BSL 2: Endophytes in commercial micropropagation - friend or foe?

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Abstract

Medicinal and aromatic plants are superorganisms like all plant species- naturally colonized by bacteria, fungi and protists. Micropropagated plants are facing different challenges under *in vitro* and *ex vitro* conditions: Mixotrophic growth under low light conditions on artificial nutrient media, poor gas exchange in small vessels, abiotic stress, bad rooting, transplanting stress, low survival rate during acclimatization in greenhouse. The use of endophytes in micropropagation can improve plant growth, yield, and health and induce tolerance to abiotic and biotic stress. A tool for the use of competent endophytes in micropropagation under *in vitro* and *ex vitro* conditions is "biotization" of plantlets with useful bacterial and fungal inocula. Fungal inocula which are used commercially are e.g. arbuscular mycorrhizal fungi) in form of spores and extraradical mycelium on different carrier materials like expanded clay, vermiculite, sand or peat. Furthermore representatives of the root fungal genus *Trichoderma* are applied as spores formulated in powder. Plant-growth promoting rhizobacteria of the important genera *Bacillus*, *Pseudomonas*, *Azospirillum* and *Azotobacter* in form of lyophilised endospores/bacterial cells in powder or liquid formulation are also available on the market.

Keywords: Endophytes, rhizobacteria, mycorrhiza, inoculation, micropropagation

Introduction

Herman (1996a; 1996b) was one of the first to think on using those microorganisms as stress eleviating factor. In 1998 Jerzy Nowak (Nowak, 1998) reviewed the benefits of *in vitro* "biotization" of plant tissue cultures with microbial inoculants, but the methods hardly found application in commercial tissue culture. First reports of microorganisms living in micropropagated plants (medicinal plant Grotkass et al., 2000, reviewed by Cassels, 1991 and Leifert et al., 1994) were seen as problem-causing inhabitants and contaminants. Evolving imaging and molecular techniques (-omics technologies) helped to discover the properties of endophytes, phytopathogens and other microorganisms from plant and soil habitats and will allow us to better understand mutualism and pathogenicity, culminating in Hardoim et al. (2015), who give ecological and evolutionary considerations for defining functioning of microbial endophytes.

But there are still challenges. It is not easy to discriminate between endo- and epiphytes, technically and scientifically. More than assumed 80 % of endophytes are ‚un-culturable', which only means that there still isn't enough knowledge on necessary culture conditions. Reports on tripartite systems, e.g. mycoviruses in fungal endophytes regulating the hypervirulence of pathogenic fungi (Ahn and Lee, 2001) or the interkingdom transfer of the acne causing agent from human to grapevine (Campisano et al., 2014), make the systems even more complex, thus difficult to understand - but understanding is the key for commercial use as commercial use of endophytes must be safe, predictable and cost-effective.

Results

Various fungi are used in micropropagation, such as arbuscular mycorrhizal fungi (Vestberg et al., 2004, Kapoor et al. 2008), ectomycorrhizal fungi (for review see Rai, 2001) and ericoid mycorrhizal fungi (Jansa and Vosatka, 2000), *Beauveria bassiana* (Akello et al., 2007), *Piriformospora indica* and other members of the Sebacinales (Sharma et al., 2014), *Fusarium oxysporum* (Ting et al., 2008), *Ophistoma* similar fungal species (Mucciarelli et al., 2003), *Phialocephala fortinii* (Vohnik et al., 2003) and *Trichoderma harzianum* (and other *Trichoderma* species) (Franken, 2012, Vestberg et al., 2004).

Examples for bacteria are *Acetobacter diazotrophicus* (AZLIN et al., 2007), *Achromobacter xylosoxidans* (BENSON et al., 2014), *Azospirillum brasilense* (CREUS et al., 1997; LARABURU and LLORENTE, 2015a), *Azotobacter chroococcum* (LARRABURU et al., 2007), *Bacillus subtilis* (WILHELM et al., 1997; VESTBERG et al., 2004), *B. megaterium* (TRIVEDI and PANDEY, 2007), *Burkholderia phytofirmans* (AIT BARKA et al., 2000), *B. vietnamiensis* (GOVINDARAJAN et al., 2006), *Enterobacter* sp. (MIRZA et al., 2001), *Klebsiella variicola* (WEI et al., 2014), *Microbacterium* sp.(QUAMBUSCH et al., 2014), *Pseudomonas fluorescens* (VESTBERG et al., 2004; THOMAS et al., 2010) and *P. putida* (LIFSHITZ et al., 1987).

Increased plant biomass was reported (shoot and root fresh weight and dry weight, plant height, leaf area, rhizom weight) (RAI et al., 2001; KAPOOR et al., 2008), ‚better' rooting in vitro (number, length) (LARRABURU and LLORENTE, 2015b), ‚better' acclimatization (survival rate, plant performance) (DUFFY et al., 1999; OVANDO-MEDINA et al., 2007), earlier flowering and increased flower number (VARMA and SCHUEPP, 1995), induction of stress resistance (NOWAK and SHULAEV, 2003), biocontrol effects (AIT BARKA et al., 2000, HARISH et al., 2008). Also, altered secondary metabolite profiles were reported (ZABETAKIS, 1997).

In the presentation, more information will be given on how microorganisms can be isolated, identified, cultured and inoculated. Furthermore, methods for characterization of endophytic microbes will be shown, esp. bacteria by different biochemical properties in order to determine a possible plant growth promotion potential especially for medicinal and aromatic plants.

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