

Sanitation of carrot seeds infected by *Ca. Liberibacter solanacearum* through a thermal treatment and assesment of its efficacy by a viability qPCR protocol

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Candidatus *Liberibacter solanacearum* is a non culturable bacterium that may affect several important crop species, including host plants belonging to the *Solanaceae* and *Apiaceae* families[1]. In carrots, celery and other *Apiaceae* this pathogen is seed-borne[2], though its transmissibility is currently a matter of debate[3]. In order to ensure an excellent seed quality, we implemented a heat treatment protocol in order to ensure freedom of viable germs possibly present in carrot seeds. Since a qPCR protocol is not able to discriminate viable from dead bacteria infecting any plant matrix, and cultural methods are not feasible either, we implemented a viability qPCR protocol, where seed extracts are treated with monoazides (PMA or EMA). Through the development of a calibration curve, we were able to assess that the naturally infected seed lots used in the experimets harboured bacterial germs in the order of 10^6 cells/g of seed. Our results showed that a thermal treatment at 50°C for 72 hours was able to sanitise carrot seeds: this was confirmed by the viability qPCR detection method that always gave negative results to the analysis. A further confirmation of seed sanitation was obtained by sowing the sanitised seeds under controlled conditions: such seeds were allowed to germinate and produce seedlings. Analysis of seedlings was negative for the presence of *Ca. Liberibacter solanacearum* 30, 60 and 90 days after sowing. Finally, seed quality assays done on thermo-treated seeds confirmed that their viability and their ability to develop strong seedlings did not differ from that of untreated seeds.

[1] Munyaneza J.E. *et al.*, 2010. *Plant Dis.* 94, 639–639

[2] Bertolini E. *et al.*, 2014. *Plant Pathol.* 64, 276–285

[3] Loiseau M., *et al.*, 2017. *Plant Dis.* 101(12), 2104–2109