yellowing/ Honduras	16SrIV-A	DQ318238	47.40
Texas Phoenix palm phytoplasma/USA	16SrIV-A	EF050087	47.30
Lethal yellowing phytoplasma/Jamaica	16SrIV-A	EF186805	47.50
Lethal yellowing phytoplasma/USA	16SrIV-A	EF186804	60.60
Coconut lethal yellowing/USA	16SrIV-A	EF193382	60.50
Mexican periwinkle virescence/ Mexico	16SrXIII	EF193365	60.30
'*Candidatus* Phytoplasma meliae'/Argentina	16SrXIII	KU850947	77.90
'*Candidatus* Phytoplasma meliae'/South Korea	16SrXIII	KU850945	78.20
'*Candidatus* Phytoplasma fragariae'/USA	16SrXIII	KJ144893	78.20

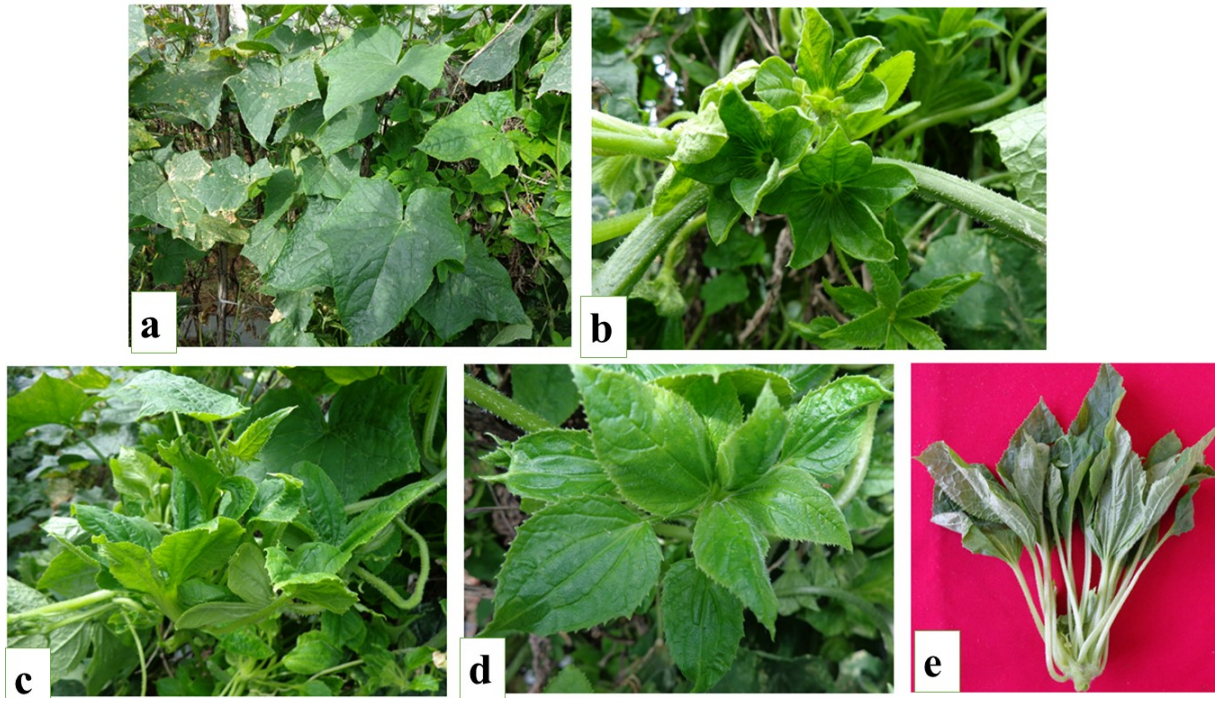
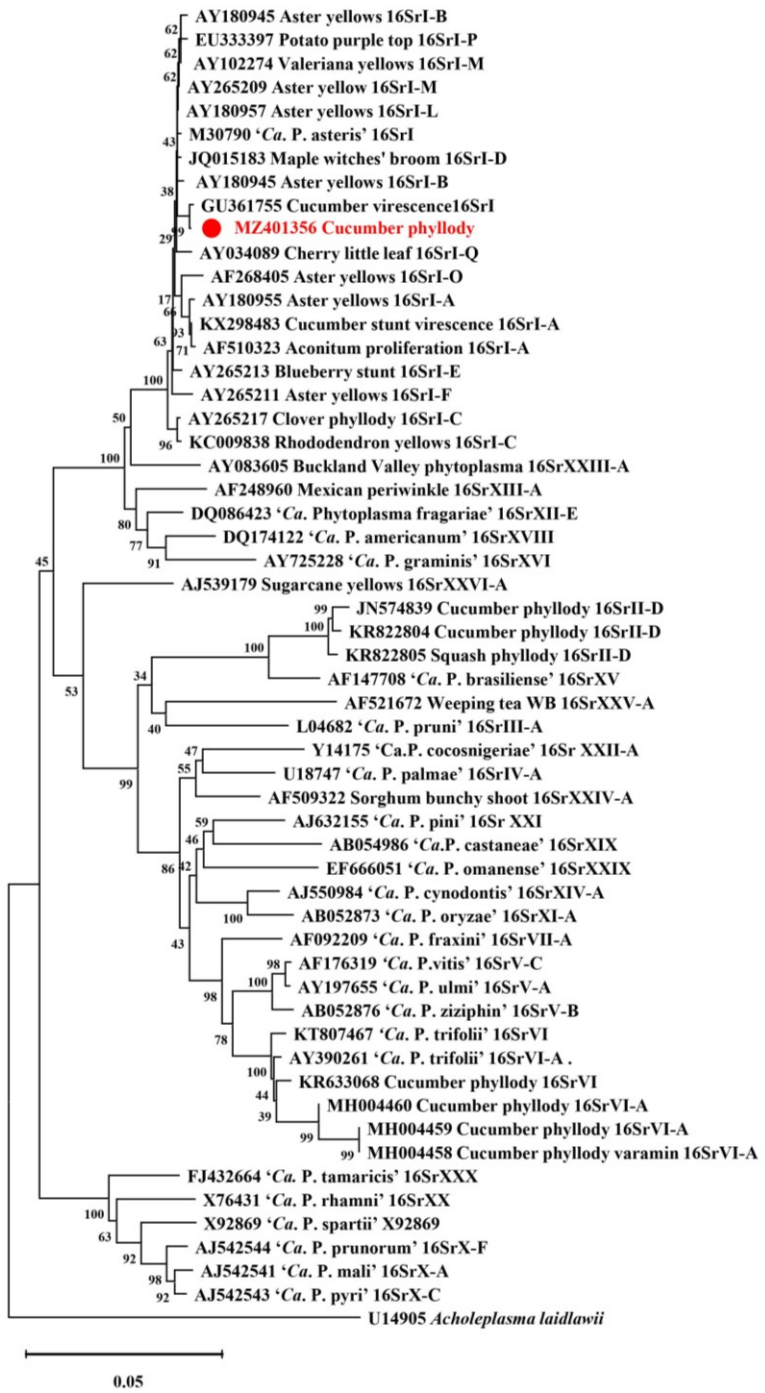
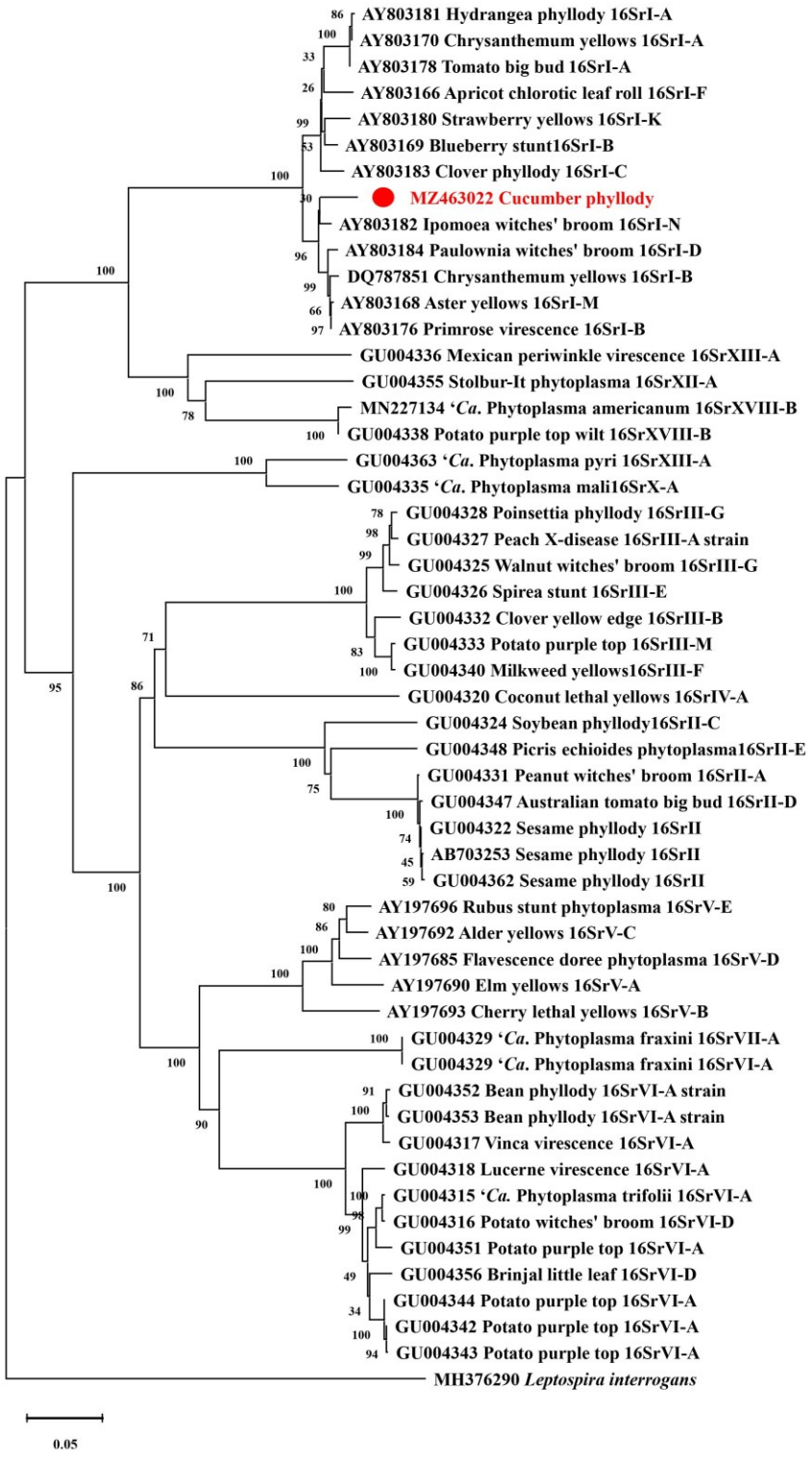


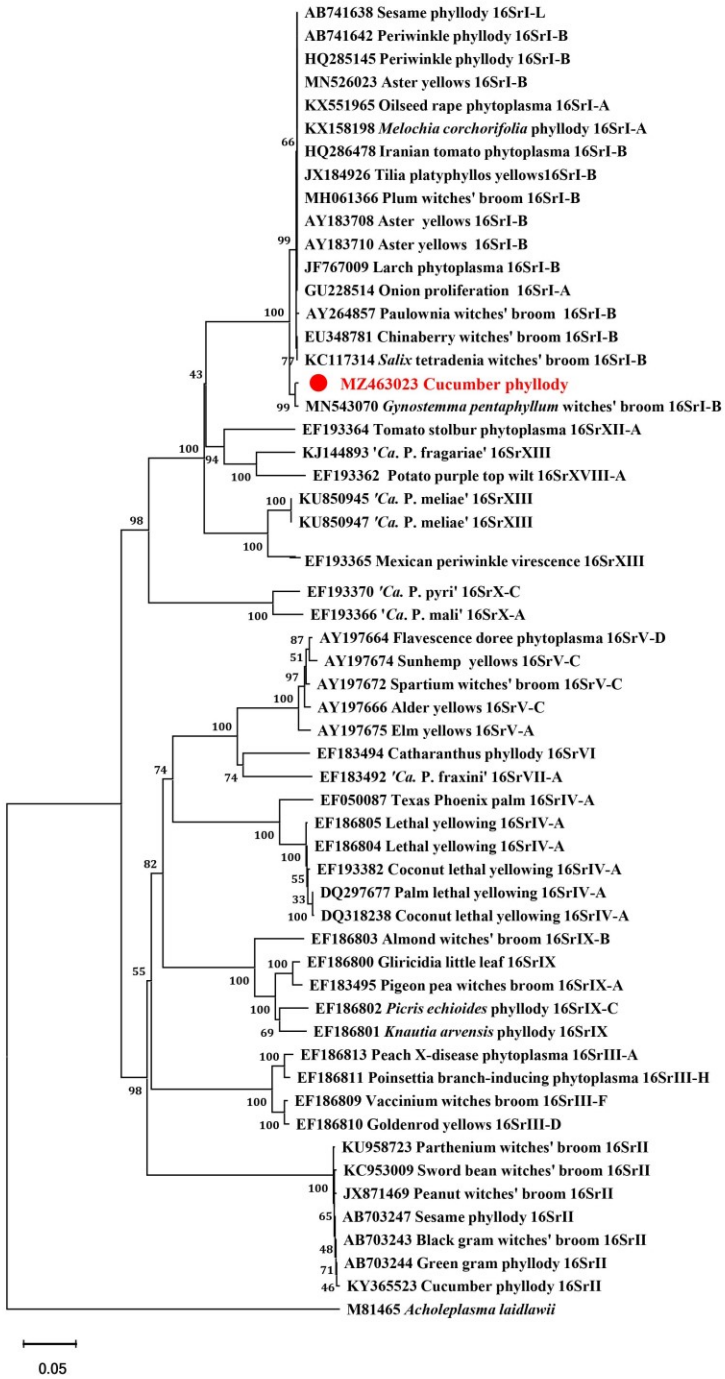
Fig: 1 Symptoms on cucumber (*Cucumis sativus*) plant var. Rashmi under field condition **a**; flower virescence with phyllody **b**; excessive shoot proliferation **c**; Little leaf **d**; cucumber plant with floral virescence and small leaves, internode shortening **e**.


AY180945 Aster yellows 16SrI-B
EU333397 Potato purple top 16SrI-P
AY102274 Valeriana yellows 16SrI-M
AY265209 Aster yellow 16SrI-M
AY180957 Aster yellows 16SrI-L
M30790 'Ca. P. asteris' 16SrI
JQ015183 Maple witches' broom 16SrI-D
AY180945 Aster yellows 16SrI-B
GU361755 Cucumber virescence16SrI
MZ401356 Cucumber phyllody
AY034089 Cherry little leaf 16SrI-Q
AF268405 Aster yellows 16SrI-O
AY180955 Aster yellows 16SrI-A
KX298483 Cucumber stunt virescence 16SrI-A
AF510323 Aconitum proliferation 16SrI-A
AY265213 Blueberry stunt 16SrI-E
AY265211 Aster yellows 16SrI-F
AY265217 Clover phyllody 16SrI-C
KC009838 Rhododendron yellows 16SrI-C
AY083605 Buckland Valley phytoplasma 16SrXXIII-A
AF248960 Mexican periwinkle 16SrXIII-A
DQ086423 'Ca. Phytoplasma fragariae' 16SrXII-E
DQ174122 'Ca. P. americanum' 16SrXVIII
AY725228 'Ca. P. graminis' 16SrXVI
AJ539179 Sugarcane yellows 16SrXXVI-A
JN574839 Cucumber phyllody 16SrII-D
KR822804 Cucumber phyllody 16SrII-D
KR822805 Squash phyllody 16SrII-D
AF147708 'Ca. P. brasiliense' 16SrXV
AF521672 Weeping tea WB 16SrXXV-A
L04682 'Ca. P. pruni' 16SrIII-A
Y14175 'Ca.P. cocosnigeriae' 16Sr XXII-A
U18747 'Ca. P. palmae' 16SrIV-A
AF509322 Sorghum bunchy shoot 16SrXXIV-A
AJ632155 'Ca. P. pini' 16Sr XXI
AB054986 'Ca.P. castaneae' 16SrXIX
EF666051 'Ca. P. omanense' 16SrXXIX
AJ550984 'Ca. P. cynodontis' 16SrXIV-A
AB052873 'Ca. P. oryzae' 16SrXI-A
AF092209 'Ca. P. fraxini' 16SrVII-A
AF176319 'Ca. P.vitis' 16SrV-C
AY197655 'Ca. P. ulmi' 16SrV-A
AB052876 'Ca. P. ziziphin' 16SrV-B
KT807467 'Ca. P. trifolii' 16SrVI
AY390261 'Ca. P. trifolii' 16SrVI-A .
KR633068 Cucumber phyllody 16SrVI
MH004460 Cucumber phyllody 16SrVI-A
MH004459 Cucumber phyllody 16SrVI-A
MH004458 Cucumber phyllody varamin 16SrVI-A
FJ432664 'Ca. P. tamaricis' 16SrXXX
X76431 'Ca. P. rhamni' 16SrXX
X92869 'Ca. P. spartii' X92869
AJ542544 'Ca. P. prunorum' 16SrX-F
AJ542541 'Ca. P. mali' 16SrX-A
AJ542543 'Ca. P. pyri' 16SrX-C
U14905 Acholeplasma laidlawii
0.05

(a) 16Sr RNA


AY803181 Hydrangea phyllody 16SrI-A
AY803170 Chrysanthemum yellows 16SrI-A
AY803178 Tomato big bud 16SrI-A
AY803166 Apricot chlorotic leaf roll 16SrI-F
AY803180 Strawberry yellows 16SrI-K
AY803169 Blueberry stunt16SrI-B
AY803183 Clover phyllody 16SrI-C
MZ463022 Cucumber phyllody
AY803182 Ipomoea witches' broom 16SrI-N
AY803184 Paulownia witches' broom 16SrI-D
DQ787851 Chrysanthemum yellows 16SrI-B
AY803168 Aster yellows 16SrI-M
AY803176 Primrose virescence 16SrI-B
GU004336 Mexican periwinkle virescence 16SrXIII-A
GU004355 Stolbur-It phytoplasma 16SrXII-A
MN227134 'Ca. Phytoplasma americanum 16SrXVIII-B
GU004338 Potato purple top wilt 16SrXVIII-B
GU004363 'Ca. Phytoplasma pyri 16SrXIII-A
GU004335 'Ca. Phytoplasma mali16SrX-A
GU004328 Poinsettia phyllody 16SrIII-G
GU004327 Peach X-disease 16SrIII-A strain
GU004325 Walnut witches' broom 16SrIII-G
GU004326 Spirea stunt 16SrIII-E
GU004332 Clover yellow edge 16SrIII-B
GU004333 Potato purple top 16SrIII-M
GU004340 Milkweed yellows16SrIII-F
GU004320 Coconut lethal yellows 16SrIV-A
GU004324 Soybean phyllody16SrII-C
GU004348 Picris echioides phytoplasma16SrII-E
GU004331 Peanut witches' broom 16SrII-A
GU004347 Australian tomato big bud 16SrII-D
GU004322 Sesame phyllody 16SrII
AB703253 Sesame phyllody 16SrII
GU004362 Sesame phyllody 16SrII
AY197696 Rubus stunt phytoplasma 16SrV-E
AY197692 Alder yellows 16SrV-C
AY197685 Flavescence doree phytoplasma 16SrV-D
AY197690 Elm yellows 16SrV-A
AY197693 Cherry lethal yellows 16SrV-B
GU004329 'Ca. Phytoplasma fraxini 16SrVII-A
GU004329 'Ca. Phytoplasma fraxini 16SrVI-A
GU004352 Bean phyllody 16SrVI-A strain
GU004353 Bean phyllody 16SrVI-A strain
GU004317 Vinca virescence 16SrVI-A
GU004318 Lucerne virescence 16SrVI-A
GU004315 'Ca. Phytoplasma trifolii 16SrVI-A
GU004316 Potato witches' broom 16SrVI-D
GU004351 Potato purple top 16SrVI-A
GU004356 Brinjal little leaf 16SrVI-D
GU004344 Potato purple top 16SrVI-A
GU004342 Potato purple top 16SrVI-A
GU004343 Potato purple top 16SrVI-A
MH376290 Leptospira interrogans
0.05

b) sec Y gene



(C) rp gene

Figure: 2 Phylogenetic tree constructed with MEGA X Software by utilizing 16S rRNA **(a)** partial sec *Y* **(b)** and partial rp **(c)** gene sequences of cucumber phyllody phytoplasma (in red) with phytoplasma strains listed in Tables 1, 2, and 3. The tree was rooted with *Acholeplasma laidlawii* (GenBank accession number U14905), *Leptospira interrogans* (GenBank accession number MH376290) and *Acholeplasma laidlawii* (GenBank

accession number M81465) respectively. A bootstrap analysis with 1,000 replicates was performed and the bootstrap percent values above 50 are showed along the branches.