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Suppression of seed setting and viability in phytoplasma-infected *Parthenium* weed in nature through differential gene expression

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ABSTRACT

The phytoplasma-mediated witches' broom disease in *Parthenium hysterophorus* L., a notorious weed, inhibits flower formation and percentage of seed setting. Even the seeds produced from infected plants showed poor viability and reduced rate of germination. Expression analysis of three orthologous genes in *P. hysterophorus*, namely, *APETALA-1* (*PhAP1*), ubiquitin binding protein (*PhDA1*), and RING-type E3 ubiquitin ligase (*PhATL80*) showed significant variation in their transcription levels. Expression of *PhAP1*, *PhDA1*, and *PhATL80* was higher in apical shoot bud and inflorescence than in other studied tissues. Infected plants showed suppressed expression of *PhAP1* and *PhATL80* in the inflorescences while no significant variation was observed in *PhDA1* expression. This resulted in floral to vegetative transition, causing phyllody and virescence, and lesser seed setting. These findings suggest that in naturally phytoplasma-infected *P. hysterophorus*, expression of gene(s) participating in floral development as well as seed setting at onset of the reproductive phase is suppressed.

INTRODUCTION

Weeds are serious problem for agriculture production (Chauhan *et al.* 2012, Aly and Dubey 2014). *Parthenium hysterophorus* L., belonging to family Asteraceae, is a devastating weed, which causes extensive losses in crop yield besides affecting biodiversity and environment in tropical and subtropical regions of the world (Adkins and Shabbir, 2012, Sushilkumar 2014)). It is difficult to control this weed in its habitat due to high seed setting as well as seed dormancy. This plant also contains allergenic growth inhibitors which cause respiratory problems, contact dermatitis, and so on in humans and livestock (Patel 2011, Sushilkumar 2005). It is also an alternative host for the sap-sucking insects like mealy bugs, leafhoppers and aphids. Currently, *P. hysterophorus* population is controlled by either conventional methods (hand pulling, crop rotation, mulching) or herbicides (2,4-D, Atrazine, metribuzin, Glyphosate, *etc*). Mexican beetle *Zygogramma bicolorata* has emerged most effective biological control agent to suppress Parthenium during rainy season (Sushilkumar 2009). Controlling this weed through conventional methods or the use of herbicides is not only cumbersome and expensive, the latter also pose a threat to the environment.

Life cycle of this weed starts with seed germination and proceeds through rosette formation, flowering, and seed setting and ends with seed dispersion. Thus, flower development, seed setting, and seed germination are considered as critical steps in the perpetuation of this weed. Several genes like *APETALA 1* (*AP1*), *LEAFY*, *DA1*, *ATATL80* (*RING-type E3 ubiquitin ligase*), *PTB1* (*POLLEN TUBE BLOCKED*), and so on play a significant role in inflorescence development and seed setting in model plants like *Arabidopsis thaliana* and *Oryza sativa* (Mandel *et al.* 1992, Gustafson-Brown *et al.* 1994, Wagner *et al.* 1999, William *et al.* 2004, Li *et al.* 2008, Benlloch *et al.* 2011, Li *et al.* 2013). The expression of *AP1* gene is up regulated during initiation of flowering, transition from vegetative to flowering stage and floral whorl development (Mandel *et al.* 1992, Gustafson-Brown *et al.* 1994, Benlloch *et al.* 2011). Mutants with pseudo-*AP1* showed secondary shoot and shoot-like development of flowers (Liljegren *et al.* 1999), and induced expression of *AP1* has been reported to convert shoot meristem into floral meristem in *A. thaliana* (Mandel and Yanofsky 1995). Seed development is a coordinated process following pollination and fertilization, and it involves development of the

embryo, endosperm, and maternal tissues (Adamski *et al*. 2009). The genes like *AUXIN RESPONSE FACTOR2*, *APETALA2*, and Arabidopsis *DA1* (*AtDA1*) play a key role in seed setting (Adamski *et al*. 2009). Among these, *AtDA1* (which codes ubiquitin binding receptor proteins) is involved in final seed setting and also determines the seed size (Fang *et al.* 2012, Xia *et al.* 2013, Li *et al.* 2008). It works with other ubiquitin-related proteins *DA2*, *EOD1*, and *UBP15* (Du *et al.* 2014). Another member of RING-type E3 ubiquitin ligase gene family like *POLLEN TUBE BLOCKED 1* (*PTB1*-a) of rice and *ATATL80* of *Arabidopsis thaliana* also play a similar role in regulation of seed setting (Suh and Kim 2015, Li *et al.* 2013).

Phytoplasma is a phloem-restricted phytopathogenic bacteria, which causes witches' broom disease in several crops with symptoms like sepal hypertrophy, bigger buds, phyllody, excessive proliferation of shoots, inflorescence clustering, virescence, and development of small leaves (Bertaccini 2007, Yadav *et al*. 2015a). The phytoplasma are transmitted through insects, and at the onset of invasion, the host's morphology and life cycle are so modified to be conducive both for vector activity (Sugio *et al.* 2011) and bacterial colonization (MacLean *et al.* 2014, Su *et al.* 2011). Being obligate parasitic bacteria, these phytoplasma are restricted to the phloem sieve tubes and induce witches' broom disease in *Parthenium* species by altering floral development (Li *et al*. 2011, Bertaccini 2007, MacLean *et al*. 2014, Pracros *et al.* 2007). During reversal of flowering to vegetative phases in the infected host plants, phytoplasma induces transcriptional reprogramming of flowering-related genes. In tomato, phytoplasma induce the expression of *LEAFY* ortholog (*FA*) and suppress the *APETALA3* (*SlDEF*) and *AGAMOUS* (*TAG1*) (Pracros *et al*. 2006, Pracros *et al*. 2007). Hypermethylation-mediated transcriptional reprogramming and inhibition in expression of *SlDEF* (*APETALA3*) in tomato and *APETALA3* and *AGAMOUS* in *A. thaliana s* have also been observed (Pracros *et al.* 2007, Finnegan *et al.* 1996). Further phytoplasma produced a novel effector protein (SAP54), which interacts with MADS-domain transcription factor (*MTF*) and degrades it with the help of *RADIATION SENSITIVE23* (*RAD23*) genes associated with proteasomes (MacLean *et al.* 2014). Degradation of *MTF*, a key regulator of flower development, led to differential expression of *SEPALLATA3* and *APETALA1* in *Arabidopsis* and thus modifies floral development (MacLean *et al.* 2014). Similarly, suppression of *CrSEP3*, a *SEPALLALA3* ortholog, and *Chalcone synthase*, and the loss of floral pigmentation in *Catharanthus roseus* have also been observed during phytoplasma infection (Su *et al.* 2011).

Phytoplasma infection also causes differential expression of genes related to photosynthesis and elevated expression of Sucrose Synthase and Alcohol Dehydrogenase-I in *Vitis vinifera* (Bertamini and Nedunchezhian 2001, Hren *et al.* 2009). Alteration in the fate of inflorescence, flowering, fertilization, flower fertility, and seed setting have been observed in different species (Mathur 1989, Keshwal 1982).

Thus, understanding the molecular mechanism for inhibition of fertilization and seed setting in naturally phytoplasma-infected *P. hysterophorus* would be useful in developing a new strategy to control this weed. This study reports on differential expression of three orthologous candidate genes, regulating flower development in naturally phytoplasma-infected *P. hysterophorus* plants. Further, the effect of natural phytoplasma infection on seed setting, seed viability, and rate of seed germination in *P. hysterophorus* are also reported.

MATERIALS AND METHODS

The *P. hysterophorus* plants with symptoms of witches' broom disease were identified in the nursery of the Central University of Rajasthan, India, and tagged. Sepal hypertrophy, big buds, phyllody, excessive proliferation of shoots, inflorescence clustering, virescence, and development of small leaves were used to confirm the disease. Further, the presence of phytoplasma on the plants was affirmed by amplification of 16S rRNA using primers (Forward 5'GACTGCTAAGACTGGATAGG 3' and Reverse 5' CGAACGTATTCACCGCGAC 3', **Table 1**) and sequencing of the 16S rRNA clones (Xcelris Labs Ltd). About 100 mature flowers of healthy and infected plants were collected and used in the assessment of seed setting, seed viability (Verma and Majee 2013) and germination test. For germination assay, the seeds were kept on distilled water-soaked blotting paper (Axiva, India) for eight to ten days, and the number of seeds that germinated was observed. This experiment was performed with three biological replicates, and the experiment was repeated twice. Student 'T' test was used to analyse the result.

Total RNA was isolated from different plant parts of infected and healthy plants using total RNA purification kit (Jena Biosciences, Germany) followed by cDNA preparation with Verso cDNA

Table 1. List of primer sequences used in study

Genes	Forward primer (5' - 3')	Reverse primer (5' - 3')
Used in amplification/cloning		
16S rRNA	GACTGCTAAGACTGGATAGG	CGAACGTATTCACCGCGAC
PhAP1	GCTCTAGAATCTTCACCCATATAGTGC	CGGGATCCTTAGGAGGATAGAGAACAAG
PhDA1	GCTCTAGATCTGATCACGAGTTCTCCATGTC	CGGGATCCGGTTCACCTTCCATGGCTTC
PhATL80	GCTCTAGATCCGATTTCGTCGTCATCC	CGGGATCCCGGAAACTCGCCGCACTTC
Used in qRT-PCR analysis		
PhAP1	TCACCCATATAGTGCCTGTGA	GGAGCGATATGAGCGATATTC
PhDA1	TCCATGTCTGACAACCGTCC	ACAGCTGCAACATCTAGGAG
PhATL80	ACGGATCAACCACCAATTGC	GTGTCAACGCACGTGACATG
Actin	GAAGAGAACCTCAGGGCAAC	CGAGCAAGAGCTTGAGACTG

synthesis kit (Thermo Scientific, USA). The phytoplasma infestations of infected plants were affirmed by reverse transcriptase (RT) PCR analysis of phytoplasma 16S rRNA (Li *et al.* 2011) using synthesized primers (**Table 1**).

Since a complete decoded genome sequence or transcriptome of *P. hysterophorus* is lacking, the sequence of corresponding genes from *Arabidopsis thaliana* (TAIR database) were used as query nucleotide for BLAST analysis with EST sequence of *Parthenium argentatum* (http://comp genomics. ucdavis.edu /cgp_wd_assemblies.php#4232) and *Dahlia trinity* using BioEdit software (**Supplementary file S1**). The conserved regions were identified by aligning the retrieved EST sequences with those of other members of *Compositae* family (http://www.genome.jp/tools/ clustalw/). The primers were designed from the most conserved region for cloning and expression analysis (**Table 1**).

The cDNAs amplified from healthy plants were directly cloned into TA cloning vector (T-Vector pMD20, Takara) and validated by re-sequencing of the clones. These partially cloned EST sequences were deposited at NCBI with accession number KY745903 (*PhAP1*), KY745904 (*PhDA1*), and KY745905 (*PhATL80*). These sequences showed significant similarity with their corresponding genes obtained from *P. argentatum* and *D. trinity*, respectively (**Supplementary file S2-4**).

Expression of orthologous genes of *P. hysterophorus*, namely, *APETALA-1* (*PhAP*1), Ubiquitin binding protein (*PhDA1*), and *RING-type E3 ubiquitin ligase* (*PhATL80*), was studied in healthy plants during developmental stages and in the shoot bud, leaf, and inflorescence of phytoplasma-infected plants. qRT-PCR of these genes was performed in biological triplicate and experimental duplicate condition by using diluted cDNA products (fivefold with deionized water) as a template. Each qRT-PCR reaction was performed at Roche Real-Time PCR Detection System (Roche, Light Cycler® 96) by using the DyNamoColorFlash SYBR Green qPCR kit (Thermo Scientific Cat No. F-416L) as prescribed in the manufacturer's manual. Each reaction mixture (10 µL) was prepared by adding 5 µL of 2X SYBR Green PCR Master mix, 1 µL (10 pmol) of forward and reverse primers, and 1 µL of diluted cDNA. The amplification condition was as follows: 95°C for 7 min followed by 45 cycles of 95°C for 10s, 60°C for 15s and 72°C for 15s. Expressions of the three genes were normalized with an internal reference gene *Parthenium argentatum* Actin (>Contig12870). Results of qRT-PCR were analysed by the '$2^{-\Delta\Delta Ct}$' method (Livak and Schmittgen 2001).

RESULTS AND DISCUSSION

In the present study, the incidence of phytoplasma in *P. hysterophorus* was affirmed by morphological symptoms of witches' broom, such as phyllody and virescence (**Figure 1a**). These findings were corroborated with earlier observations (Li *et al.* 2011) that altered distribution of phytohormones and repression of genes related to floral development (Tan and Whitelow 2001, Sugio *et al.* 2011, Su *et al.* 2011) could cause the observed symptoms.

Further, the presence of phytoplasma in plant tissue was validated by the amplification of 16S rRNA unique to this phytopathogen group (**Figure 1b**). The 16S rRNA sequence of the phytoplasma strain obtained in the present study revealed more than 99% similarity with that of phytoplasma strain *YJJ2*, which belongs to phytoplasma 16SrII-A group (**Supplementary file S5**) and causes witches' broom disease in *P. hysterophorus* (Gene ID.EU779826.1; Li *et al.* 2011). Further, phylogenetic analysis of 40 reported 16S rRNA sequences along the present one showed its clustering with the phytoplasma sequences belonging to 16SrII group (**Figure 1c**), and most of them cause witches' broom disease in different species, including *Hibiscus* sp., bamboo, cactus, and peanut (Montano

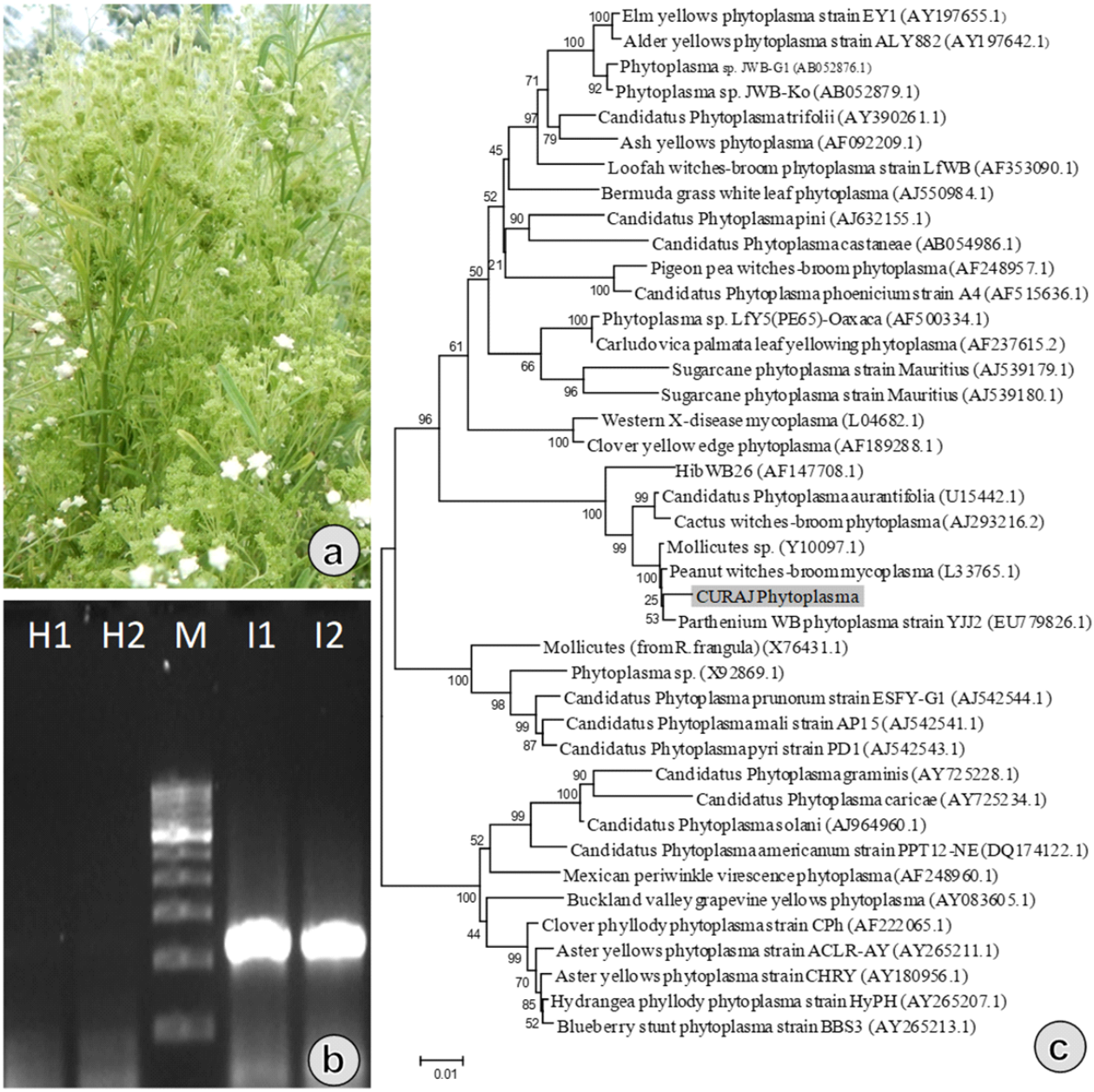


Figure 1. Identification and confirmation of witches' broom disease in *P. hysterophorus* L. through infective phytoplasma (a) Photography of infected plants, (b) Confirmation of phytoplasma infection by RT-PCR analysis 16S rRNA, and (c) Phylogenetic map of phytoplasma's 16S rRNA sequences with available sequence of the other strain (Alignments were made using CLUSTAL Omega multiple sequence alignment tool. The phylogenetic tree was constructed by the neighbourjoining approach using the MEGA5.10 program with default settings. CURAJ_Phytoplasma is shown in the yellow bracket. Numbers at the branch points indicate bootstrap values based on 1000 bootstrap replicate)

et al. 2001, Yadav *et al.* 2015b, Li *et al.* 2012, Chung *et al.* 2013). Phytoplasma-infected plants of *P. hysterophorus* showed about ten-fold reduction in number of seeds per inflorescence compared to healthy plants (**Figure 2a-e**) as reported earlier in this species (Taye *et al.* 2002). The seeds of infected plants appeared mottled black whereas those of healthy plants were dark black in appearance (**Figure 2g-h**). The viability assessment revealed that less number of viable seeds were produced in the infected plants as compared to healthy plants (**Figure 2f**), and the seed viability ratio of healthy plants was more than 80% in comparison to about 12% in infected plants. Delayed response to seed germination and lowered rate of seed germinations were noticed for the seeds of infected plants, the latter being about five fold lower (**Figure 2i-k**).

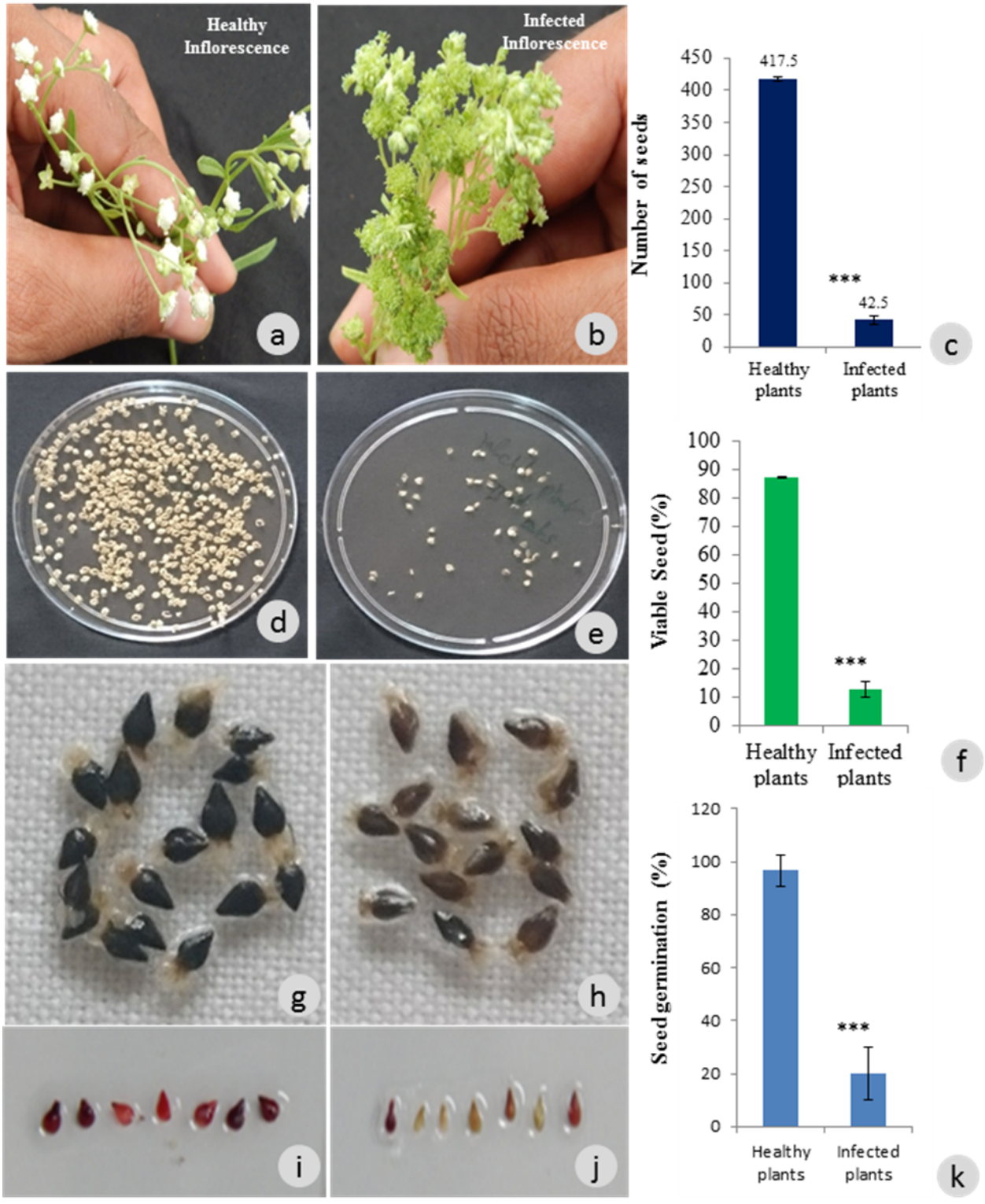


Figure 2. Effect of phytoplasma infection on seed settings, viability, and germination rate in *P.hysterophorus* L. plants (a–b) Photograph of inflorescence of healthy and infected plants, (c–e) Differences in seed setting,(f–j)Nature and viability of sets seeds in healthy and infected plant, and (k) Seed germination percentage in healthy and infected plants; hundreds of inflorescences of healthy and infected plants were collected for the experiment. Mean ± SE were obtained from three independent plants. Bars labelled with stars indicate the significant differences as determined by Student T-Test (P ≤ 0.001)

In this study, the expression of putative orthologous genes related to flower development, *PhAP1*, and seed setting, *PhDA1* and *PhATL80*, varied in at different stages of growth and development in healthy plants. Expression of *PhAP1* was maximum in inflorescence (139-fold), followed by apical bud (34-fold) than in seedling leaves (**Figure 3a**). Expression of *PhDA1* was significant and induced more than two fold in buds and inflorescence than in other part like seedling leaf and leaves of vegetative and flowering stages (**Figure 3b**). The expression of *PhATL80* was found significantly induced in all the tissues tested in the study compared to seedling leaves (**Figure 3c**). The maximum expression of *PhATL80* was in apical bud (55-fold) followed by inflorescence (38-fold) compared to seedling leaves (**Figure 3c**). Among the three selected genes, the expression of *PhAP1* was maximum in inflorescence whereas the expression of *PhATL80* and *PhDA1* were also augmented at onset of the reproductive phases. Their expression profiles suggest their role in flowering and development of *P. hysterophorus* as reported earlier in various species (Mandel *et al.* 1992, Gustafson-Brown *et al.* 1994, Li *et al.* 2008, Benlloch *et al.* 2011, Li *et al.* 2013, Fang *et al.* 2012, Xia *et al.* 2013, Suh and Kim 2015).

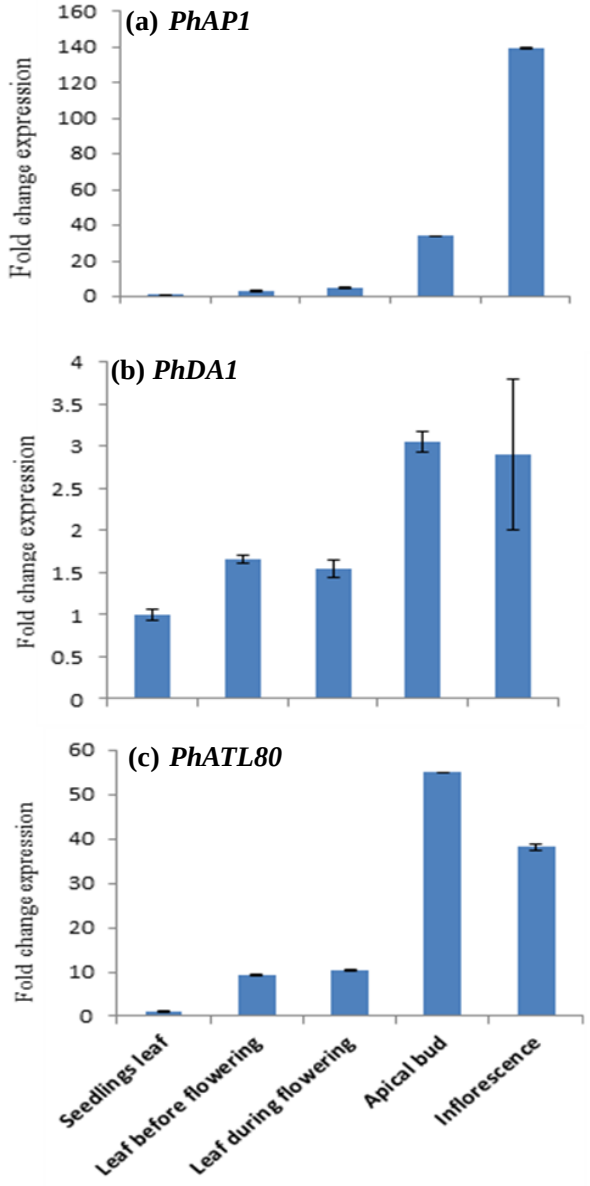


Figure 3. Expression pattern of *PhAP1*, *PhDA1*, and *PhATL80* genes at different stages and tissues of *P. hysterophorus* L. plant; expression of (a) *PhAP1*, (b) *PhDA1*, and (c) *PhATL80* in seedling leaves, leaf before flowering, leaf during flowering, apical bud, and inflorescence

The expressions of these three genes (*PhAP1*, *PhDA1* and *PhATL80*) were tested in leaves, apical buds, and inflorescences of infected plants along with

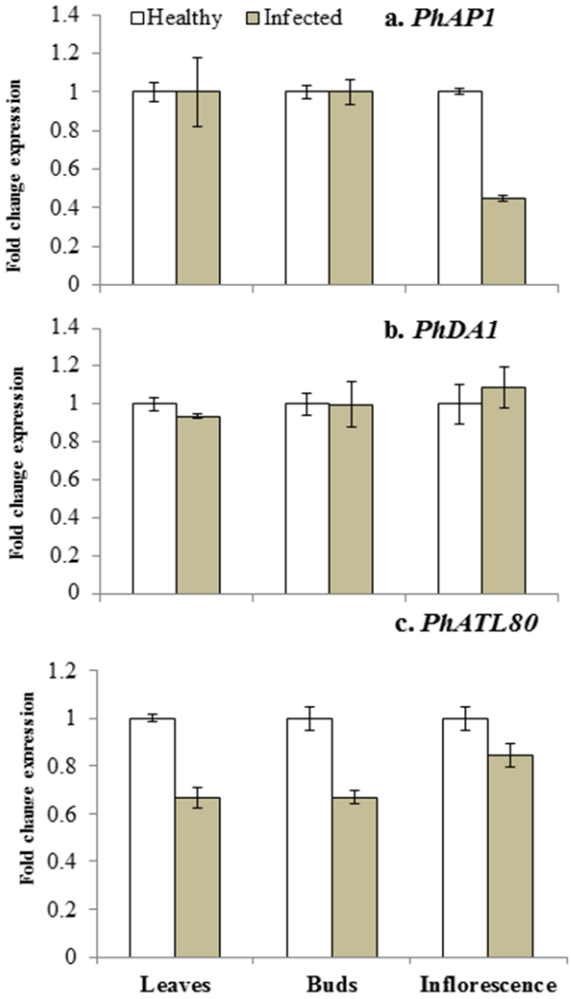


Figure 4. Expression pattern of *PhAP1*, *PhDA1*,and *PhATL80* genes in different tissues of phytoplasma-infected *P. hysterophorus* L. plant. Expression of (a) *PhAP1*, (b) *PhDA1*, and *PhATL80* (c) in buds, leaf, and inflorescence of infected and healthy plants

healthy plants. The expression of *PhAP1* was significantly suppressed in the inflorescences of infected plants and remained unchanged in buds and leaves during reproductive phases (**Figure 4a**). In contrast to *PhAP1*, the expression of *PhDA1* in buds, leaves, and inflorescences of the infected plants remained equivalent to that in healthy ones **(Figure 4b).** Like *PhAP1*, expression of *PhATL80* was significantly suppressed in buds, leaves, and inflorescence, but the suppression level was less than that of *PhAP1* (**Figure 4c**).

Zhang *et al.* (2015) have shown that *Arabidopsis DA1* is a negative regulator in seed size development, and its mutant produces larger seeds than the wild type. However, the over expression of *GsoDA1* gene did not affect seed size in *Glycine soja* and was associated with salinity resistance, which suggests its diverse function across the species (Zhao *et al.* 2015). The role of *DA1*in endo-re-duplication with cell and organ growth during leaf development has also been provided (Peng *et al.* 2015). In the present study, expression of *PhDA1* was almost unaffected in infected leaves, buds, and flowers of *P. hysterophorus*, showing that phytoplasma infection is unable to modulate expression of this gene and causes no alteration in seed size. Transcription factor like *AP1* regulate the flower formation along with *LFY* and mutation of these genes causes conversion of flower into shoot (Weigel *et al.* 1992, Riechmann *et al.* 1996, Parcy *et al.* 1998). In the present study, the expression *PhAP1* was significantly suppressed in the inflorescences of infected plants, which could putatively be associated with the reversal of flowering to vegetative phase causing phyllody. *ATATL80* (AT1G20823.1) are plasma membrane (PM)-localized ubiquitin (Ub) ligase and are involved in maintaining the phosphorus content as well as seed yield and biomass in *Arabidopsis* (Suh and Kim 2015). As expected, the expression of *PhATL80* was also significantly suppressed in buds, leaves, and inflorescences of the infected *P. hysterophorus* plants causing reduction in seed yield. The mutant line of *Arabidopsis* for this gene *ATATL80* showed earlier bolting than wild type, and overexpression of this gene resulted in late flowering and lower seed yield (Suh and Kim 2015). This implies the important role that *ATATL80* gene plays in flowering as well as seed setting in *Arabidopsis*. In *P. hysterophorus*, in contrast to *Arabidopsis*, phytoplasma infestation causes suppression of *PhATL80* gene resulting in floral to vegetative transition, causing phyllody and virescence, and lesser seed setting (Suh and Kim 2015).

Supplementary file S1. Systemic representation of different steps in isolation and fetching of candidate genes of *Arabidopsis thaliana* from Compositae EST database (http:// compgenomics. ucdavis. edu/ cgp_ wd_assemblies. php# 4232).

(1) Selection and isolation of candidate gene sequences from TAIR (e.g. AP1 (AT1G69120.1), DA1 (AT1G19270.1), ATATL80 (AT1G20823.1) gene sequences were isolated from TAIR website (https://www.arabidopsis.org))

⇩

(2) Selection and isolation of EST sequence of *Parthenium argentatum* and *Dahlia trinity* from Compositae Genome database (http:// compgenomics. ucdavis. edu/ cgp_ wd_assemblies. php# 4232) and creation of local nucleotide data base file with BioEdit software (http://www.mbio.ncsu.edu/bioedit/bioedit.html) and considering them as source file.

⇩

(3) Nucleotide BLAST analysis with *A. thaliana*'s selected sequences (as query sequences) and *P. argentatum* and *Dahlia trinity* EST Library (as source file) with the help of BioEdit software (http:// www.mbio.ncsu.edu/bioedit/bioedit.html) with filteration of maximum percentages of similarity.

⇩

(4) Fetching of corresponding EST sequence of *Parthenium argentatum* and *Dahlia trinity* from Compositae Genome database (e.g. *AtAP1* - matched with corresponding contig >1078 of *P. argentatum AtDA1* - matched with corresponding contig >guayule_c124924 *P. argentatum*, and *ATATL80* matched with Dahlia.faS >comp1908_c0_seq3 of *Dahlia trinity*)

⇩

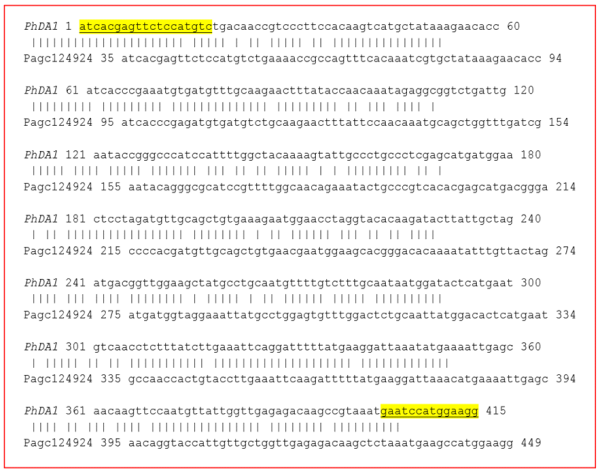
(5) Further to get the conserved region and designing of primers for cloning, the corresponding retrieved sequences were separately aligned with other members of compositae family's 454 derived EST sequences (http://www.genome.jp/tools/clustalw/).

Supplementary file S2. Alignment of the selected nucleotide sequence of the *P. argentatum* contigs1078 (Pag1078) with cloned sequence from *Parthenium hysterophorus* (*PhAP1*);analysis was performed with BioEdit software (http://www.mbio.ncsu.edu/bioedit/ bioedit.html);underlined, coloured letters show the primer regions of the selected sequences for cloning.

```
PhAP1  1 atcttcacccatatagtgcctgtgatttctttgtaaaagctcagcccttgatttaagttt 60
         ||||||||||||||||||||||||||||| |||||||||||||||||||||| |||||||| ||
Pag1078 381 atcttcacccatatagtgcctgtgattcctttgtaaaagctcagccctagatttaagctt 440

PhAP1  61 gttgtactcaagggtccaacttcttggagtagcatcagcggcaactagctgtctctcggt 120
          ||| ||||||||||||||||||| ||||||||||||||||||||||||||||||||||| ||
Pag1078 441 gttatactcaagggtccaacttctcggagtagcatcagcggcaactagctgtctctcagt 500

PhAP1  121 ataagaatatcgctcatatcgctccaagatgctgtccatgcaagagtcagtagaaaactc 180
           |||||||||||||||||||||||||||| |||||||||||||||||||||| ||||||||
Pag1078 501 ataagaatatcgctcatatcgctccaatatgctgtccatgcaagagtcagttgaaaactc 560

PhAP1  181 aaagagttttcctttgttggagaagacaattaaggcaacttcagcatcacaaagaacaga 240
           ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Pag1078 561 aaagagttttccttttgttggagaagacaattaaggcaacttcagcatcacaaagaacaga 620

PhAP1  241 aatttcatgggctttcttcaataagccacctctttctcttggagaaagtaacttgcctgtt 300
           |||||||||||||||||||||||||||||||| ||||||||||||||||||||||||||
Pag1078 621 aatttcatgggctttcttcaataagccacctctcctcttggagaaagtaacttgcctgtt 680

PhAP1  301 gatcttgttctctatcctcctaag 324
           |||||||||||||||||||||||||
Pag1078 681 gatcttgttctctatcctcctaag 704
```

Score = 486 bits (306),
Expect = e-141
Identities = 315/324 (97%)
Strand = Plus / Plus

Supplementary file S3. Alignment of the selected nucleotide sequence of the *P. argentatum*guayule_c124924 (Pagc124924) with cloned sequence from *Parthenium hysterophorus* (*PhDA1*);analysis was performed with BioEdit software (http://www.mbio.ncsu.edu/bioedit/bioedit.html); underlined coloured letters show the primer regions of the selected sequences for cloning



Score = 446 bits (281),
Expect = e-129
Identities = 348/415 (83%)
Strand = Plus / Plus

Supplementary file S4. Alignment of the selected nucleotide sequence of the *Dahlia trinity* comp1908_c0_seq3 (Dtcomp1908) with cloned sequence from *Parthenium hysterophorus* (*PhATL80*); analysis was performed with http://www.ebi.ac.uk/Tools/msa/clustalw2/; underlined coloured letters show the primer regions of selected sequences for cloning

Supplementary file S5. Alignment of the selected nucleotide sequence of the phytoplasma (EU779826.1_CoWBP) and clones 16S rRNA of phytoplasma (CURAJ Phytoplasma);analysis was performed with BioEdit software (http://www.mbio.ncsu.edu/bioedit/bioedit.html); underlined coloured letters shows the primers region of selected sequences for cloning

Score = 2351 bits (1186),
Expect = 0.0
Identities = 1211/1218 (99%),
Strand = Plus / Plus

In conclusion, these findings suggest that in naturally phytoplasma-infected *P. hysterophorus*, expression of gene(s) participating in floral development as well as seed setting at onset of the reproductive phase is suppressed. Further, we also suggest that a chemically or biologically active compound which can suppress the expression of these genes could be used to control seed setting and spreading of *P. hysterophorus*.

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Aly R, Dubey NK. 2014. Weed Management for parasitic weeds. pp. 315-345. In: *Recent Advances in Weed Management*). Springer, New York, NY.

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