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First report of stem fasciation symptom in Crape jasmine (*Tabernaemontana divaricata*) not associated with phytoplasma infection

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Abstract
Crape jasmine (*Tabernaemontana divaricata*) exhibiting stem fasciation symptom was observed in November 2022 at Indian Agricultural Research Institute, Regional Station campus in Pune (Maharashtra) India. As fasciation is a typical symptom induced due to phytoplasma infection in plants, PCR and nested PCR using phytoplasma-specific primer pairs was carried out to determine the possible association of a phytoplasma with the symptomatic sample. Amplification was not obtained in the symptomatic sample during direct PCR with two sets of primers and also in the nested PCR assay. This is the first report of occurrence of stem fasciation in *T. divaricata* and the observed symptom is not associated with a phytoplasma infection.

Keywords: Stem fasciation, phytoplasma, crape jasmine, pinwheel flower, India

Introduction
Tabernaemontana divaricata (L.) R.Br. belongs to family Apocynaceae and is commonly called pinwheel flower, crape jasmine and Nero's crown. It is an evergreen shrub which occurs widely throughout the Indian subcontinent, Southeast Asia and China. The plant is cultivated as an ornamental and found in lawns, gardens and along the roadsides. It is extensively utilized in various traditional systems of medicine for the treatment of several diseases such as asthma, epilepsy, fever, inflammation, diarrhoea and skin disorders (Ghosh *et al.*, 2021) [9]. In western India, its latex is applied to wounds to prevent inflammation (Van Beek *et al.*, 1984) [18].
Fasciation which is also called cresting is a condition of abnormal growth in vascular plants. It is generally manifested morphologically as a change from the normal round or polygonal stem or axis to a flattened or ribbon like expansion of the main stem axis (White, 1948) [21]. This phenomenon is not rare among plants and has been reported to occur in 107 families, but it is not often reported in the case of plants having woody stems. It is very common in Rosaceae, Liliaceae, Crassulaceae, Compositae, Ranunculaceae, Euphorbiaceae, Leguminosae and Cactaceae (Barannon, 1914; White, 1948) [1, 21]. The phenomenon is prevalent in plant species having indeterminate growth patterns of their vegetable organs and inflorescence. In agriculture and horticulture, the occurrence of fasciation is a concern as it reduces the value of traded plants (Porbeni and Fawole, 2013; Wilson *et al.*, 2001) [16, 20].
Fasciation has been associated with a range of abiotic and biotic factors among which phytoplasma-associated fasciation has been reported from several plant species such as lillies (Bertaccini *et al.*, 2005) [3], pomegranate (Gao *et al.*, 2018) [8], flax (Biswas *et al.*, 2014) [4], China ixeris (Li *et al.*, 2013) [12], asparagus (Fránová and Petrzik, 2010) [7] and crotolaria (Kumar *et al.*, 2010) [11]. On the contrary, there are also reports showing fasciated plants testing negative for phytoplasma presence in PCR assays in the case of herbaceous plants such as apennines genepì (Pace *et al.*, 2020) [14] and sesame (Wilson *et al.*, 2001) [20].
In light of the above, the present study was conducted to determine whether phytoplasma infection is associated with the stem fasciation observed in a *T. divaricata* plant.

Materials and Methods
Sample collection
A *T. divaricata* plant exhibiting stem fasciation symptom was observed in November 2022 at the campus of Indian Agricultural Research Institute, Regional Station, Pune (Maharashtra), India. Morphological differences between the fasciated and symptomless plants were recorded.

The symptomatic and two asymptomatic samples were collected with the objective of determining a possible association of phytoplasma with the diseased sample.

DNA extraction and PCR assays

Total genomic DNA was extracted from the fasciated stem and leaf petioles (100 mg) of the symptomatic plant and leaf petioles of the asymptomatic samples using DNeasy Plant Mini Kit (Qiagen, Germany) as per the manufacturer's protocol. DNA extracted from 16SrVI-D phytoplasma infected bitter gourd plant was used as the positive control (Verma *et al.*, 2022) [19]. The extracted DNA was quantified using Bio Spectrometer (Eppendorf, Germany).

Direct PCR was carried out using P1/P7 universal primers (Deng and Hiruki, 1991; Schneider *et al.*, 1995) [5, 17] for amplifying the phytoplasma ribosomal DNA (rDNA). Each reaction mixture (25 µl) for the PCR assay contained 12.5 µl Dream Taq PCR Master Mix (2X) (Thermo Fisher Scientific, MA), 0.5 µl of each forward and reverse primer (10 pmol/ µl), 9.5 µl nuclease free water and 2 µl DNA (0.1 µg). The PCR conditions were: initial denaturation at 94 °C for 4 minutes, followed by 30 cycles of denaturation at 94 °C for 1 minute, annealing at 53 °C for 1 minute, primer extension at 72°C for 2 minutes and a final extension at 72 °C for 10 minutes.

Direct PCR was also carried out using P1/P6 universal primers (Deng and Hiruki, 1991) [5] with annealing at 55°C for 1 minute.

Products of the direct PCR (0.3 µl) were utilized as templates for nested PCR assay with R16F2n/R2 primers (Gundersen and Lee, 1996) [10]. The thermal cyclic conditions for the nested PCR assay were: initial denaturation at 94 °C for 5 minutes followed by 30 cycles of denaturation at 94 °C for 1 minute, annealing at 60 °C for 2 minutes, primer extension at 72°C for 3 minutes and a final extension at 72 °C for 10 minutes.

Gel electrophoresis of the PCR products was carried out in a 1% (w/v) agarose gel stained with GelRed nucleic acid stain (Biotium, CA) which was then observed using a Gel Documentation system (Syngene, UK).

Results and Discussion

Morphological differences were clearly visible in the fasciated *T. divaricata* plant as compared to the symptomless plants. The stem had been converted into a broad, flattened and ribbed structure which was completely different from the usual circular stem of the normal plant of the same species (Fig 1.).

Fig 1: *Tabernaemontana divaricata* plant showing stem fasciation symptom (left), healthy plant (right)

The occurrence of the stem fasciation had also altered the phyllotaxy. Similar changes in phyllotaxy owing to stem fasciation have been previously reported in *Cocculus hirsutus* (Menispermaceae) (Yadav *et al.*, 2023) [22], *Phaseolus multiflorus* (Fabaceae) (Bausor, 1937) [2] and succulents (El-Banna *et al.*, 2013) [6].

Direct PCR assay using P1/P7 and P1/P6 primers yielded amplicons of the expected size 1.8 kb and 1.5 kb respectively in the positive controls (Fig 2.). However, no amplification was observed in the symptomatic and asymptomatic samples. In the nested PCR assay using R16F2n/R2 primers, expected amplification product of 1.2 kb was obtained in the positive control but not in the symptomatic and asymptomatic samples (Fig 2.).

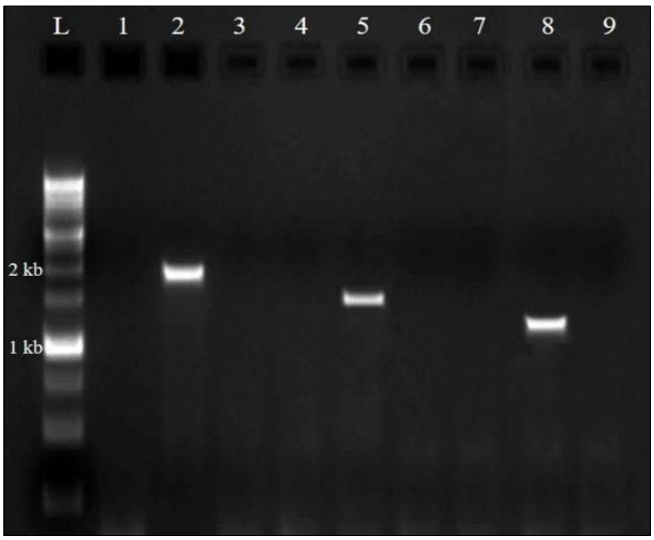

Fig 2: Agarose gel pictures of direct and nested PCR assays using P1/P7 (lane 1 – 3), P1/P6 (lane 4 – 6) and R16F2n/R2 (lane 7 – 9) primers. L: 1 kb molecular weight marker (Gene Dire X, Taiwan); lane 1, 4, 7: asymptomatic sample; lane 2, 5, 8: positive control (Bitter gourd); lane 3, 6, 9: symptomatic sample

The present study found no association of phytoplasma infection with the occurrence of stem fasciation in the *T. divaricata* plant based on molecular assays. Non-association of fasciation with phytoplasma infection has been previously reported in Apennines genepì (Pace *et al.*, 2020) [14] and sesame (Wilson *et al.*, 2001) [20].

The fasciation observed in *T. divaricata* could possibly be due to infection of a bacterium such as *Rhodococcus fascians* which is known to induce fasciation in a wide spectrum of plant species (Park *et al.*, 2021) [15]. It could also be attributed to several other factors such as hormonal imbalances in the meristematic cells, random genetic mutation, environmental factors including temperature fluctuations, mineral deficiency, infection of nematodes or physical damage to the growing tip (Omar *et al.*, 2014) [13].

To the best of our knowledge, this is the first record of occurrence of stem fasciation phenomenon in *Tabernaemontana divaricata* and the observed symptom is not associated with a phytoplasma infection. Further studies need to be undertaken to determine the cause of occurrence of this anomaly.

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Author contributions

All authors contributed equally to this research study. RV and ST collected and the samples. AV carried out DNA extraction, PCR and nested PCR assays. AV and ST prepared the manuscript draft. ST and RV proofread and made revisions to the manuscript.

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