Parthenium phyllody in Ethiopia: Epidemiology and host range of phytoplasmas within important cultivated crops

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Fig. 1: *Parthenium hysterophorus*. Healthy plant (white arrow) and phyllody diseased plant (red arrow)

INTRODUCTION

Parthenium hysterophorus L. is an annual herb of the *Asteraceae* family originating from Central America. It was introduced to tropical regions worldwide in the 1950s and became a major weed in Ethiopia. Parthenium phyllody is an important disease of *P. hysterophorus* (up to 75% field incidence). Diseased plants are characterized by excessive branching, reduced plant height and leaf size, as well as modification of the inflorescences into leaf-like structures that lead to sterility (Fig. 1).

More than 700 plant diseases are associated with phytoplasmas. Phytoplasmas are transmitted by insect vectors of the order *Hemiptera*, mainly by leafhopper species (Family *Cicadellidae*). Furthermore, species within three other families of fulgorids (*Cixiidae*, *Delphacidae*, *Derbidae*, *Flatidae*) are confirmed as phytoplasma vectors as well as two psyllid species.

This study aims to determine the host range of the pathogen within agricultural crops cultivated in Ethiopia as well as to test whether Parthenium acts as a reservoir from which the pathogen can be transmitted to cultivated plants via insect vectors found in Ethiopia.

Fig. 5: left: Cages for transmission studies, each containing 2 healthy Parthenium plants and 10 leafhoppers after acquisition feeding on diseased Parthenium plants. right: Planthoppers collected from phyllody diseased *P. hysterophorus*

METHODS

Parthenium and cultivated plants showing phyllody symptoms (Fig. 2-4) were collected from locations heavily affected by the weed. After DNA extraction phytoplasma specific DNA fragments were amplified by polymerase chain reaction, PCR, (Parthenium, peanut, and sesame) or nested PCR (grass pea) respectively. The PCR products were further characterized by Restriction Fragment Length Polymorphism (RFLP) analysis. Amplified fragments were sequenced allowing species identification of the pathogens.

In order to characterize the potential risk of vector insects, planthoppers were captured from phyllody diseased Parthenium plants (Fig. 5), analysed for phytoplasma infection, and classified by morphological and molecular methods. Furthermore, transmission studies with leafhoppers of the species *Orosius cellulosus* Lindberg of the family *Cicadellidae* were carried out (Fig. 5).

Fig. 2: Healthy grass pea with fertile inflorescences (left) and infected plant with phyllody symptoms (right)

Fig. 3: Sesame seed pods of a haealthy sesame plant (left) and deformed, sere and empty seed pods of an infected plant (right)

Fig. 4: Infected peanut plant with leaves of normal size and shape (white arrow) and small chlorotic leaves (red arrow)

RESULTS

DNA fragments specific for phytoplasmas could be detected in *Parthenium hysterophorus* as well as in peanut (*Arachis hypogaea*), sesame (*Sesamum indicum*), and grass pea (*Lathyrus sativum*) (Fig. 6). After *AluI*-digestion of PCR-amplicons of Parthenium, sesame, peanut, and a *Vinca rosea* infected by faba bean phyllody (FBP positive control) showed identical restriction profiles (Fig. 7), indicating a close relationship to FBP of the Peanut witches´ broom group.

Comparison of rDNA sequences of P1/P7 amplicons revealed that phytoplasmas detected in Parthenium plants were also present in sesame and peanut. Sequence identities of 1488 bp of the 16S rDNA sequence were above 99%, covering strains infecting sesame and peanut in other countries. Ethiopian Parthenium, sesame and peanut phytoplasma exhibited sequence similarities of 98% to phytoplasmas within the 16SrII species group (Peanut witches´-broom group) including phytoplasmas originating from Ethiopian papaya, faba bean phyllody (FBP), and the reference species *Candidatus* Phytoplasma aurantifolia, causing witches´-broom disease of lime (Fig. 8).

The planthoppers collected from phyllody diseased Parthenium plants could be assigned to the genus *Hilda* of the family *Tettigometridae*. There were positive detections of phytoplasmas in almost every planthopper sample investigated. Because of the high similarity of the sequences from the 16S rDNA-gene, these phytoplasmas also belong to the phylogenetic clade 16Sr-II. Hence, members of *Tettigometridae* were described as potential vectors of phytoplasmas for the first time.

In transmission studies a successful acquisition of phytoplasmas by *O. cellulosus* was shown by means of positive detection of the pathogen in several probed leafhoppers. Furthermore, detection of phytoplasmas in a single bait plant suggests that this species is suitable for transmitting phytoplasmas. However, as the Parthenium plants used as baits developed no characteristic symptoms a successful transmission of phytoplasmas by *Hilda* sp. and *O. cellulosus* still has to be proven.

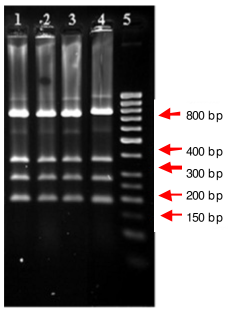

Fig. 7: PCR-RFLP-detection of phytoplasmas; *AluI*-digestion of PCR-Products:

1 = Sesame
2 = Parthenium
3 = Peanut
4 = positive control (FBP)
5 = 50 bp DNA-ladder, Fermentas

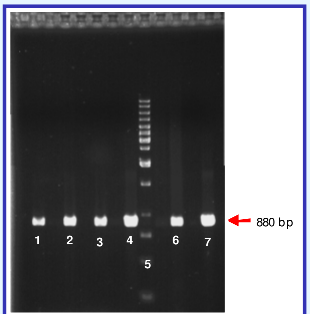

Fig. 6: PCR-Amplifications (samples 1-4) and nested PCR-Amplifications (samples 6,7) of healthy plants (left) and plants showing phyllody symptoms (right):

1 = Parthenium
2 = Peanut
3 = Sesame
4 = negative and positive control
5 = 1 kb-ladder, Fermentas
6 = Grass pea
7 = negative and positive control

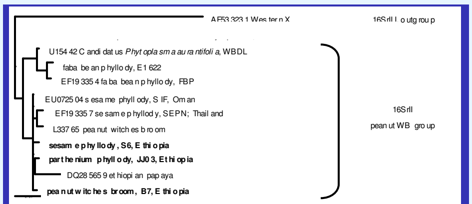

Fig. 8: Phylogenetic tree of 1488 bp long 16S rDNA phytoplasma sequences calculated by PHYLIP using neighbour-joining algorithm

CONCLUSION

Phytoplasma detected in Parthenium and Ethiopian crops are closely related and potential vector insects are native in Ethiopia. This suggests that Parthenium represents a pathogen reservoir for the phytoplasmas affecting agricultural crops in the country. Since phytoplasma infections can lead to sterility of the inflorescences, severe losses in yield of agricultural crops could be expected. Thus, control of Parthenium and putative vectors transmitting phyllody disease is important.

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