

## *Brief Report*

### **NOVEL ISOLATE OF *Spiroplasma citri* FROM LEAFHOPPER *Circulifer haematoceps* FROM FARS PROVINCE**

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Strains of *Spiroplasma citri* have been classified into five groups based on electrophoretic mobility (EM) of spiralin protein and RFLP analysis of spiralin gene (Foissac *et al.* 1996). In the present study, two isolates of *S. citri* qualifying for formation of a sixth group are reported. Infection of sesame plants by *S. citri* has been reported previously (Salehi and Izadpanah 2002). Samples of vector (*Circulifer haematoceps*) leafhoppers (Salehi *et al.* 1993) were collected in various regions in Fars province of Iran. The insects were caged on periwinkle (*Catharanthus roseus*) plants. Presence of *S. citri* in collected plant samples and vector-inoculated plants was verified by ELISA using a locally produced polyclonal antiserum against a citrus isolate of *S. citri*. ELISA-positive samples were used to isolate *S. citri* in culture medium LD10 (Lee and Davis 1984). *S. citri* grown in culture was used to amplify spiralin gene by PCR using specific primer pair F<sup>1</sup>/R<sup>1</sup> (5'-TAATTTTAATAACATTTGCTT-3'/5'-GTTCTAAATAAGAAAAGTTT-3'). The amplified products were cloned in *E. coli* and sequenced. RFLP analysis showed that most isolates fell into group I of *S. citri* strains but two isolates originally obtained from *C. haematoceps* in a sesame field and propagated in periwinkle (*Catharanthus roseus*) did not match any of the five groups. The spiralin gene in both isolates (Acc. No. FJ755921 and FJ755922.) had only 93 percent homology with that of GII3 strain and could code for 244 amino acids (aa) instead of 241 aa in strains of groups 1-4 and 242 aa in a group 5 (Palmyre) strain. RFLP analysis showed that the leafhopper isolates had one additional *Mbo*I restriction site at nucleotide 456 plus changes in the position of certain other restriction sites. These results were confirmed by electrophoresis in polyacrylamide gel in which the spiralin protein from both insect isolates showed a different EM and banded at 28.8 kDa compared to 32 kDa for palmyre isolate and 24-28.5 reported for groups 1-4. By the current criteria (Foissac *et al.* 1996) the two new isolates qualify for forming a new group (group 6) of *S. citri* strains from Iran.