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EVIDENCE OF SEED TRANSMISSION OF PHYTOPLASMA INFECTING KING CHILLI (*CAPSICUM CHINENSE* JACQ.) IN ASSAM

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ABSTRACT

King chilli (*Capsicum chinense* Jacq.) is an economically, culturally, and medicinally important crop of Northeast India. Besides various maladies, phytoplasma infection has emerged as a serious threat to its cultivation of this important cash crop very recently, spreading to newer locations quickly. The present study was undertaken to investigate the possibility of seed-borne transmission of phytoplasma in king chilli. Survey was conducted during 2024 in three major king chilli growing districts of Assam namely, Jorhat, Sivasagar and Nagaon. Symptomatic plants exhibiting leaf growing reduction, yellowing, stunting, and witches' broom were collected and phytoplasma infection was confirmed through conventional and nested PCR using universal 16S rRNA gene primers, followed by sequencing. The detected phytoplasma shared 99.76% sequence identity with '*Candidatus* Phytoplasma citri' and was classified under the 16SrII-D subgroup. Seeds collected from phytoplasma-positive and healthy plants were sown under insect-proof conditions to assess germination and possibility of seed transmission of the phytoplasma. Seeds collected from infected mother plants showed 100% germination, similar to those collected from healthy plant. PCR analysis of 80 seedlings germinated from infected seeds revealed phytoplasma presence in five seedlings, indicating a seed transmission rate of 6.25%. This study highlights the role of seed-borne inoculum in phytoplasma spread and emphasizes the need for phytosanitary measures and healthy seed production to protect king chilli cultivation in Northeast India.

Keywords: King chilli, *Candidatus*, 16SrII-D, seed transmission.

Introduction

King chilli (*Capsicum chinense* Jacq.), popularly known as ghost pepper or *bhut jolokia* in Assamese, is considered a natural hybrid between *Capsicum chinense* and *Capsicum frutescens*. This locally important chilli is widely cultivated in Assam, Nagaland, and Manipur, and to a lesser extent in other parts of Northeast India, where it carries deep cultural and culinary significance. It has an impressive Scoville Heat Unit (SHU) rating of 1,001,304, leading to its recognition as the seventh hottest chilli pepper in the world by the Guinness World Records. Compared to other chilli species, King chilli contains exceptionally

high levels of 'capsaicin' the active compound responsible for its pungency (Baruah *et al.*, 2014; Sanatombi and Sharma, 2008) with concentrations ranging from 3–5 per cent (Borgohain and Devi, 2007). Beyond its extreme heat, this chilli is highly valued for its cultural, culinary, economic, and medicinal significance in the region. It is rich in vitamins, minerals, and antioxidants and also supports digestion, enhance metabolism, and provide pain-relieving effects. Owing to its therapeutic properties, it has been traditionally used to treat ailments such as spondylitis, headaches, night blindness, and digestive problems (Sarwa *et al.*, 2012), and is also known to alleviate chronic congestion (Bhagowati and Changkija, 2009).

According to reports of capsaicin's diverse pharmacological activities and clinical uses (Ochoa-alejo and Ramirez-malagon, 2001), it may be used for a number of medical conditions, including pain management, cancer prevention, weight loss, gastrointestinal benefits, anti-inflammatory properties, and antioxidant activity. As a result, King chilli is a highly sought-after chilli variety due to its commercial extraction of capsaicin and its potential for use in pharmaceutical industries (Higashiguchi *et al.*, 2006). During the production season, green King chilli is sold at a moderate price of 300–400 INR per kg, though prices rise sharply during the off-season (Malangmeih and Rahaman, 2016; Meetei *et al.*, 2016; Malakar *et al.*, 2019). However, the crop is prone to various pests and diseases hampering its growth and development, inflicting adverse effect in its production and yield. Among the various devastating diseases causing serious hinderance, the association of phytoplasma with king chilli was first observed and reported by Dutta *et al.* (2022), and its increasing incidence now poses a significant threat to the successful cultivation of the crop. Phytoplasmas are mostly dependent on insect transmission for their spread and survival (Hogenhout *et al.*, 2008) and is also efficiently transmitted through dodder (Rao, 2021). Insects from the families Cicadellidae, Cixidae, Psyllidae, Delphacidae, and Derbidae are the vectors of phytoplasmas (Weintraub and Beanland, 2006). Seed transmission of phytoplasmas has been demonstrated under laboratory conditions in several plant species (Khan *et al.*, 2002; Calari *et al.*, 2011; Satta *et al.*, 2019). Given the economic and cultural importance of king chilli in Northeast India, this study was undertaken to determine whether such seed transmission also occurs in king chilli.

Materials and Methods

King chilli plants exhibiting phytoplasma disease symptoms during 2024 were collected from fields located in the districts of Jorhat, Sivasagar and Nagaon in Assam. These symptomatic plants were subsequently tested to confirm the presence and identity of phytoplasma. Seeds were then collected from fully ripened fruits of phytoplasma-positive king chilli plants as well as from healthy plants. After air-drying the seeds for one day, 80 seeds each from diseased and healthy lots were sown separately in two different pro-trays containing a standardized potting mixture, and the trays were maintained under protected insect proof conditions inside a net house. Germination percentages were recorded for both healthy and infected seed lots. At 35 days after germination, non-destructive sampling was performed, and DNA was

extracted. Total genomic DNA from the seedlings was isolated using the CTAB protocol (Kollar *et al.*, 1990) with some modifications. For each batch, extraction was conducted from 0.5 g of leaf tissue comprising equal portions of leaf veins and leaf lamina. Conventional PCR was performed to detect phytoplasma in the extracted DNA using the universal primer pair P1/P6 (Deng and Hiruki, 1991), followed by nested PCR using the primer pair R16F2n/R16R2 (Gundersen and Lee, 1996). The PCR reaction mixture consisted of 100 ng DNA template (1 μ l), 5 μ l of 2 \times Emerald master mix (Takara®), 0.5 μ l each of forward and reverse primers (10 pmol concentration), and 3 μ l sterile distilled water. The amplification program for conventional PCR included denaturation at 94°C for 45 sec, annealing at 54.9°C for 2 min, and extension at 72°C for 3 min for a total of 32 cycles, followed by a final extension at 72°C for 10 min. For nested PCR, the product from the conventional PCR was diluted in the ratio of 1:15 with sterile water, and 1 μ l of this dilution was used as the template. Nested PCR followed the same cycling conditions as the conventional assay, except that the annealing temperature was adjusted to 54.8°C for 2 min. All amplifications were conducted using a thermal cycler (Gene Amp® PCR System 9700, Applied Biosystems, USA). The resulting PCR products were separated on a 1% agarose gel alongside a 100 bp DNA ladder (HiMedia) and visualized using a gel documentation system (Gel Doc™ EZ Imager, Bio-Rad, USA). Positive samples were subsequently outsourced for sequencing.

Results and Discussion

A wide range of phytoplasma-associated symptoms were observed in king chilli plants collected from the field. The predominant symptoms included leaf size reduction, yellowing, stunting, and witches' broom. The phytoplasma detected in these plants was sequenced and deposited in GenBank under the accession number PV891971, where it showed 99.76% identity to '*Candidatus* Phytoplasma citri'. RFLP analysis of the amplified products placed the isolate within the 16SrII-D subgroup (data not shown).

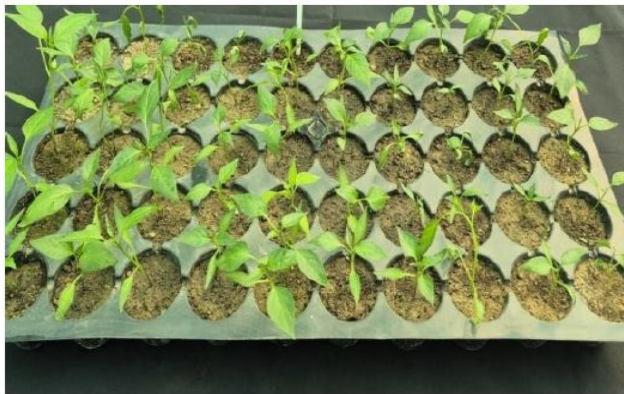
Seeds obtained from mother plants, that tested positive for the 16SrII-D ribosomal group exhibited 100% germination (Table-1, Figure-1). Among the 80 seedlings screened, five tested positive for phytoplasma, producing an amplicon of ~1.5 kb in direct PCR and ~1.25 kb in nested PCR (Figure-2). The seed-borne transmission efficiency was therefore estimated at 6.25 per cent. The PCR-positive samples have been sent for sequencing, and results are currently awaited.

The present study provides insights into the association of phytoplasma with king chilli (*Capsicum chinense* Jacq.) in Assam and its potential role in seed-borne transmission, an aspect that has remained largely unexplored for this economically and culturally important crop. Field-grown king chilli plants collected from Jorhat, Sivasagar, and Nagaon exhibited characteristic phytoplasma-associated symptoms such as leaf size reduction, yellowing, stunting, and witches' broom, consistent with the symptoms reported by Dutta *et al.*, 2022 in phytoplasma infected king chilli plants. These symptoms also align with the typical manifestations reported in other phytoplasma-infected crops (Hemmati *et al.*, 2021). A key focus of this study was the assessment of seed-borne transmission, a mode of transmission that is often debated in phytoplasma research due to the pathogen's restriction to phloem tissues and dependence on insect vectors (Hogenhout *et al.*, 2008). Interestingly, seeds collected from

diseased plants exhibited 100% germination, indicating that phytoplasma infection of the mother plant does not impair seed viability. These findings align with Mateeti *et al.* (2022), which confirmed seed transmission of phytoplasma in tomato, reporting a 85 per cent germination in seedlings positive for the 16SrX-B group and 74 per cent germination for the seedlings positive for the 16SrV group. Similar observations were also made by Zamharir and Shameli (2022) where they reported 1.8 per cent of seeds from infected soybean plants testing positive.

Table 1 : Transmission of phytoplasma in King chilli plants through dodder inoculation method

	No of seed taken	Seed germinated	Germination percentage	PCR positive plants	Disease transmission (%)
Healthy	80	80	100	-	-
Infected	80	80	100	5	6.25

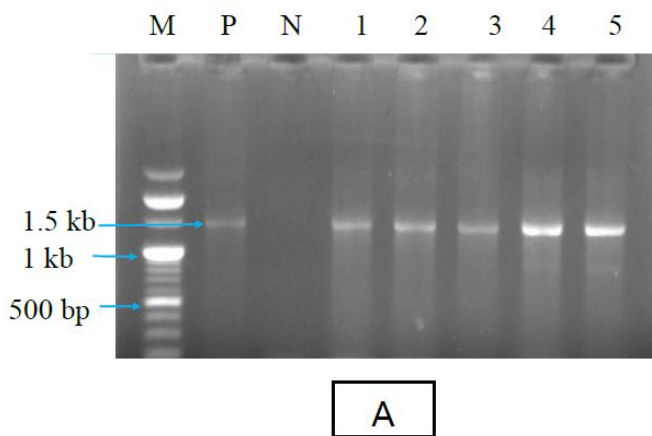


A

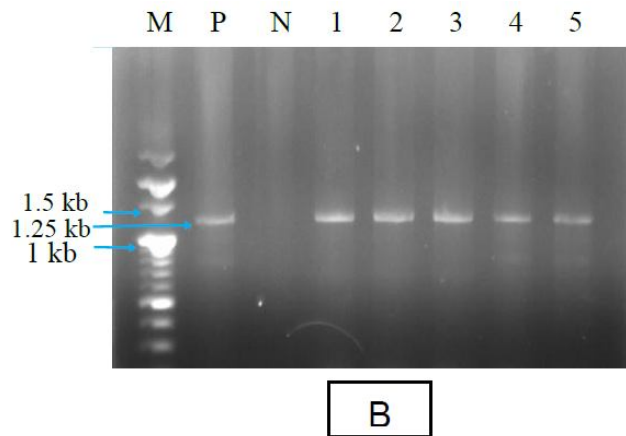


B

Fig. 1 : Germinated seedlings after 30 days of sowing : A) Seedlings from healthy seed; B) Seedlings from infected seeds



A



B

Fig. 2 : PCR amplification of 16S rRNA of phytoplasma from king chilli seedling after germination, Lane M. 100bp DNA ladder; Lane P. Positive control (Phytoplasma infected plant); Lane N. Negative control (Healthy plant); Lane 1-5. Seedlings germinated from seeds of phytoplasma infected king chilli plant. A) Direct PCR; B) Nested PCR

Conclusion

The present findings indicate that phytoplasmas associated with king chilli can be transmitted through

seeds, even though the transmission rate observed was relatively low. These observations indicate that although most seeds from phytoplasma-infected king

chilli plants remain viable and germinate successfully, the pathogen can still be propagated through seed and potentially spread over long distances, as the farmers most commonly collect the seeds from their fields for the next year crop. Even with a relatively low seed transmission rate (6.25%), the presence of competent insect vectors can readily amplify the pathogen and trigger disease outbreaks in nearby cultivated and natural ecosystems. Therefore, understanding the dynamics of phytoplasma seed transmission is critical for developing effective seed production and disease management strategies aimed at minimizing the impact of phytoplasma-associated disorders in culturally and economically important crops such as king chilli.

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