Studies on Phyllody in *Parthenium hysterophorus* and detection of phytoplasma within important crops cultivated in Ethiopia

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

INTRODUCTION

Parthenium hysterophorus is an annual weed that, due to its competitiveness and adaptability to different climatic and soil conditions, is widely spread in Australia, South Asia and parts of East Africa. It was introduced to Ethiopia in the 1980ies and became the major invasive weed in both arable and grazing lands. In Ethiopia a disease caused by phytoplasmas was commonly observed in Parthenium (up to 75% field incidence). Diseased plants are characterized by excessive branching, reduced plant height and leaf size, as well as modification of floral structures into leaf-like structures that lead to sterility (Fig. 1a,b,c).

More than 700 plant diseases are associated with phytoplasmas. Phyllody symptoms caused by phytoplasmas were already found on different crops, e.g. sunn hemp, lupin, field pie, soybean, and cowpea. This suggests that Parthenium phyllody also affects a wide range of legume species and other crops in Ethiopia.

METHODS

In order to test whether Parthenium plants harbour phytoplasma, which may also infect important agricultural crops in Ethiopia, weeds and cultivated plants showing phyllody symptoms were collected. *P. hysterophorus* as well as grass pea (*Lathyrus sativum*, Fig. 2) and sesame plants (*Sesamum indicum*, Fig. 3) showed extensive phyllody symptoms. Peanut plants (*Arachis hypogaea*, Fig. 4) with phyllody, witches´-broom (small chlorotic leaves, proliferating shoots, shortened internodes) as well as die-back symptoms were also included in the study.

Phytoplasma infection of plants was assessed by polymerase chain reaction (PCR). Specific fragments from Parthenium, peanut, and sesame were amplified by PCR using either the primers fU5/rU3 or P1/P7 (Fig.5). For the detection of specific DNA fragments in grass pea a nested PCR was carried out by subsequent application of the primer pairs. P1/P7 generated PCR products were further characterized by Restriction Fragment Length Polymorphism (RFLP) analysis. Amplified fragments were sequenced allowing species identification of the pathogens.

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

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

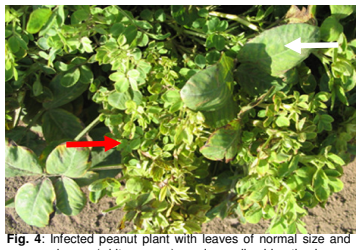

Fig. 4: Infected peanut plant with leaves of normal size and shape (white arrow) and small chlorotic leaves (red arrow)

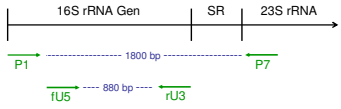

Fig. 5: Phytoplasma rRNA operon with 16S rRNA gene, 23S rRNA gene, and spacer region (SR).
The arrows indicate the position of the P1/P7 (size of 1800 bp) and fU5/rU3 primer (size of 880 bp).

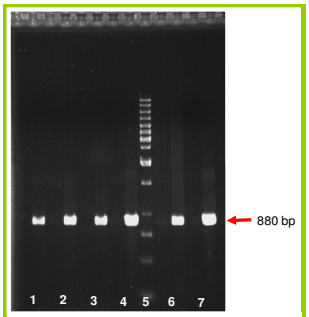

Fig. 6: PCR-Amplifications (fU5/rU3; samples 1-4) and nested PCR-Amplifications (P1/P7 and fU5/rU3; samples 6,7) of healthy plants (left) and plants showing phyllody sympomts (right):
1 = Parthenium
2 = Peanut
3 = Sesame
4 = negative and positve control
5 = 1 kb-ladder, Fermentas
6 = Grass pea
7 = negative and positive control

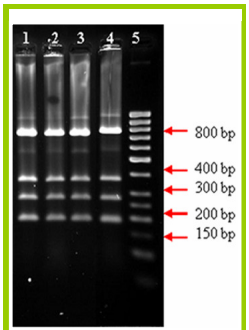

Fig. 7: PCR-RFLP-detection of phytoplasmas; *AluI*-digestion of PCR-Products (P1/P7):
1 = Sesame
2 = Parthenium
3 = Peanut
4 = positive control
5 = 50 bp DNA-ladder, Fermentas

RESULTS

DNA fragments specific for phytoplasma 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*-digesting the PCR-Amplicons (P1/P7) of Parthenium, sesame, peanut, and *Vinca rosea* infected with faba bean phyllody (FBP) serving as positive control have identical restriction profiles (Fig. 7), indicating a relationship between these phytoplasma strains.

First 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 a phytoplasma originating from Ethiopian papaya, faba bean phyllody (FBP), which serves as type-strain of the Peanut witches`-broom disease, and the reference species *Candidatus* Phytoplasma aurantifolia, causing witches´-broom disease of lime (Fig. 8).

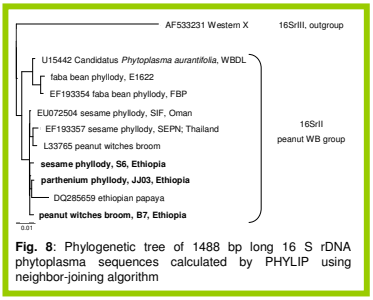

Fig. 8: Phylogenetic tree of 1488 bp long 16 S rDNA phytoplasma sequences calculated by PHYLIP using neighbor-joining algorithm

CONCLUSION

Phytoplasmas detected in Ethiopian crops are closely related, which 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 florescences, severe losses in yield of agricultural crops could be expected.

In order to evaluate the impact of diseases caused by phytoplasma, further investigations on weeds and important Ethiopian agricultural crops such as faba bean, lentil, chick pea, fenugreek, papaya, orange and tangerine have to be conducted.

ACKNOWLEDGEMENTS:

This research project was financially supported by Stiftung für tropische Agrarforschung, DAAD, BHGL, Verein der Freunde und Förderer der LGF der Humboldt Universität zu Berlin, Bayer CropScience and Syngenta.