

## DISTRIBUTION OF CITRUS HUANGLONGBING DISEASE AND ITS VECTOR IN SOUTHERN IRAN\*

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### Abstract

Huanglongbing (HLB) has been previously reported in southern Iran. In the present study, additional information is given on the distribution of HLB agent and its vector. The psyllid *Diaphorina citri*, vector of 'Candidatus Liberibacter asiaticus' was found in all surveyed citrus growing areas of Sistan- Baluchistan, Hormozgan, Kerman (Jiroft and Kahnooj) and Fars (Darab and Lar) provinces. Using direct and nested PCR with specific primer pairs 103 out of 140 psyllid samples from Ghasre Ghand (Sistan- Baluchistan province), Minab and Roodan (Hormozgan province) were positive for Asian form of HLB. By the same PCR methods positive reactions were obtained from 22 Valencia and two local sweet orange and 1 mandarin trees from Nikshhar and Sarbaz (Sistan- Baluchistan province) and 16 Valencia sweet orange from Roodan and Senderk (Hormozgan province). Using PCR with designed primers a 909 bp segment of *opm* gene of two HLB isolates from Sistan- Baluchistan and Hormozgan was amplified, cloned and sequenced (Acc. No. HQ267229 and HQ267230, respectively). The two isolates showed 100% sequence identity and were identified as *Ca. L. asiaticus* but not *Ca. L. africanus*. Graft inoculation of grapefruit (*Citrus paradisi*) and Valencia sweet orange seedlings with scions from a symptomatic Valencia sweet orange from Sarbaz caused blotchy mottle, a specific symptom of HLB. *D. citri* is reported for the first time from Fars province. Based on the results of the present study HLB disease appears to be widely distributed in citrus growing regions of Sistan- Baluchistan and Hormozgan provinces.

**Keywords:** Citrus, Huanglongbing, Greening, Candidatus Liberibacter asiaticus, Asian citrus psyllid, Southern Iran.

See Persian text for figures and tables (Pages ۱۹۵-۲۰۸).

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## References

- BOVÉ, J. M. 2006. Huanglongbing: a destructive, newly-emerging, century-old disease of citrus. **J. Plant Pathol.** 88: 7-37.
- BOVÉ, J.M., CALAVAN, E.C., CAPOOR, S.P., CORTEZ, R.E. and SCHWARZ, R.E. 1974. Influence of temperature on symptoms of California stubborn, South African greening, Indian citrus decline and Philippines leaf mottling diseases. **Proc. 6<sup>th</sup> Conf. IOCV. Riverside, CA**, pp. 12-15,
- BOVE, J.M., DANET, J.L., BANANEJ, K., HASSANZADEH, N., TAGHIZADEH,M., SALEHI, M. and GARNIER, M. 2000. Witches' broom disease of lime in Iran. **Proc. 14<sup>th</sup> . Conf. IOCV. Riverside, CA**,pp. 207-212.
- CAPOOR, S.P., RAO, D.G. and VISWANATH, S.M. 1967. *Diaphorina citri* Kuway, a vector of the greening disease of citrus in India. **Ind. J. Agric. Sci.** 37: 572-576.
- CHEN, J., PU, X, DENG, X., LIU, S., PU, X., LI, H. and CIVEROLO, E. 2009. A phytoplasma related to *Candidatus* Phytoplasma asteris detected in citrus showing huanglongbing (yellow shoot disease) symptoms in Guangdong, P. R. China. **Phytopathology** 99: 236- 242 .
- COCHRAN, L. C. and SAMADI, M. 1976. Distribution of stubborn disease in Iran. **Proc. 7<sup>th</sup> Conf. IOCV. Riverside, CA**, pp. 10- 12.
- COLETTA-FIHO, H. D., TARGON, M. L. P. N.,TAKITA, M. A., De NEGRI, J. D., POMPEU, J. MACHADO, M. A., Do AMARAL, A. M., and MULLER,G. W. 2004. First report of the causal agent of huanglongbing (“*Candidatus* Liberibacter asiaticus”) in Brazil. **Plant Dis.** 88:1382.
- DAGRAÇA, J.V. 1991. Citrus greening disease. **Annu. Rev. Phytopathol.** 29: 109-36.
- DAGRAÇA, J.V. and KORSTEN, L. 2004. Citrus huanglongbing: review, present status and future strategies. Pp. 229-245. *In*: S.A.M.H. Naqvi (Ed.), **Diseases of Fruits and Vegetables**, Kluwer Academic Pub., Dordrecht, The Netherlands.
- DE BARRO, P. J., SHERRATT, T. N., BROOKES, C. P., DAVID, O. and MACLEAN, N.1995. Spatial and temporal variation in British field populations of the grain aphid *Sitobion avenae* (F.) (Hemiptera: Aphididae) studied using RAPD-PCR. **Proc. R. Soc. Lon. Ser-B.** 262:321-327.
- DING, F., WANG, G., YI, G., ZHONG, Y., ZENG, J. and ZHOU, B. 2005. Infection of wampl and lemon by the citrus huanglongbing pathogen (*Candidatrus* Liberibacter asiaticus) in China. **J. Plant Pathol.** 87: 207- 212.
- DING, F., YI, G. J. and WANG, G.P. 2004. Research on the PCR and Nested – PCR detection of citrus Huanglongbing pathogen. **Acta Hort. Sci.** 31: 803 – 806.
- FAGHIHI, M. M., SALEHI, M., BAGHERI, A. and IZADPANA, K. 2009. First report of citrus huanglongbing disease on orange in Iran. **Plant Pathol.** 58:793.
- GARNIER, M., DANEL, N. and BOVÉ, J.M. 1984. The greening organism is a Gram negative bacterium. **Proc. 9<sup>th</sup> Conf. IOCV, Riverside, CA**, pp.115-124.
- GARNIER, M. and BOVÉ, J.M. 1993. Citrus greening disease and the greening bacterium. **Proc. 12<sup>th</sup> Conf. IOCV, Riverside, CA**, pp. 212-219 .
- GARNIER, M. and BOVÉ, J.M. 1983. Transmission of the organism associated with citrus greening disease from sweet orange to periwinkle by dodder. **Phytopathology** 73: 1358-1363.
- GARNIER, M., GAO, S.J., HE, Y., VILLECHANOUX, S., GARDNER, J. and BOVÉ, J.M. 1991. Study of the greening organism (GO) with monoclonal antibodies: serological identification, morphology serotypes and purification of the GO. **Proc. 11<sup>th</sup> Conf. IOCV, Riverside, CA**, pp. 428-435.
- HALBERT, S.E. 2005. The discovery of huanglongbing in Florida. **Proc. 2<sup>nd</sup> Intl. Citrus Canker and Huanglongbing Res. Workshop**, Florida Citrus Mutual, Orlando, 2005, H - 3.
- HALBERT, S.E. and MANJUANTH, K.L. 2004. Asian citrus psyllids (Sternorrhycha: Psyllidae) and greening disease of citrus: a literature review and assessment of risk in Florida. **Florida Entomol.** 87: 330-53.
- HOLMES, D. S. and GUIGLEY, M. 1981. A rapid boiling method for the preparation of bacterial plasmids. **Ann. Biotech.**114:193-197.

- HUNG, T. H., HUNG, S. C., CHEN, C. N., HSU, M. H. and SU, H.J. 2004 Detection by PCR of *Candidatus Liberibacter asiaticus*, the bacterium causing citrus greening in vector psyllids: application to the study of vector-pathogen relationship. **Plant Pathol.** 53:96-102
- JAGOUEIX, S., BOVE J.M. and GARNIER, M. 1996. PCR detection of the two ‘*Candidatus*’ *Liberibacter* species associated with greening disease of citrus. **Mol. Cell. Probes** 10: 43-50.
- JAGOUEIX, S., BOVE, J. M., and GARNIER, M. 1994. The phloem- limited bacterium of greening disease of citrus is a member of the alphasubdivision of the *Proteobacteria*. **Intl. J. Syst. Bacteriol.** 44: 379-386.
- LAFLECHE, D. and BOVÉ, J.M., 1970. Structures de type mycoplasmedans les feuilles d’orangers atteints de la maladie du greening. **Comptes Rendus de l’Académie des Sciences, Paris**, 270: 1915-1917.
- LOPES, S.A., FRARE, G.F., BERTOLINI, E., CAMBRA, M., FERNANDES, N.G., AYRES, A.J., MARIN, D.R. and BOVÉ, J. M. 2009. *Liberibacter* associated with citrus huanglongbing in Brazil: *Candidatus Liberibacter asiaticus* is heat tolerant, *Candidatus Liberibacter americanus* is heat sensitive. **Plant Dis.** 93: 257- 262.
- LUIS PANTOJA, M., COLLAZO CORDERO, C., LLAUGER RIVERON, R., PEÑA BARZAGA, L. and LOPEZ HERNANDEZ, D. ET AL. 2008. Huanglongbing in Cuban Citriculture. Book of Programs and Abstracts, **11<sup>th</sup> Intl. Citrus Cong., Wuhan, China**, Pp. 50-51.
- MANJUNATH, K., HALBERT, S., RAMADUGU, C., WEBB, S. and LEE, R. 2008. Detection of *Candidatus Liberibacter asiaticus* in *Diaphorina citri* and its importance in the management of citrus huanglongbing in Florida. **Phytopathology** 98: 387-396.
- MCCLEAN, A.P.D. and OBERHOLZER, P.C.J. 1965b. Citrus psylla, a vector of the greening disease of sweet orange. **South Africa J. Agric. Sci.** 8: 297-298.
- MOHKAMI, A., SATARI, R., LORI, Z., EHSANI, A. and NAZEMI, A. 2011. First report of citrus huanglongbing in the Orzooiyeh region in Kerman province. **Iran. J. Plant Pathol.** 47: 29 (In Fasi With English Summary).
- PLANET, P., JAGOUEIX, S., BOVÉ, J.M. and GARNIER, M. 1995. Detection and characterization of the African citrus greening liberobacter by amplification, cloning and sequencing of the rplKAJL-rpoBC operon. **Current Microbiol.** 30: 137-141.
- RAHIMIAN, H. 1983. Distribution and symptoms of citrus stubborn disease in South East of Iran. **Proc. 7<sup>th</sup> Iran. Plant Protec. Cong., Karaj, Iran.** 70 (Abst.).
- SALEHI, M., HEYDARNEJAD J. and IZADPANA, K. 2005. Molecular characterization and grouping of 35 phytoplasmas from central and southern provinces in Iran. **Iran. J. Plant Pathol.** 41:62-65 (In Fasi With English Summary).
- SALEHI, M., IZADPANA, K. and RAHIMIAN, H. 1993. Citrus stubborn and its vector in the Fars province. **Proc. 11<sup>th</sup> Iran. Plant Protec. Cong., Rasht, Iran.** 230 (Abst.).
- SAMBROOK, J., FRITSH, E. F. and MANIATIS, T. 1989. **Molecular Cloning: A Laboratory Manual**, 2<sup>nd</sup> ed., Cold Spring Harbor Laboratory Press., Cold Spring Harbor, New York.
- TEXEIRA, D.C., AYRES, A.J., KITAJIMA, E.W., TANAKA, F.A.O., DANET, J.L., JAGOUEIX-EVEILLARD, S., SAILLARD, C. and BOVÉ, J.M. 2005a. First report of a Huanglongbing-like disease of citrus in Sao Paulo State, Brazil, and association of a new liberibacter species, “*Candidatus Liberibacter americanus*”, with the disease. **Plant Dis.** 89: 107.
- TEIXEIRA, D. C., DANET, J.L., EVEILLARD, S., MARTINS, E.C., DE JESUS JUNIOR, W.C, YAMAMOTO, P.T., LOPES, S.A., BASSANEZI, R.B., AYRES, A.J., SAILLARD, C. and BOVÉ, J.M. 2005b. Citrus huanglongbing in São Paulo State, Brazil: PCR detection of the “*Candidatus*” *Liberibacter* species associated with the disease. **Mol. Cell. Probes** 19: 173-179.
- TEIXEIRA, D. C., EVEILLARD, S., SIRANG-PUGNET, P., WULFF, N.A., SAILLARD, C., AYRES, A.J. and BOVÉ, J.M. 2008a. The *tufB-secE-nusG-rplKAJL-rpoB* gene cluster of the liberibacters: sequence comparisons, phylogeny and speciation. **Intl. J. Syst. Evol. Microbiol.** 58: 141-1421.
- TEIXEIRA, D. C., WULFF, N. A., MARTINS, E. C., KITAJIMA, E. W., BASSANEZI, R., AYRES, A. J., EVEILLARD, S., SAILLARD, C. and BOVÉ, J. M. 2008b. A phytoplasma closely related to the

- Pigeon pea witches' broom phytoplasma (16Sr IX) is associated with citrus huanglongbing symptoms in the State of São Paulo, Brazil. **Phytopathology** 98: 977-984.
- TEIXEIRA, D.C., LOPES, S.A., YAMAMOTO, P.T., EVEILLARD, S., MARTINS, E.C., DE JESUS JUNIOR, W.C., BASSANEZI, R.B., AYRES, A.J., DANET, J.L., SAILLARD, C. and BOVÉ, J.M. 2005c. PCR detection of the two liberibacter species associated with citrus huanglongbing (HLB) in São Paulo State, Brazil, **Proc. 16<sup>th</sup> Conf. IOCV, Riverside, CA**, pp. 432-438.
- TEIXEIRA, D.C., SAILLARD, C., EVEILLARD S., DANET J.L., AYRES, A.J. and BOVÉ, J.M. 2005d. '*Candidatus Liberibacter americanus*', associated with citrus huanglongbing (greening disease) in São Paulo State, Brazil. **Intl. J. Syst. Evol. Microbiol.** 55: 1857-1862.
- VAN VUUREN S. P. 1993. Variable transmission of African greening to sweet orange. **Proc. 12<sup>th</sup> Conf. IOCV, Riverside, CA**. Pp. 264-268.
- VILLECHANOUX, S., GARNIER, M., LAIGRET, F., RENAUDIN, J. and BOVE, J.M. 1993. The genome of the non-cultured, bacterial-like organism associated with citrus greening disease contains the nusG-rplKJL-rpoBC gene cluster and the gene for a bacteriophage type DNA polymerase. **Current Microbiol.** 26: 161-166.
- VILLECHANOUX, S., GARNIER, M., RENAUDIN, J. and BOVÉ, J.M. 1992. Detection of several strains of the bacterium-like organism of citrus greening disease by DNA probes. **Current Microbiol.** 24: 89-95.
- YI, G. J., HUO, H. Q., CAI, C. C., QIU, Y. P. HUANG, Z. R. and CHEN, D. C. 1999. DNA isolation procedure for AFLP amplification reaction of Litchi. **J. South China Agric. Univ.** 20: 123 - 124.
- ZHOU, L.J., GABRIEL, D. W., DUAN, Y. P., HALBERT, S. E. and DIXON, W. N. 2007. First report of dodder transmission of huanglongbing from naturally infected *Murraya paniculata* to citrus. **Plant Dis.** 91: 227.
- ZHOU, L.J., DUAN, Y., GABRIEL, D. and GOTTWALD, T.R. 2008. Seed transmission of *Candidatus Liberibacter asiaticus* in periwinkle and dodder resulted in low bacterial titer and very mild disease in periwinkle. **Phytopathology** 98: S181.