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SHORT COMMUNICATION

Association of '*Ca*. Phytoplasma asteris' (16SrI group) with flattened stem and witches' broom symptoms of *Petunia hybrida* in India

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Phytoplasmas are mollicutes associated with diseases of several plant species (2) and known to cause economic losses in ornamental plants (3). General yellowing and stunting of plants, proliferation of shoots, phyllody, virescence and reduced size of flowers and reddening of leaves are the common symptoms observed in phytoplasma infected ornamental plants (3). So far, 42 phytoplasmas belonging to 9 groups are identified on ornamental plants worldwide (3). Little work has been done on occurrence and identification of phytoplasma in ornamental plants in India (3). In this study, we characterized phytoplasma associated with *Petunia hybrida*, a commonly grown flower showing flattened stem and witches' broom symptoms at Indian Agricultural Research Institute (IARI) campus, New Delhi, India.

During January 2013 surveys of floriculture fields, IARI, Division of Floriculture, New Delhi, India, about two per cent *Petunia hybrida* plants were found to exhibit flattened stem and witches' broom type of symptoms without flower buds (Fig. 1). The symptomatic and healthy samples were collected and analysed for phytoplasma presence. The nucleic acid from flattened stem tissues of symptomatic *Petunia* plants was extracted (1) and amplification of phytoplasma ribosomal DNA (rDNA) was performed with the universal phytoplasma primer pairs P1/P7 (6). Further nested PCR was done with primer pair R16F2n/R16R2 (7). The DNA isolated from periwinkle infected with toria phyllody phytoplasma (group 16SrIX, pigeon pea witches' broom phytoplasma) maintained in greenhouse was used as positive control. The DNA extracted from asymptomatic *Peutnia* stem tissue was used as negative control.

Reactions were carried out in a Mastercycler (Eppendorf) and the cycling protocol used for the first round PCR was with initial denaturation at 94°C for 5 min, followed by 35 cycles consisting of denaturation at 94°C for 45 sec, annealing at 55°C for 1 min. and extension at 72°C for 2 min, with extension in the final cycle for 10 min. Two µl of product of the first round of PCR was used in nested PCR using internal primer pairs R16F2n/R16R2. Reaction mixture and condition of nested PCR were similar as above except for the annealing at 56°C for 1 min. Twenty-five microlitres of each PCR product was subjected to electrophoresis using 1.0% (w/v) agarose gel, stained with ethidium bromide and observed under UV transilluminator.

Nested PCR product (1.2 kb amplicon) was purified using the Wizard[R] SV Gel and PCR Clean-up System (Promega) and amplified product was sequenced directly

Fig.1. Witches'broom (a) and flattened stem (b) symptoms on naturally infected plants of *Petunia hybrida*

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in both directions using the nested PCR primers. The sequences were aligned using CLUSTAL W method of Bio-Edit software (Bio-edit Sequence Align Editor) and the consensus sequence was submitted to GenBank and used in BLAST search. The test sequence and reference phytoplasma strains sequences retrieved from GenBank were used to construct phylogeny through MEGA 5.0 version software (10).

PCR amplification did not yield expected 1.8 kb product of the 16S rDNA region of samples from symptomatic flat stem *Petunia* plants with primer pair P1/P7 but 1.8kb amplicon products was obtained with toria phyllody phytoplasma infected leaf (positive control) (data not shown). However, 1.2 kb amplicon in nested PCR products were observed with R16F2/R2 primers in both the flat stem DNA samples and the positive control of toria phyllody phytoplasma infected *Catharanthus roseus* leaf tissue (Fig. 2). No DNA was amplified by nested PCR assay from the template DNA isolated from any of the non-symptomatic healthy samples (Fig. 2). From sequencing and alignment of R16F2/R2 amplicon a 1249 bp DNA sequence of *Petunia hybrida* phytoplasma, designated as *Petunia* flattened stem phytoplasma (PFSP) was obtained and submitted in GenBank (Ac no.KF 728947).

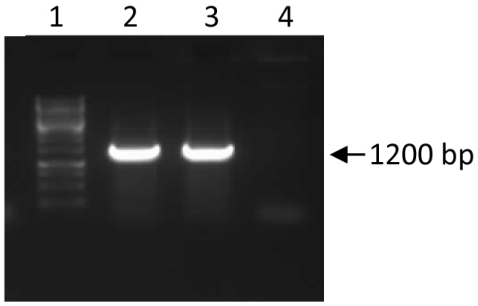

Fig 2. Nested PCR of Petunia flat stem phytoplasma. Lane 1: 1kb DNA ladder (G biosciences) lane 2: Positive control Toria phyllody phytoplasma (Acc.No.HM559247), lane 3: nested PCR products *Petunia flat stem* infected tissues; Lane 4: Negative control from healthy *Petunia* stem sample.

BLAST analysis of 16S rRNA partial gene sequence of PFSP isolate showed maximum identity (99%) with phytoplasmas associated with mulberry yellow dwarf from China (GQ249410), sesame phyllody from India (KC920747), Iranian lettuce from Iran (JQ015290), alfalfa stunt from Lithuania (GU289675), aster yellows from Serbia and Canada (HM4671274 and FJ824597) and bamboo witches' broom from China (FJ853161), which are all members of '*Ca.* P. asteris' (16SrI group, aster yellows group).

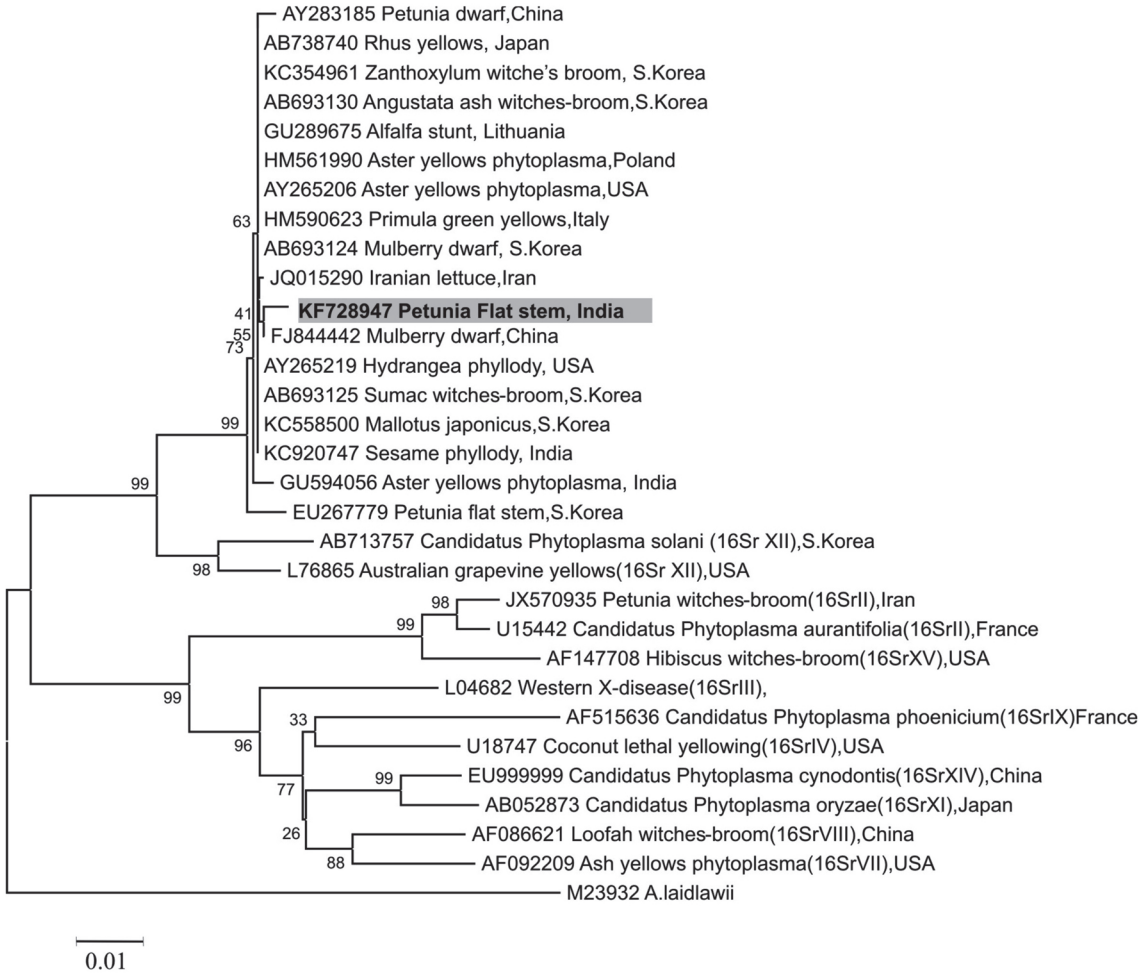

Fig. 3. Phylogenetic relationship between the *Petunia* flat stem phytoplasma – India isolate and reference phytoplasma strains based on Mega 5.0 software. Accession numbers are specified in the tree.

Phylogenetic analysis (Fig. 3) of the 16S rRNA gene partial sequence of *Petunia* flattened stem and witches' broom phytoplasma in the present study clustered together phytoplasma associated with *Petunia* dwarf phytoplasma, alfalfa stunt, aster yellows, mulberry dwarf, Iranian lettuce witches' broom, *Petunia* flat stem and sesame phyllody, all belonging to '*Ca.* P. asteris' of 16Sr-I group. Earlier, natural occurrence of different groups of phytoplasma on *Petunia* has been reported from different countries as, 16Sr III group causing little leaf from Australia (8), 16Sr I group, causing flat stem, yellows and witches' broom from Korea, Iran and India (4), not published Ac no. AY 283185, not published, Ac no. JX570935; and 16SrXII group from Iran (5). In India so far only leaf yellows symptoms were recorded on *Petunia* species caused by '*Ca* P. asteris' (16Sr I) group (9), hence our study confirmed the association of '*Ca* P. asteris' with flat stem symptomatic *Petunia* species in India and tentatively nomenclature as *Petunia* flat stem phytoplasma-Indian isolate.

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