

Disease Notes

First Report of Soybean Witches'-Broom Disease Caused by Group 16SrII Phytoplasma in Soybean in Malawi and Mozambique

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Soybean (*Glycine max* L.) is an important grain legume cultivated on approximately 1.24 million ha in Africa (1). Malawi ranks fourth in area of production in Africa, with 75,000 ha in 2009 (1). Soybean is also gaining importance in Mozambique and several other southern African countries due to diversification programs. During a field survey conducted in March 2010, soybean plants with phyllody and witches'-broom disorders typical of phytoplasma infection were observed in three of five fields surveyed in Lilongwe (Chitedze Research Station) and Salima (Channa, Chitala) districts in Malawi and three of four fields surveyed in Zambezia Province in Mozambique. Symptoms consisted of shoot proliferation, reduced leaflets, shortened internodes, proliferated auxiliary shoots producing witches'-brooms, virescence, and phyllody. Incidence of symptomatic plants was <1% in Malawi and 10 to 15% in Mozambique. Yield loss was 100% in affected plants. Five leaf samples each from symptomatic and asymptomatic plants were collected from six fields; total genomic DNAs were isolated and used as templates in PCR using phytoplasma-universal primer pair P1 and P7 for 16S-23S ribosomal RNA encoding region (3). PCR amplicons (1,709 bp) were produced from only templates derived from symptomatic plants. Amplicons from a symptomatic plant each from Malawi (Channa, Salima District) and Mozambique (Mutequelse, Zambezia Province) were directly sequenced in both directions and submitted to the GenBank (Accession Nos. HQ840717 and HQ845208). Nucleotide sequences of the two African soybean witches'-broom (SoyWB) phytoplasma strains were 100% identical. The virtual restriction fragment length polymorphism (RFLP) pattern derived from these sequences using *PhyClassifier* software (4) was similar to the reference pattern of the 16Sr group II, subgroup C (cactus phytoplasma, Accession No. AJ293216), with a pattern similarity coefficient of 0.99. A BLASTn search revealed that the African SoyWB phytoplasma sequences had a nucleotide sequence identity of 99% with those of soybean phytoplasma from Thailand (Accession No. EF193353), cactus phytoplasma from China (Accession No. EU099561), and several other members of 16SrII group. Phylogenetic analysis revealed the clustering of these strains with members of 16SrII group. In 1984, the occurrence of phyllody and witches'-broom symptoms in soybean in Mozambique was reported (2), however, no comprehensive details on the pathogen are available. To our knowledge, this is the first report of phyllody and witches'-broom disease in soybean in Malawi and the first molecular evidence of association of a 16SrII-C group '*Candidatus* phytoplasma' with the disease in Malawi and Mozambique. Phyllody and witches'-broom is a destructive disease, and its widespread occurrence can adversely affect soybean production in sub-Saharan Africa. Identification of alternative hosts and vector species would improve our understanding of the disease's epidemiology and contribute to development of appropriate tactics to prevent escalation of this problem into a major disease.

References: (1) FAOSTAT. <http://faostat.fao.org/site/567/default.aspx>. Retrieved 28 December 2010. (2) P. Plumb-Dhindsa and A. M. Mondjane. *Trop. Pest Manage.* 30:407, 1984. (3) L. B. Sharmila et al. *J. Plant Biochem. Biotech.* 13:1, 2004. (4) Y. Zhao et al. *Int. J. Syst. Evol. Microbiol.* 59:2582, 2009.

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Figure 1. Witches'-broom disease affected (left) and healthy (right) soybean plants.



Figure 2. Phylogenetic relationship of phytoplasma causing phyllody and witches'-broom disease in soybean in Malawi and Mozambique (highlighted) relative to various GenBank sequences inferred by the Neighbor-Joining method using MEGA4 software based on the Clustalw alignment of the 1709 base pairs of 16S - 23S ribosomal RNA encoding region. Bootstrap values (1,000 replications) are shown as percentages at the branch points. Branches corresponding to partitions reproduced in less than 50% bootstrap replicates are collapsed. GenBank accession number, host, disease and country are listed.

