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Identification and characterization of a phytoplasma disease of jute (*Corchorus olitorius* L.) from south-western Turkey

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ABSTRACT

A phytoplasma disease was detected in naturally occurring jute (*Corchorus olitorius* L.) plants in an experimental sesame field at the Akdeniz University campus, Antalya, Turkey during the growth periods of 2011 and 2012. Infected plants showed symptoms of phyllody, abnormal development of flowers, yellowing, reduced leaf size and short internodes. DNA extractions were made from symptomatic and symptomless plants' root and leaf tissues. Direct PCR assays with P1/P7 universal phytoplasma primers detected a 1.8 kb product and a 1.2 kb product was obtained using R16F2n/R16R2 primers with nested PCR. Phytoplasmas were detected from all symptomatic plants as well as off-season three dry leafless jute plants collected in 2012. DNA extracted from roots rather than leaf tissues showed better amplification in direct PCR assays. Three samples were cloned and their F2nR2 region was sequenced for identification. Jute phytoplasmas were clustered with the 16SrII (Peanut witches'-broom) group members in BLAST search. For subgroup characterization, virtual RFLP profiles were generated in *i*Phyclassifier program. As a result, phytoplasmas were identified as related strains of the 16Sr II-D reference strain, '*Candidatus* Phytoplasma australasia' (Y10097). Actual RFLP profiles obtained with *Alu*I, *Rsa*I, *Mse*I, *Taq*I enzymes, revealed a similar pattern with virtual RFLP confirming subgroup characterization. In our previous findings, phyllody phytoplasmas infecting sesame plants in this experimental field were classified in the 16SrII-D subgroup. This study shows that phytoplasmas infecting jute and sesame plants were identified in the same phytoplasma group and naturally grown jute plants could possibly serve as inoculum source for next year infections for phyllody disease in sesame. To our knowledge this is the first report of molecular detection of a phytoplasma disease infecting jute in Turkey.

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1. Introduction

Phytoplasmas are wall-less, phloem-limited, unculturable plant pathogenic bacteria that cause diseases in many plants worldwide (Lee et al., 2000; IRPCM, 2004). Insect vectors feeding from phloem sap mainly leafhoppers (Cicadellidae) and planthoppers (Fulgoromorpha) and psyllids (Psyllidae), play a major role in their transmission to plant hosts (Lee et al., 2000; Garnier et al., 2001; Weintraub and Beanland, 2006). In detection of phytoplasmal diseases, symptomatology, serology and microscopy techniques were employed in early detection methods (Salehi and Izadpanah, 1992; Kersting, 1993; Klein, 1997; Seemüller et al., 1998; Firrao et al., 2007). After development of molecular techniques, PCR-based methods were routinely used mostly based on the amplification of 16SrRNA genes with nested PCR with phytoplasma specific primers (Ahrens and Seemüller, 1992; Gundersen and Lee, 1996; Smart et al., 1996).

Typical symptoms induced by phytoplasmas include phyllody (change of flowers to leafy structures), virescence (greening of flower tissue), witches-broom appearance (proliferation of shoots), sterility, elongated or shortened internodes, and color change in leaves and shoots (Lee et al., 2000; Bertaccini and Duduk, 2009; Marcone, 2012; Cagirgan et al., 2013). Stage of plant growth during infection could affect symptom appearance, cause detrimental effectst on plant health in general, and cause economic loss in crop production (Lee et al., 2000).

Jute (*Corchorus olitorius* L.) is a fiber crop grown mainly in India and Bangladesh (Islam and Alauddin, 2012). In Turkey, jute plants occur naturally in the Mediterranean region and are considered as a

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Table 1
Jute samples collected from Akdeniz University campus field for DNA extraction and detection of phytoplasmas with direct and nested PCR.

No	Sample collection year	Sample/plant tissue[a]	Symptoms	Direct PCR P1/P7	Nested PCR R16F2n/R16R2
1	2011	J1R	Yes	Positive	Positive
2	2011	J1L	Yes	Weak positive	Positive
3	2011	J2R[b]	Yes	Positive	Positive
4	2011	J2L	Yes	Positive	Positive
5	2011	J3R	Yes	Positive	Positive
6	2011	J3L	Yes	Weak positive	Positive
7	2011	J4R	Yes	Positive	Positive
8	2011	J4L	Yes	Negative	Positive
9	2011	J5R	Yes	Positive	Positive
10	2011	J5L	Yes	Negative	Positive
11	2011	J6R	Yes	Positive	Positive
12	2011	J6L	Yes	Positive	Positive
13	2011	J7R	Yes	Positive	Positive
14	2011	J7L	Yes	Negative	Positive
15	2012	J1R	No	Negative	Negative
16	2012	J1L	No	Negative	Negative
17	2012	J2R	No	Negative	Negative
18	2012	J2L	No	Negative	Negative
19	2012	J3R	Yes	Weak positive	Positive
20	2012	J3L	Yes	Negative	Positive
21	2012	J4R	Yes	Positive	Positive
22	2012	J4L	Yes	Negative	Positive
23	2012	J5R	Yes	Positive	Positive
24	2012	J5L	Yes	Negative	Positive
25	2012	J6R	No	Negative	Negative
26	2012	J6L	No	Negative	Negative
27	2012	J7R	No	Negative	Negative
28	2012	J7L	No	Negative	Negative
29	2012	J8L[b]	Yes	Weak positive	Positive
30	2012	J9L[b]	Yes	Negative	Positive
31	2012	J10R	Unknown[c]	Positive	Positive
32	2012	J11R	Unknown	Positive	Positive
33	2012	J12R	Unknown	Positive	Positive

[a] J: Jute, R:Root, L:Leaf, DNA extracted separately from root and leaf tissues of each jute plant.
[b] These samples were cloned and sequenced for the F2nR2 region of the 16S ribosomal RNA gene.
[c] Unknown: J10R, J11R and J12R were dried leafless plants collected in the campus field on 01.02.2012.

weed rather than a fiber crop (Cagirgan et al., 2014). Symptoms of phyllody in jute were first reported in India by Rabindran et al. (1988). Recently, in Turkey, the disease was characterized based on symptomatology that infected plants had shortened internodes, increased number of leaves in reduced size, yellowing and more anthocyanin accumulation in leaves, and abnormal growth of ovary with phylloid ovules (Cagirgan et al., 2014). Recently Biswas et al. (2014) reported the presence of 16Sr V-C group phytoplasmas causing symptoms of little leaf and bunchy top appearance in jute.

During 2011 and 2012 in experimental sesame trials conducted at the Akdeniz University campus, Antalya, jute plants as weed were observed with phyllody-like symptoms: abnormally developed flowers, smaller leaves with short internodes, and occasional yellowing. Although presence of phyllody was reported by Cagirgan et al. (2014) based on symptomatology, detection and characterization of phytoplasmas infecting jute should be further investigated.

The objective of this study was, therefore, to identify and characterize the phytoplasmas from infected jute plants grown as weed in Turkey.

2. Materials and methods

2.1. Plant material and DNA extraction

Symptomatic and symptomless jute plants were collected from an experimental sesame field located at the Akdeniz University

Table 2
GenBank accession numbers and subgroup of phytoplasma strains used in construction of the phylogenetic tree.

Phytoplasma designation	Group-subgroup	Accession number	Reference
Jute phyllody JPhy1	16SrII-D	KM103728	This study
Jute phyllody JPhy2	16SrII-D	KM103729	This study
Jute phyllody JPhy3	16SrII-D	KM103730	This study
Sesame phyllody GPS16	16SrII-D	KF612969	Özdemir et al., 2013
Sesame phyllody GPS18	16SrII-D	KF612970	Özdemir et al., 2013
Sesame phyllody GPS10	16SrII-D	KF612966	Özdemir et al., 2013
Candidatus Phytoplasma australasia	16SrII-D	Y10097	White et al., 1998
Tomato big bud	16SrII-D	EF193359	Martini et al., 2007
Peanut witches'-broom	16SrII-A	L33765	Gundersen et al., 1994
Sesame phyllody	16SrII-A	EF193357	Martini et al., 2007
Faba bean phyllody	16SrII-C	X83432	Seemüller et al., 1998
Candidatus Phytoplasma aurantifolia	16SrII-B	U15442	Zreik et al., 1995
Lime witches'-broom strain LWB	16SrII-B	EF186828	Martini et al., 2007
Picris echiodes phyllody	16SrII-E	Y16393	Seemüller et al., 1998
Italian alfalfa witches'-broom	16SrII-E	EF193356	Martini et al., 2007
Acholeplasma laidlawii	–	M23932	Weisburg et al., 1989

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