



## The two most common endophytic bacteria hampering date palm large scale propagation: identification and eventual relationship with the Brittle Leaf Disease

L. FKI<sup>1</sup>, O. CHKIR<sup>1</sup>, M.A. TRIKI<sup>2</sup>, N. BOUAZIZ<sup>1</sup>, and N. DRIRA<sup>1</sup>

<sup>1</sup>Laboratory of Plant Biotechnology, Faculty of Sciences of Sfax- Route Sokra BP 1171, 3000 Sfax, Tunisia,

University of Sfax, Tunisia, <sup>2</sup>Olive Tree institute, Route Elmatar, Sfax, Tunisia



5th International DPG-Berlin-Symposium



In the last three decades, *in vitro* technology has been widely applied for the large-scale propagation of plants since benefits arising from this technology are numerous (Figs 1 and 2). *In vitro* plants are generally true-to-type and pathogen-free. Moreover, *in vitro* highly regenerable tissues can be considered as being the most suitable plant material to create cryobanks and to carry out gene transfer experimentations. However, several constraints may hamper the regeneration of *in vitro* plants such as the recalcitrance and/or the genetic instability of some species or cultivars; and in some cases the difficulty to establish clean *in vitro* tissue cultures.

This study aimed: (1) to identify the most fastidious endophytic bacterial strains affecting date palm *in vitro* tissue culture, (2) to determine if populations of indigenous bacterial endophytes in tissue cultures are influenced by host genotype and disease such as the brittle leaf disease, (3) to determine their toxicity against plantlets and *in vitro* cultures which are in proliferation phase, and (4) to find the factors that must be taken into consideration to establish a clean tissue cultures.



Fig. 1. Date palm micropropagation via somatic embryogenesis



Fig. 2. Date palm micropropagation via caulogenesis

Two strains of endophytic bacteria hampering date palm micropropagation were isolated from two cultivars ('Deglet Nour' and 'Barhee'). The isolates, which were orange-yellow and white of colors, were identified by API galleries as *Photobacterium damsela* and *Yersinia ruckeri*, respectively (Fig. 3A).



Fig. 3. The two most common endophytic bacteria hampering date palm micropropagation

Significant differences in bacterial population densities were observed in the cultures of the two genotypes and in cultures established from healthy and Brittle Leaf Disease-affected plants. Indeed, the percentage of contaminated tissue in 3-year-old *in vitro* tissue culture established from 'Deglet Nour' and 'Barhee' were 15 % and 30 %, respectively. Moreover, we found that percentage of contaminated tissue in 3-year-old *in vitro* tissue cultures established from healthy and BLD-affected palms *cv.* Deglet Nour were 15% and 2%, respectively.

Embryogenic and organogenic cultures were seriously damaged by these fastidious bacteria. They slowly turned yellow and brown and then died within three months (Fig. 3B). On the other hand, the two bacteria were not toxic for *in vitro* plants as growth rates were similar in *in vitro* plant growing with and without bacteria (Fig. 3C). In our study we show that only juvenile explants are able to produce bacteria free tissue cultures.