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Biological Hardening - A New Approach to Enhance Resistance against Biotic and Abiotic Stresses in Micropropagated Plants

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ABSTRACT

Micropropagated plantlets are physiologically different from normal plants showing reduced photosynthetic activity, lower wax deposits, poorly functioning stomata, under developed root system and very few leaf and root hairs. These problems can be significantly overcome by inoculating beneficial microorganisms into micropropagated plantlets. In addition, the beneficial microorganisms protect the micropropagated plantlets from varied biotic and abiotic stresses such as saline, drought and flooding. Recently biological hardening (biopriming) is associated with the induction of resistance in tissue culture propagules using beneficial microorganisms against biotic and abiotic stresses upon transplanting and during early growth after transplanting. Among the different beneficial microbes, use of plant growth promoting rhizobacteria (PGPR) in plant nurseries have advantage in accelerating the production process by minimizing the time required for lignification of micropropagated plantlets. Research findings from several laboratories demonstrated the bacteria mediated improvement in host physiology and their studies indicated the sustainability of microbes and their utilities in micropropagated plantlets especially for banana (*Musa* spp) even after transplanting into field conditions.

Keywords: biopriming, biotic and abiotic stress tolerance, rhizosphere and endophytic bacteria, tissue culture plantlets

CONTENTS

INTRODUCTION

Plants in their natural environment are colonized by both external and internal microorganisms. Some microorganisms, particularly beneficial bacteria and fungi, can improve plant performance under stress environments and consequently enhance yield (Lazarovits and Nowak 1997; Creus *et al*. 1998; Kavino *et al*. 2008). Plants inoculated by microorganisms develop systemic resistance (systemic acquired resistance, SAR, or induced systemic resistance, ISR) and/or benefit from their antagonistic abilities towards pathogens (cross protection) (Ramamoorthy *et al*. 2001; Walters *et al*. 2005). Although, the inoculation of seeds with beneficial microorganisms has been practiced for more than 50 years, the inoculation of tissue culture propagules to enhance plant performance is relatively new (Nowak and Shulaev 2003). Plant tissue culture is based on axenic (contaminant-free) culture systems. Hence, endophytic pathogenic microorganisms are treated as problem causing contaminants, and various procedures have been developed to eliminate them. Recently, microbial inoculants, such as bacterial and mycorrhizal, have been evaluated as propagule priming agents both as *in vitro* co-cultures and on transplanting (Nowak and Shulaev 2003; Weber *et al*. 2007).

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Invited Review

Upon exposure to stress, the pre-sensitized or primed plant adapt better and faster than non-primed plants (Conrath *et al*. 2002). The organisms under most scrutiny for potential use in agriculture and horticulture are beneficial bacteria belonging to the genera *Pseudomonas* and *Bacillus* (Powell and Rhodes 1994; Choudhary and Johri 2009; Lugtenberg and Kamilova 2009). Similarly, the use of plant growth-promoting bacteria for biocontrol of plant diseases and the principles and mechanisms of action involved in the management of plant diseases are discussed in detail by Compant *et al*. (2005). This use of microbial inoculants, primarily bacteria as propagule priming agents both as *in vitro* co-cultures and on transplanting (Nowak and Pruski 2002), often referred as "biopriming", is an emerging trend in biotechnology aimed at reducing chemical input in plant production, while increasing plant fitness, productivity and their resistance against pest and diseases, in the context of sustainable horticulture (Conrath *et al*. 2006). In this review, the main emphasis has been given on the biohardening of tissue culture plants using beneficial microbes and their utility in horticultural cropping system.

BIOPRIMING FOR GROWTH AND DEVELOPMENT OF PLANTS

PGPR has both indirect and direct impact on plant growth and development (Solano *et al*. 2008; Walters and Fountaine 2009). The various effects of beneficial microbes on crop plants and their method of inoculation have been given in **Table 1**. The indirect promotion of plant growth occurs when beneficial bacteria prevent some of the deleterious effects of a phytopathogenic organism by one or more mechanisms (Raaijmakers *et al*. 2009; Wang *et al.* 2009). On the other hand, the direct promotion of plant growth by PGPR generally entails providing the plant with a compound that is synthesized by the bacterium or facilitating the uptake of nutrients from the environment (Glick 1995; Glick *et al*. 1999; Dubuis *et al*. 2007; Adesemoye *et al.* 2009). Plant growth benefits due to the addition of PGPR include increase in germination rate, root growth, leaf area, chlorophyll content, magnesium, nitrogen and protein content, hydraulic activity, tolerance to drought and salt stress, shoot and root weights and delayed leaf senescence which ultimately enhanced the yield of crop plants (Lucy *et al.* 2004; van Loon 2007).

Micropropagated plants are now utilized as an integral component of the on going eradication and rehabilitation program in the developing countries as a control approach to viral diseases, which are commonly spread through propagative materials as well as to get higher yield. Unfortunately, tissue culture plantlets are more susceptible to pest and disease all over the world. In this context, biopriming mediates the metabolic response of *in vitro* grown plant material to microbial inoculants, leading to the developmental and physiological changes, enhancing biotic and abiotic stress resistance of the derived propagules (Nowak 1998; Nakkeeran *et al*. 2005; Bernal *et al*. 2008; Harish *et al*. 2009a). Tissue culture techniques provide an opportunity for the introduction of nitrogen fixing endophytes into clonally propagated plants for sustainable production systems (Reis *et al*. 1999). These microorganisms can offer during the *in vitro* culture and also in the acclimatization phase, a potentially efficient method to improve vigor and adaptation of plantlets for transplanting (Nowak 1998).

The use of plant growth promoting rhizobacteria (PGPR) in plant nurseries has the advantage of accelerating the production process by minimizing the time required for the lignifications of plantlets with the purpose of obtaining hardened plants which is essential for their future development after transplant into the field (Caesar and Burr 1987; Ramamoorthy *et al*. 2002a). Potato, tomato, pepper, and other vegetable nodal explants in dual cultures with a *Pseudomonas* sp. strain PsJN showed significant growth stimulation under sterile tissue culture conditions and during early growth after transplanting (Nowak *et al*. 1995; Bharathi *et al*. 2004). Inoculated plants of potato were taller with more nodes, higher dry matter content, better developed root systems, more leaf hairs, increased amounts of chlorophyll and starch and were more lignified (Frommel *et al*. 1991). Non inoculated plantlets desiccated rapidly when removed from tissue culture conditions, whereas bacterized plants remain turgid because they had functional stomata and could regulate water loss (Frommel *et al*. 1991). Soil less transplant media amended with a formulation of PGPR designated LS 213 has been shown to improve plant vigour, reduce disease severity and increase yield of tomato, pepper (Kokalis-Burelle *et al*. 2002, 2006), muskmelon and watermelon (Kokalis-Burelle *et al*. 2003) in Florida. Strawberry cv. 'Camarosa' transplant plugs amended with LS 213 (PGPR formulation) resulted in a greater enhancement of growth and yield (Kokalis-Burelle 2003). In *Prunus* rootstocks, *Pseudomonas* strains could promote the growth of rootstocks when applied to the potting mix under greenhouse conditions (Bonaterra *et al*. 2003). Shoot growth increase upon treatment with *B. subtilis* strain EBW4 were reported in apple trees (Utkhede and Smith 1992). Inoculation of efficient bacterial strains in micropropagated pineapple plantlets before transplanting increased the shoot and root dry weight and leaf area (Mello *et al*. 2000). Bacterial suspension of *Bacillus* sp. when applied at the beginning of the weaning phase in banana cv. 'Grand Naine' (AAA) significantly improved the banana growth and development and foliar mineral contents (Vega *et al*. 2004). Bacterized potato plantlets were greener, had elevated levels of cytokinins, PAL, and free phenolics (Nowak *et al*. 1997). Micropropagated banana plantlets which were immersed in bacterial mixtures during planting significantly improved the growth characters (Albuquerque *et al*. 2003). Ryu *et al*. (2003) reported that treatment of tomato transplants by a biological preparation containing industrial formulated spores of *Bacillus subtilis* GB 03, *B. amyloliquefaciens* IN 937a and a chitosan significantly increased the growth of tomato transplants irrespective of the concentrations or potting medium used compared to the carrier and a non treated control. The use of bacterial strains in combination with IBA applications significantly increased the rooting of cuttings sour cherry (Esitken *et al*. 2003) and hazelnut (Bassil *et al.* 1991).

The mechanisms involved in growth promotion are increased production of auxin, gibberellin, cytokinin, ethylene (Kloepper and Schroth 1981; García de Salamone *et al.* 2001; Bottini *et al*. 2004; Glick *et al.* 2007; Remans *et al.* 2008; Ortíz-Castro *et al*. 2009), the solubilization of phosphorus and oxidation of sulfur, increase in nitrate availability, the extra cellular production of antibiotics (Whipps 2001), lytic enzymes, hydrocyanic acid, increase in root permeability, strict competition for the available nutrients and root sites (Enebak and Carey 2000), symbiotic N_2 fixation, mobilization of insoluble nutrients (Subba Rao 1982) and volatile components (Ryu *et al*. 2004). Some bacteria solubilize organic phosphate by secreting phosphatase or inorganic phosphate from soil particles by releasing organic acids and this could make phosphorus as well as micronutrients more readily available for plant growth in some soils (Kloepper *et al*. 1991). In potato plantlets grown *in vitro*, strain PsJN increased cytokinin content by inducing synthesis in the early stages of plant growth and development (Lazarovits and Nowak 1997). Thus, it appears that rhizobacteria also affect hormone metabolism and reactivity within the plant itself.

PHYSIOLOGICAL RESPONSE OF MICROPROPAGATED PLANTLETS

Biopriming for abiotic stress tolerance in plants

Upon exposure to stress, the pre-sensitized or primed plants adapt better and faster than the non-primed plants (Goellner and Conrath 2008) and rhizosphere bacteria have also been found to help plants tolerate abiotic stresses (Liddycoat *et al*.

Table 1 Beneficial microorganisms used as inoculants in various plantlets and its significance on plant characters.

Bio control agents	**Crop (micropropagated)**	**Method of inoculation**	**Significance**	**Reference**
Bacillus sp. and *Pseudomonas corrugata*	Tea	*Ex vitro*	Improving the survival rate of seedlings	Pandey *et al.* 2000
Enterobacter sp.	Sugarcane	*Ex vitro*	Growth promotion	Mirza *et al.* 2001
Burkholderia vietnamiensis	Sugarcane	*In vitro* co culture	Improving the growth and yield	Govindarajan *et al.* 2006
Pseudomonas putida, *Pseudomonas fluorescens*	Sugarcane	*Ex vitro*	Growth promotion	Mehnaz *et al*. 2009
Fungal endophyte (*Sordariomycete* sp.)	Peppermint	*In vitro* & *In vivo*	Growth promotion	Mucciarelli *et al.* 2003
Ericoid mycorrhiza (*Oidiodendron* sp.)	Rhododendrons	*In vitro* & Post vitro	Growth promotion	Jansa and Vosatka 2000
Glomus mosseae, *Bacillus coagulans* and *Trichoderma harzianum*	*Ficus benjamina*	*Ex vitro*	Growth promotion	Srinath *et al*. 2003
Arbuscular mycorrhizal fungi (*Glomus* sp.)	*Capsicum annum*	Acclimatization and post acclimatization	Improving the physiological traits	Estrada-Luna and Davies 2003
Bacillus megaterium, *B. subtilis* and *Pseudomonas corrugata* as individual	*Picrorhiza kurrooa*	Acclimatization	Growth promotion	Trivedi and Pandey 2007
Pseudomonas sp. PsJN	Tomato	Root dipping	Growth promotion	Pillay and Nowak 1997
Pseudomonas fluorescens and *Pantoea agglomerans*	*Prunus* rootstock	Application through irrigation (liquid)	Growth promotion	Bonaterra *et al.* 2003
Trichoderma harzianum, *Glomus catenulatum* and *Bacillus subtilis*	Strawberry	Applied at weaning stage	Growth promotion and disease control	Vestberg *et al.* 2004
Arbuscular mycorrhizal fungi (*Glomus* sp.) and *Pseudomonas putida*	Strawberry	Co inoculation	Growth promotion	Vosatka *et al.* 1992
Glomus fasciculatum	Avocado	Applied at hardening stage	Growth promotion	Vidal *et al.* 1992
Pseudomonas sp. PsJN	Watermelon and cantaloupe	*In vitro*	Growth promotion	Liu *et al*. 1995
Pseudomonas sp. PsJN	Grape	*In vitro*	Growth promotion and disease control	Barka *et al.* 2000
Pseudomonas sp. PsJN	Grape	*In vitro*	Growth promotion and disease control	Barka *et al.* 2002
Pseudomonas sp. PsJN	Potato	*In vitro* co culture	Growth promotion	Frommel *et al.* 1991
Pseudomonas sp. PsJN	Potato	*In vitro*	Growth promotion	Nowak *et al*. 1995
Pseudomonas sp. PsJN	Potato	*In vitro*	Growth promotion and disease control	Nowak 1998
Pseudomonas fluorescens	Potato	*In vitro*	Growth promotion	Duffy *et al.* 1999
*Burkholderia sp.*strain PsJN	Tomato, cucumber and sweet pepper	*In vitro* co-culture	Enhancing the transplant performance	Nowak *et al.* 2004
Fusarium oxysporum strain V5w2 (fungal endophyte)	Banana	Applied at hardening stage	Pest control (*Cosmopolites sordidus* and *Radopholus similis*)	Dubois *et al.* 2004
Bacillus sp. strain INR7,T4 & IN937b	Banana	Applied at hardening stage	Growth promotion	Vega *et al.* 2004
Glomus manihotis and *Bacillus* sp. strain INR7, T4 and IN937b	Banana	Applied at acclimatization stage	Growth promotion and nutrition	Rodríguez-Romero *et al.* 2005
Streptomyces violaceusniger strain g10	Banana	Applied at acclimatization stage	Disease control (*Fusarium* wilt)	Getha *et al*. 2005
Beauveria bassiana	Banana	Applied at acclimatization stage	Pest control (*Cosmopolites sordidus*)	Akello *et al*. 2007
Burkholderia spp. and *Herbaspirillum* spp.	Banana	Applied at acclimatization stage	Disease control (*Fusarium* wilt)	Weber *et al*. 2007
Fusarium oxysporum strain V5w2 and III4w1	Banana	Applied at acclimatization stage	Pest control (*Cosmopolites sordidus* and *Radopholus similis*)	Paparu *et al.* 2007
Serratia sp. strain UPM39B3 and *Fusarium oxysporum* strain UPM31P1	Banana	Applied at acclimatization stage	Growth promotion and disease control (*Fusarium* wilt)	Ting *et al*. 2008
Bacillus sphaericus UPMB10	Banana	*In vitro*	Growth promotion and nutrition	Maziah *et al*. 2010
Mixture of endophytes (proteobacteria)	Banana	*Ex vitro*	Growth promotion and disease control	Lian Jie *et al*. 2009
Azospirillum brasilense strain Sp7 and *Bacillus sphaericus* st.UPMB10	Banana	*Ex vitro*	Growth promotion and nutrition	Baset Mia *et al.* 2009
Two isolates of *Bacillus* spp. (B21 and B31) and two isolates of *Pseudomonas* (P52 and P58) + two non-pathogenic *Fusarium oxysporum* isolates (E3 and E4), two *Trichoderma atroviride* isolates (E1 and E2)	Banana	*Ex vitro*	Growth promotion and reduced nematode incidence	Chaves *et al*. 2009
Endophytic *Fusarium oxysporum* isolates Emb2.4o and V5w2	Banana	*Ex vitro*	Growth promotion and pest control (*Cosmopolites sordidus* and *Radopholus similis*)	Paparu *et al*. 2009
Beauveria bassiana (Balsamo) Vuillemin	Banana	*Ex vitro*	Growth promotion	Akello *et al*. 2009
F. oxysporum strain 162, *Paecilomyces lilacinus* strain 251 and the antagonistic bacteria *Bacillus firmus*	Banana	*Ex vitro*	Pest control (*Radopholus similis*)	Mendoza and Sikora 2009

2009; Yang *et al*. 2009). The bacterized potato plantlets transplanted directly from culture vessels to the field had significantly better survival than the non-bacterized controls (Nowak *et al*. 1999). A mixture of three strains of rhizobacteria improved the post-transplanting performance of strawberries when bacterized with post *in-vitro* conditions (Vosatka *et al*. 2000). In tea, hardening of tissue-cultured plants with bacterial inoculations enhanced the survival percentage (up to 100, 90 and 86%) as against control plants (0, 52 and 36%) in rainy, winter and summer seasons respectively (Pandey *et al*. 2000). Acclimatization of micropropagated plants (tomato, pepper and vinca) inoculated with PGPR showed a higher survival rate and a better quality of hardened off specimens (Carletti 2000). The post *vitro* mycorrhization and bacterization of micropropagated strawberry and potato with certain combinations of bacteria and mycorrhiza enhanced greenhouse production of minitubers and a mixture of three strains of rhizobacteria improved the post transplanting performance of strawberries (Vosatka *et al*. 2000). In banana, bioprimimg with cocktails of *Pseudomonads* strains significantly enhanced the survival percentage of banana cv. 'Virupakshi (AAB)' under rainfed ecosystems (Kavino 2005). Conspicuously, some PGPR possesses the enzyme 1-aminocyclopropane-1-carboxylate (ACC) deaminase (Jacobson *et al*. 1994; Glick *et al*. 1997) and this enzyme can cleave the plant ethylene precursor ACC, and thereby lower the level of ethylene in a developing or stressed plant (Sheehy *et al*. 1991; Mayak *et al*. 2004b). By facilitating the formation of longer roots, these growth promoting bacteria may enhance the survival of plant seedlings under various biotic and abiotic stresses. In addition, plants that are treated with ACC deaminase-containing PGPR are dramatically more resistant to the deleterious effects of ethylene that is synthesized as a consequence of stressful conditions such as flooding (Grichko and Glick 2001), heavy metals (Grichko *et al*. 2000), the presence of phytopathogens (Wang *et al*. 2000), drought and high salt (Mayak *et al*. 2004a, 2004b). Recently, Saravanakumar and Samiyappan (2007) demonstrated the role of ACC deaminase of *P. fluorescens* strain TDK1 against salt stress in groundnut plants under field conditions.

Biopriming for biotic stress tolerance in plants

The use of PGPR has been reported for the control of various fungal, bacterial and viral pathogens (Gutterson 1990; Wei *et al*. 1991; Kavino *et al*. 2007; Udaya Shankar *et al*. 2009; Verhagen *et al*. 2010). Kurze *et al*. (2001) evaluated a chitinolytic rhizobacterium, *Serratia plymuthica* strain HRO-C48, as a bare root transplant dip for strawberries and had good success in reducing disease caused by *Verticillium* and *Phytophthora* and increasing yields. Bacterial strains 84 and 4B when introduced to banana roots of tissue cultured plants at de-flasking stage significantly improved plant growth and reduced infection of *Fusarium oxysporum* f.sp. *cubense* in the rhizome under greenhouse conditions (Smith *et al*. 2003). Roots of apple seedlings soaked in the bacterial suspension of selected antagonistic PGPR strains before planting and supplemented by adding and mixing the suspension into the soil and repeated irrigation treatments with the antagonists reduced the replant disease in apple seedlings (Bir'o *et al*. 1998). Application of *Bacillus* spp. through transplant plug delivery system significantly improved the growth and development of drip irrigated pepper and reduced the bacterial spot disease incidence in the field (Vavrina 2004). Similarly, application of Fluorescent pseudomonads increased plant growth promotion in tomato and hotpepper (Ramamoorthy *et al*. 2002b). Vegetable transplant plugs of tomato and cucumber when treated with bio preparations (*Bacillus* spp. with chitin) significantly reduced the disease severity of bacterial spot and late blight of tomato and angular leaf spot of cucumber respectively (Amruthesh *et al*. 2003). In grapes, when *in vitro* bacterized plantlets were challenged with *Botrytis cinerea*, the symptoms of grey mold failed to develop compared to non-bacterized controls (Barka *et al*. 2002). Micropropagated rooted banana plantlets which were immersed in bacterial mixtures significantly controlled the *Fusarium* wilt disease at the time of planting (Albuquerque *et al*. 2003). Similarly, Müller and Berg (2008) reported the effect of biocontrol agent *Serratia plymuthica* HRO-C48 on *Verticillium* wilt in oilseed rape. Recently it has been reported that biopriming banana plants with mixtures of *Pseudomonas* strains significantly reduced the bunchy top disease incidence under greenhouse and field conditions (Harish *et al*. 2008; Kavino *et al*. 2009).

PEST AND DISEASE RESISTANCE OF BIOPRIMED PLANTS

A large number of defense enzymes have been associated with biopriming which includes phenylalanine ammonia lyase, chitinase, β-1,3-glucanase, peroxidase, polyphenol oxidase, superoxide dismutase, catalase, ascorbate peroxidase, lipoxygenase and proteinase inhibitors (Ye *et al*. 1990; Koch *et al*. 1992; Schneider and Ullrich 1994; van Loon 1997). Chitinases and β-1,3-glucanases are pathogenesis related (PR) proteins and they are activated during incompatible plant pathogen interactions (Harish *et al*. 2009b). Biopriming can also signal molecules related to salicylic acid or jasmonic acid mediated pathway which are activated by necrotizing pathogens and chemical inducers (Borges *et al*. 2009; Vicedo *et al*. 2009). These enzymes also bring about liberation of molecules that elicit the first steps of induction of resistance, phytoalexins and phenolic compounds (Keen and Yoshikawa 1983; van Loon *et al*. 1994).

Induced systemic resistance by PGPR has been achieved in large number of crops including *Arabidopsis* (Pieterse *et al*. 1996), cucumber (Wei *et al*. 1996), tobacco (Troxler *et al*. 1997), tomato (Duijff *et al*. 1997), potato (Doke *et al*. 1987), radish (Leeman *et al*. 1996), carnation (van Peer *et al*. 1991), bean (de Meyer and Hofte 1997), sugarcane (Viswanathan and Samiyappan 1999), chilli, brinjal (Ramamoorthy and Samiyappan 2001; Bharathi *et al*. 2004), mango (Vivekananthan *et al*. 2004) and banana (Kavino *et al*. 2007; Harish *et al*. 2008) against broad spectrum of pathogens including fungi (Doke *et al*. 1987; Leeman *et al*. 1995), bacteria (Liu *et al*. 1995a; 1995b) and viruses (Maurhofer *et al*. 1994; Kandan *et al*. 2005).

Peroxidase

Peroxidases (PO) have been implicated in the regulation of plant cell elongation, phenol oxidation, polysaccharide cross-linking, IAA oxidation, cross linking of extension monomers, oxidation of hydroxyl–cinnamyl alcohols into free radical intermediates and wound healing (Vidhyasekaran *et al*. 1997). Bradley *et al*. (1992) reported that the increased PO activity has been correlated with resistance in many species including barley, cucurbits, cotton, tobacco, wheat and rice and these enzymes are involved in the polymerization of proteins and lignin or suberin precursors into plant cell wall, thus constructing a physical barrier that could prevent pathogen penetration of cell walls and movement through vessels. Plant root colonization by PGPR was associated with PO activity. These enzymes are also part of the response of plant defense to pathogens (Hammerschmidt and Kuc 1995) and they may decrease the quality of these plants as host for insects. High level expression of PO was reported in *P. fluorescens* strain Pf1 treated chilli plants challenged with *Colletotrichum capsici* (Bharathi *et al*. 2004). The higher PO activity was noticed in cucumber roots treated with *P. corrugata* challenged with *Pythium aphanidermatum* (Chen *et al*. 2000). Multifold increase in PO activity was observed in the *P. fluorescens* strain Pf1 + *B. subtilis* + Neem + Chitin formulation treated plants over control in chilli against CMV (Bharathi 2001). The timely induction and greater accumulation of PO in tea plants primed with *P. fluorescens* strain Pf1 effectively reduced the incidence of blister blight disease under field conditions besides increasing the yield (Saravanakumar *et al*. 2007). Recently, Kavino *et al*. (2008) reported greater accumulation of PO in banana plants treated with endophytic and rhizosphere bacterial strains which showed enhanced resistance to *Banana bunchy top virus* (BBTV).

Polyphenol oxidase (PPO)

PPO usually accumulated upon wounding in plants. Biochemical approaches to understand PPO function and regulation are difficult, because the quinonoid reaction products of PPO covalently modify and cross-link the enzyme. PPO can be induced *via* octadecanoid defense signal pathway (Constabel *et al*. 1995). Chen *et al*. (2000) reported that PPO was stimulated by PGPR or by the pathogen, but the wounds on split roots did not influence PPO activity compared to intact control in 13 days. PGPR untreated canes after pathogen inoculation showed comparatively lesser induction of PPO isoforms than the PGPR treated sugarcane (Viswanathan 1999). Expression of new PPO isoform was observed in *P. fluorescens* strain Pf1 treated tomato plants challenged with *F. oxysporum* f. sp. *lycopersici* (Ramamoorthy *et al*. 2002b). In tomato, PPO is induced by caterpillar feeding, jasmonates and mechanical damage but not by mites or leafminers (Thaler *et al*. 1996). Similarly, increased activity of PPO was observed in tomato by fluorescent pseudomonads in response to infection by *Tomato spotted wilt virus* (Kandan *et al*. 2002). More induction of PPO activity in *P. fluorescens* strain Pf1 treated chilli plants in response to *C. capsici* correlated with reduced infection of anthracnose disease (Bharathi *et al*. 2004). Recently, Kavino *et al*. (2007; 2008) demonstrated the greater activity of defense related enzymes including PPO in biohardened banana plantlets showing resistance to BBTV. Thus, the activation of defense related enzymes is found to greatly influence the resistance mechanisms in bioprimed plants against insect pests and diseases.

Phenylalanine ammonia lyase (PAL)

PAL catalyzes the deamination of L-phenylalanine to *trans*-cinnamic acid which is the first step in the biosynthesis of large class of plant natural products based on the phenylpropane skeleton, including lignin monomers as well as certain classes of phytoalexins. PAL activity also generates precursors of lignin biosynthesis and other phenolic compounds that accumulate in response to pathogen infection (Klessig and Malamy 1994). PAL is the key enzyme in inducing the synthesis of salicylic acid (SA) which induces systemic resistance in many plants. *Bacillus amyloliquefaciens* strain EXTN-1-treated tobacco plants showed augmented, rapid transcript accumulation of defense related genes including PR-1a, PAL and 3-hydroxy-3-methylglutaryl CoA reductase (HMGR) following inoculation of *Pepper mild mottle virus* (PMMoV) (Ahn *et al.* 2002). When cucumber roots were treated with *Pseudomonas corrugata* 13 or *P. aureofaciens* 63-28, PAL activity was stimulated in root tissues in two days and this activated accumulation lasted for 16 days after bacterization (Chen *et al*. 2000).

Scavengers of reactive oxygen species

One of the biochemical changes occurring in plants subjected to various environmental stress conditions is the production of reactive oxygen species (ROS) such as superoxide radicals (O_2-), hydrogen peroxide, single oxygen and hydroxyl radicals (OH) (Iturbe-Ormaetxe *et al*. 1998; Cho and Park 2000). The ROS have a role in lipid peroxidation, membrane damage and consequently in plant senescence (Fridovich 1986; Thompson *et al*. 1987) and antioxidant enzymes such as superoxide dismutase (SOD), peroxidases (PO), ascorbate peroxidases (APX) and catalases (CAT) are involved in the scavenging of ROS (Asada 1992; Foyer 1993). SOD is a metalloprotein that catalyzes the dismutation of superoxide to H_2O_2 and molecular oxygen (Allen 1995). Various antioxidant enzymes such as CAT and PO eliminate H_2O_2. CAT found predominantly in peroxisomes dismutase H_2O_2 into H_2O and O_2, whereas PO decomposes H_2O_2 by oxidation of co-substrates such as phenolic compounds and antioxidants (Sudhakar *et al*. 2001). Catalase and peroxidase are of particular interest because of their role in binding SA, which plays an important role in induced resistance (Anderson *et al*. 1998). APX is primarily located in both chloroplasts and cytosol and eliminates peroxides by converting ascorbic acid to dehydroascorbate (Asada 1992). As a member of the ascorbic acid glutathione cycle, APX is one of the most important enzymes playing a crucial role in eliminating toxic H_2O_2 from plant cells during biotic and abiotic stress (Foyer *et al*. 1994; Cho and In-Taek 2003). Kavino (2005) assayed the greater activity of antioxidant enzymes such as SOD, PO and CAT in tissue culture banana plants primed with endophytic and rhizosphere bacterial bioformulations which showed high resistance to BBTV. Similar studies were carried out by Harish *et al*. (2009) who demonstrated that the defense related proteins *viz*., chitinase and β-1,3-glucanases and defense related enzymes *viz*., PAL, PO and PPO were significantly activated in banana plants bioprimed with plant growth promoting endophytic bacteria strains against BBTV. In addition to the enzyme induction, the bioprimed banana plantlets produced higher yield when compared to untreated plants under field conditions. Similarly, Kavino *et al.* (2008) demonstrated that biopriming of banana plantlets with bioformulations containing chitin molecules and *P. fluorescens* strain CHA0 effectively reduced the incidence of BBTV by activating different defense related enzymes. Recently, Saravanakumar *et al.* (2009) reported the differential expression of PO, PPO and PAL in rice plants primed with mixtures of fluorescent pseudomonads. Thus, it is clearly evidenced from several researches that the expression of PO, PPO and PAL in crop plants mediated by plant growth promoting bacteria have resistant mechanisms to biotic and abiotic stresses.

PR proteins (chitinases and glucanases)

Evidence of β-1, 3-glucanases in disease resistance was first reported by Kauffmann *et al*. (1987). In dicots, β-1,3-glucanase genes are considered to constitute a part of the general array of defense genes induced during pathogenesis (Mauch and Staehelin 1989). Later, induction of β-1,3-glucanases was demonstrated in barley and other monocots like wheat, rice and sorghum in response to infection by the necrotrophic pathogen, *Bipolaris sorokiniana* (Jutidamrongphan *et al*. 1991). Daugrois *et al*. (1992) reported rapid induction of two β-1,3-glucanases in the incompatible interaction between bean and *C. lindemuthianum*. Purified fungal elicitor can also induce defense related proteins in the host (Martinez-Esteso *et al*. 2009). Purified acidic β-1,3-glucanases from cucumber had antifungal activity against *C. orbiculare* (Ji and Kuc 1996). Maurhofer *et al*. (1994) reported that *P. fluorescens* strain CHA0 enhanced the activity of β-1,3-glucanases along with chitinases in tobacco and offered systemic protection against *Tobacco necrosis virus*. Xue *et al*. (1998) found an 8-fold increase in β-1,3-glucanases in bean in response to binucleate *Rhizoctonia* (BNR) treatment and such treatment offered protection against pathogenic *R. solani* and *C. lindemuthianum*. Similarly, Vivekananthan *et al*. (2004) reported the more induction of β-1,3-glucanase isoforms in mango trees treated with *P. fluorescens* in response to infection by anthracnose pathogen than the untreated control. Recently, Kavino *et al*. (2007) reported the greater accumulation of glucanases in bacterized banana plantlets against BBTV infection.

Chitinases are PR-proteins which hydrolyze chitin, major cell wall component constituents for 3-10% of higher fungi and cuticle of peritrophic membrane in insects. Chitinase cleave a bond between C1 and C4 of two consecutive *N*-acetyl glucosamine (GlcNAc) either by endolytic or exolytic mechanisms. A large number of plant chitinases have been purified and characterized which are endochitinases with molecular weights ranging from 25 to 36 kDa. The production of chitinases in plants has been suggested to be a part of their defense mechanism against fungal pathogens (Schlumbaum *et al*. 1986). In recent years, several biocontrol agents have shown to induce systemic resistance in plants. Enhanced accumulation of chitinase in tobacco and bean leaves was observed in response to application of *Pseudomonas* spp. to roots (Zdor and Anderson 1992; Maurhofer *et al*. 1994). Increased chitinase activity in tobacco and maximum activity in cucumber have been observed as a result of systemic resistance by fluorescent pseudomonads against *P. syringae* pv. *tabaci* (Schneider and Ullrich 1994). Induction of four new chitinase isoforms with molecular weights of 12.0, 34.5, 53.5 and 63 kDa in *Pseudomonas* treated canes challenged with *C. falcatum* in sugarcane was observed (Viswanathan and Samiyappan 2001). Thus, the synthesis and accumulation of PR proteins upon exposure of plants to beneficial microorganisms have been found to play an important role in plant defense (Edreva 2005).

Strengthening of plant cell wall

The rapid strengthening of reaction sites of fungal and insect entry delays the infection process and allows sufficient time for the host to built up other defense reactions. Seed treatment with PGPR in bean induces the lignification of cell wall (Anderson and Guerra 1985). *Agrobacterium rhizogenes* Ri T-DNA transformed pea roots pre-inoculated with the endophytic bacterium, *B. pumilus* SE34 were protected against the root rot pathogen, *F. oxysporum* f. sp. *pisi*. They found that these cell walls were strengthened at the sites of attempted fungal penetration by opposition containing large amounts of callose and phenolic substances, effectively preventing the fungal ingress. In tomato, bacterization with same bacterial strain has brought about cell wall thickening, deposition of phenolic compounds and formation of callose resulting in restricted growth of *F. oxysporum* f. sp. *radicis-lycopersici* to the epidermal cell and outer cortex in the root system in the treated plants (M'Piga *et al*. 1997). Similar wall appositions and papillae were observed in pea roots treated with the *P. fluorescens* 63-28R upon challenge inoculation with either *F. oxysporum* f. sp. *pisi* or *P. ultimum* (Benhamou *et al*. 1996), indicating a general induction of physical defense barriers to pathogen ingress. Induction of thickening of cortical cell walls in tomato was seen after colonization of roots by *P. fluorescens* WCS417 (Duijff *et al*. 1997). *B. pumilus* strain SE 34 has also induced strengthening of cell wall structure in tomato against *F. oxysporum* f. sp. *radicis-lycopersici* (Benhamou and Theriault 1998).

DEVELOPMENT OF BIOFORMULATION

In developing formulations, several molecules have been reported to be added to enhance the survival and efficacy of the PGPR. Chitin, as a carbon source/substrate for the growth of chitinolytic bacteria, increased the chitinase production when bacteria were grown in chitin amended medium (Gooday 1990). Chitosan, a nontoxic polymer obtained from the chitin of crustacean shell wastes is not only the inhibitor of fungal growth but also activates genes encoding defense related proteins in plants (Hadwiger *et al*. 1986; Lafontaine and Benhamou 1996). In addition, chitin oligomers which are released during degradation of chitin substrate by chitinolytic bacteria are also found to elicit plant defense reactions (Benhamou and Theriault 1998). Incorporation of chitin in King's medium B (KMB) supported the multiplication of *P. fluorescens* and enhanced chitinase activity when compared to the medium without incorporation of chitin (Viswanathan and Samiyappan 2001). Tomato plant treated with chitosan showed enhanced protection against crown and root rot caused by *Fusarium oxysporum* f.sp. *radicis-lycopersici* (Lafontaine and Benhamou 1996). Similarly, banana plants treated with *P. fluorescens* strain CHA0 along with chitin showed enhanced protection against BBTV besides improving the bunch yield (Kavino *et al*. 2008).

PGPR strains and host plant specificity

This specificity appears to be related to the different composition of the rhizosphere exudates depending on the plant species which affect the levels of colonization and subsequently the efficacy of the PGPR strains or the specific compounds present in the root exudates, that may stimulate the synthesis of secondary metabolites implicated in the plant growth promotion in the bacteria (van Overbeek and van Elsas 1995). Quantitative differences in phytohormone production by bacteria and the degree of sensitivity of plants to phytohormones are being suggested as the main reasons for this phenomenon (Glick 1995). Plant species or cultivars differ in their reaction to inoculation with beneficial rhizobacteria (Fredrickson and Elliott 1987). A high specificity was observed between several growth promoting strains and the type of *Prunus* rootstock. Strains of *P. fluorescens* EPS 383 and EPS 286 were only active in Almond x Peach hybrid GF 677, whereas strains EPS 231 and EPS 588 were only active in Marianna 2624 (Bonaterra *et al*. 2003). Similar results describing strain-host plant specificity have been reported in other plant systems such as several herbaceous crops (Howie and Echandi 1983: Kloepper 1996). In strawberry, addition of LS 213 to plugs resulted in a greater enhancement of growth and yield in variety 'Camarosa' than in 'Sweet Charlie' indicating better suitability of this particular combination of bacterial isolates to variety 'Camarosa' and the differences in varietal response may occur within crops (Kokalis-Burelle 2003). Under tissue culture conditions, bacterial treatments increased the dry weight of roots of the potato cultivar Norchip by up to 600-1000% and Kennebec by 200-400% whereas it inhibited the root weight of Chaleur by 40% (Nowak *et al*. 1995). Two PGPR strains protected cucumber and tomato from *Cucumber mosaic virus* (CMV), but different levels of protection on these two plant species were noticed suggesting that some level of specificity exists in the interaction between plant and bacteria (Raupach *et al*. 1996).

IMPROVEMENT OF THE EFFICACY OF BIOFORMULATIONS

Mixtures of microbial strains

Generally, application of PGPR singly leads to inconsistent performance, because a single PGPR is not likely to be active in all kinds of soil environment and agricultural ecosystems. For plant-beneficial pseudomonads, strain mixtures and combinations with other bacteria or fungi often provided more-effective disease control than the application of an individual biocontrol pseudomonad alone (Pierson and Weller 1994; Duffy *et al*. 1996; Duijff *et al*. 1999; de Boer *et al*. 2003; Kavino *et al*. 2007). Another approach to obtain a successful microbial biocontrol consortium is to apply mixtures of biocontrol agents which display different disease-suppressive mechanisms that are complementary to each other. Cocktails of various *Pseudomonas* strains provided enhanced protection than a single organism (Thomashow and Weller 1998). Mixtures of PGPR strains significantly reduced the severity of diseases compared to the non bacterized control in tomato, pepper and cucumber (Jetiyanon and Kloepper 2002). de Boer *et al*. (2003) stated that combined *Pseudomonas* strains are effective in siderophore-mediated competition for iron and induction of systemic plant resistance to control Fusarium wilt of radish. Dunne *et al*. (1998) applied a mixture of the DAPG producer *P. fluorescens* F113 and a proteolytic rhizobacterium to enhance suppression of *Pythium* sp. mediated damping off in sugar beet.

Amendment of elicitors

Involvement of chitin or chitosan in inducing systemic resistance alone or in combination with biocontrol agents has been demonstrated in few crops. Unique biological properties of chitin oligomers including their antifungal properties on various plant pathogenic fungi like *F. oxysporum* f. sp. *radicis-lycopersici* and *P. aphanidermatum* have been well documented (Leuba and Stossel 1986; El Ghaouth *et al*. 1994; Lafontaine and Benhamou 1996). The chitin oligomers are also found as potential elicitors of plant defense reactions (Leuba and Stossel 1986; Benhamou 1992). Benhamou and Theriault (1998) found induction of resistance against Fusarium wilt by combining chitosan with an endophytic bacterium, *B. pumilus* strain SE 34 in tomato. Chitin amendment drastically reduced the number of stubby root nematodes (*Trichodorus* spp.) (Ellis *et al*. 1998). Recent reports have revealed the fact that mixing of chitin with PGPR increases the biocontrol efficacy against insect pest and pathogen in crop plants (Nandakumar 1998; Radjacommare *et al*. 2002; Bharathi *et al*. 2004). Apart from inducer of systemic resistance, chitin application enhanced the biocontrol of early leaf spot in peanut with a chitinolytic PGPR strain by providing a nutrient source for the applied bacterium and resident chitinolytic microbes (Kokalis-Burelle *et al*. 1992). Also, chitooligosaccharides possess a variety of functional properties such as antibacterial, antitumor and immuno enhancing effects (Jeon and Kim 2000). Recently, Kavino *et al*. (2008) reported that PGPR bioformulation amended with chitin molecules enhanced the resistance to BBTV infection in banana plants.

CONCLUSIONS

In-vitro biopriming of micropropagated plants with PGPRs can improve banana and plantains performance under stress environments and consequently enhances yield besides reducing the disease incidence. The defense chemicals induced upon treatment with PGPR bioformulations and growth promoting substances produced by rhizosphere and endophytic bacterial strains may play a significant role in reducing the disease incidence and thereby increasing the yield. The application of bioinoculants at the earlier stages of the propagation material will improve the health condition of the plantlets under varied environmental conditions and maintain the microbial population as rhizobacteria and/or as endophytes by compressing the deleterious microorganisms. It is concluded from the earlier demonstrations that the use of biocontrol agents in integrated management systems, either as plug and/or soil treatments or both, can significantly increase the production and productivity levels of banana and plantains and improve the soil status which ultimately enhances the health status of second season crop. In addition, the biocontrol agents contribute for the eco-friendly management of pest and diseases for the sustainable horticulture. On the other hand, the selection of versatile plant growth promoting bacteria for the biopriming process is the primary aspect in the biohardening process. In addition, the development of a bioformulation either in the form of carrier based material or liquid based formulation play an important role in the commercialization of biohardended plants. In this regard, the research work should be focused more on identifying the bioagents that are suitable for biological hardening of micropropagated materials as well as standardizing the methods of application.

To exploit the potentiality of *in vitro* priming in tissue culture propagules, and to design novel strategies for increased efficiency of plant micropropagation and plant productivity, biochemical and molecular mechanisms underlying in this process still need to be clarified. Recent developments in genomics, proteomics and metabolomics provide researchers with new molecular tools, allowing them to scrutinize earlier findings and look at the molecular interaction between plant-beneficial microbes, plant-biotic/abiotic stress, plant-beneficial microbes-biotic/abiotic stress in a much more holistic manner than ever before (Delseny *et al*. 2001; Nowak and Shulaev 2003). The global profiling of gene and protein expression in plant tissues during biopriming could identify genes and proteins differentially expressed in response to the applied agents and identify signaling networks leading to enhanced resistance to a specific abiotic or biotic stress. Metabolite profiling of plant interaction with beneficial microorganisms could identify chemicals involved in the development of mutualistic interactions and provide tools to manipulate this process in a rational manner. These tools are currently being largely used on model plant species and their application is essential for the development of effective priming methods tailored to many cultivated plant species and cultivars.

REFERENCES

Adesemoye AO, Torbert HA, Kloepper JW (2009) Plant growth-promoting rhizobacteria allow reduced application rates of chemical fertilizers. *Microbial Ecology* **58 (4)**, 921-929

Ahn II P, Park K, Kim CH (2002) Rhizobacteria-induced resistance perturbs viral disease progress and triggers defense-related gene expression. *Molecules and Cells* **13 (2)**, 302-308

Akello J, Dubois T, Coyne D, Kyamanywa S (2009) The effects of *Beauveria bassiana* dose and exposure duration on colonization and growth of tissue cultured banana (*Musa* sp.) plants. *Biological Control* **49 (1)**, 6-10

Akello J, Dubois T, Gold CS, Coyne D, Nakavuma J, Paparu P (2007) *Beauveria bassiana* Vuillemin as an endophyte in tissue culture banana (*Musa* spp). *Journal of Invertebrate Pathology* **96**, 34-42

Albuquerque VV, Terao D, Mariano RLR (2003) Growth-promotion and biocontrol of *Fusarium* wilt in micropropagated plantlets of *Musa* sp. In: Reddy MS, Anandaraj M, Eapen SJ, Sarma YR, Kloepper JW (Eds) *6th International PGPR Workshop*, Calicut, Kerala, October 5-10, 2003, pp 3-8

Allen RD (1995) Dissection of oxidative stress tolerance using transgenic plants. *Plant Physiology* **107**, 1049-1054

Amruthesh KN, Niranjan Raj S, Kiran B, Shetty HSB, Reddy MS (2003) Growth promotion by plant growth promoting rhizobacteria in some economically important crop plants. In: Reddy MS, Anandaraj M, Eapen SJ, Sarma YR, Kloepper JW (Eds) *6th International PGPR Workshop*, Calicut, Kerala, October 5-10, 2003, pp 97-103

Anderson AS, Guerra D (1985) Responses of bean to root colonization with *Pseudomonas putida* in a hydroponic system. *Phytopathology* **75**, 992-995

Anderson MD, Chen Z, Klessig D (1998) Possible involvement of lipid peroxidation in salicylic acid-mediated induction of PR-1 gene expression. *Phytochemistry* **47**, 555-566

Asada K (1992) Ascorbate peroxidase a hydrogen peroxide scavenging enzyme in plants. *Physiologia Plantarum* **85**, 235-241

Barka EA, Belarbi A, Hachet C, Nowak J, Audran JC (2000) Enhancement of *in vitro* growth and resistance to gray mould of *Vitis vinifera* co-cultured with plant growth promoting rhizobacteria. *FEMS Microbiology Letters* **186**, 91-95

Barka EA, Gognies S, Nowak J, Audran JC, Belarbi A (2002) Inhibitory effect of endophyte bacteria on *Botrytis cinerea* and its influence to promote the grapevine growth. *Biological Control* **24**, 135-142

Baset Mia MA, Shamsuddin ZH, Wahab Z, Marziah M (2009) The effect of rhizobacterial inoculation on growth and nutrient accumulation of tissue-cultured banana plantlets under low N-fertilizer regime. *African Journal of Biotechnology* **8 (21)**, 5855-5866

Bassil NV, Proebsting WM, Moore LW, Lightfoot DA (1991) Propagation of hazelnut stem cuttings using *Agrobacterium rhizogens*. *HortScience* **26**, 1058-1060

Benhamou N (1992) Ultrastructure and cytochemical aspects of chitosan on *Fusarium oxysporum* f.sp. *radicis-lycopersici*, agent of tomato crown and root rot. *Phytopathology* **82**, 1185-1193

Benhamou N, Belanger RR, Paulitz TC (1996) Induction of differential host responses by *Pseudomonas fluorescens* in *Ri* T-DNA-transformed pea roots after challenge with *Fusarium oxysporum* f. sp. *pisi* and *Pythium ultimum*. *Phytopathology* **86**, 114-128

Benhamou N, Theriault G (1998) Treatment with chitosan enhances tomato plants to the crown and root rot pathogen *Fusarium oxysporum* f. sp. *lycopersici*. *Physiological and Molecular Plant Pathology* **41**, 33-52

Bernal A, Machado P, Cortegaza L, Carmona ER, Rivero O, Zayas CM, Nodarse O, Perez A, Santana I, Arencibia AD (2008) Priming and biopriming integrated into the sugarcane micropropagation technology by Temporary Immersion Bioreactors (TIBS). *Sugar Tech* **10 (1)**, 42-47

Bharathi R (2001) Development of a rhizobacteria based bio-formulation for the management of major pests and diseases in chillies. MSc thesis, Tamil Nadu Agricultural University, Coimbatore, India, 205 pp

Bharathi R, Vivekananthan R, Harish S, Ramanathan A, Samiyappan R (2004) Rhizobacteria based bioformulations for the management of fruit rot infection in chillies. *Crop Protection* **23**, 835-843

Bir'o B, Magyar K, Várady GY, Kecskés M (1998) Specific replant disease reduced by PGPR rhizobacterium on apple seedlings. *Acta Horticulturae* **477**, 75-81

Bonaterra A, Ruz L, Badosa E, Pinochet J, Montesinos E (2003) Growth promotion of *Prunus* rootstocks by root treatment with specific bacterial strains. *Plant and Soil* **255**, 555-569

Borges AA, Dobon A, Expósito-Rodríguez M, Jiménez-Arias D, Borges-Pérez A, Casañas-Sánchez V, Pérez JA, Luis JC, Tornero P (2009) Molecular analysis of menadione-induced resistance against biotic stress in *Arabidopsis*. *Plant Biotechnology Journal* **7 (8)**, 744-762

Bottini R, Cassan F, Piccoli P (2004) Gibberellin production by bacteria and its involvement in plant growth promotion and yield increase. *Applied Microbiology and Biotechnology* **65**, 497-503

Bradley DJ, Kjellborn P, Lamb C (1992) Elicitor and wound induced oxidative cross-linking of a plant cell wall proline-rich protein: A novel, rapid defense response. *Cell* **70**, 21-30

Caesar AJ, Burr TJ (1987) Growth promotion of apple seedlings and rootstocks by specific strains of bacteria. *Phytopathology* **77**, 1583-1588

Carletti S (2000) Use of plant growth-promoting rhizobacteria in plant micropropagation. Available online: http://www.ag. auburn.edu/argentina/pdfmanuscripts/mello.pdf

Chaves NP, Pocasangre LE, Elango F, Rosales FE, Sikora R (2009) Combining endophytic fungi and bacteria for the biocontrol of *Radopholus similis* (Cobb) Thorne and for effects on plant growth. *Scientia Horticulturae* **122**, 472-478

Chen C, Belanger RR, Benhamou N, Paulitz TC (2000) Defense enzymes induced in cucumber roots by treatment with plant-growth promoting rhizobacteria (PGPR). *Physiological and Molecular Plant Pathology* **56**, 13-23

Cho U, In-Taek K (2003) Effect of cadmium on oxidative stress and activities of antioxidant enzymes in tomato seedlings. *Korean Journal of Ecology* **26 (3)**, 115-121

Cho UH, Park JO (2000) Mercury-induced oxidative stress in tomato seedlings. *Plant Science* **156**, 1-9

Choudhary DK, Johri BN (2009) Interactions of *Bacillus* spp. and plants – with special reference to induced systemic resistance (ISR). *Microbiological Research* **164**, 493-513

Compant S, Duffy B, Nowak J, Clément C, Barka EA (2005) Biocontrol of plant diseases using plant growth-promoting bacteria (PGB): principles, mechanisms of action and future prospects. *Applied and Environment Microbiology* **71 (9)**, 4951-4959

Conrath U, Beckers GJM, Flors V, García-Agustín P, Jakab G, Mauch F (2006) Priming: getting ready for battle. *Molecular Plant-Microbe Interactions* **19**, 1062-1071

Conrath U, Pieterse CMJ, Mauch-Mani B (2002) Priming in plant-pathogen interactions. *Trends in Plant Science* **7**, 210-216

Constabel CP, Bergery DR, Ryan CA (1995) Systemin activates synthesis of wound-inducible tomato leaf polyphenoloxidase *via* the octadecanoid defense signaling pathways. *Proceedings of the National Academy of Sciences USA* **92**, 407-412

Creus CM, Sueldo RJ, Barassi CA (1998) Water relations in *Azospirillum*-inoculated wheat seedlings under osmotic stress. *Canadian Journal of Botany* **76**, 238-244

Daugrois JH, Lafitte C, Barthe JP, Faucher C, Touze A, Esquerre-Tugaye MT (1992) Purification and characterization of two basic 1,3-glucanases induced in *Colletotrichum lindemuthianum* infected bean seedlings. *Archives of Biochemistry and Biophysics* **292**, 468-474

de Boer M, Bom P, Kindt F, Keurentjes JJB, van der Sluism I, van Loon LC, Bakker PAHM (2003) Control of *Fusarium* wilt of radish by combining *Pseudomonas putida* strains that have different disease-suppressive mechanisms. *Phytopathology* **93**, 626-632

de Meyer G, Hofte M (1997) Salicylic acid produced by the rhizobacterium *Pseudomonas aeruginosa* 7NSK2 induces resistance to leaf infection by *Botrytis cinerea* on bean. *Phytopathology* **87**, 588-593

Delseny M, Salses J, Cooke R, Sallaud C, Regad F, Lagoda P, Guideroni E, Ventelon M, Brugidou C, Ghesquière A (2001) Rice genomics: present and future. *Plant Physiology and Biochemistry* **39**, 323-334

Doke N, Ramirez AV, Tomiyama K (1987) Systemic induction of resistance in potato plants against *Phytophthora infestans* by local treatment with hyphal wall components of the fungi. *Journal of Phytopathology* **119**, 232-239

Dubois T, Gold CS, Coyne D, Paparu P, Mukwaba E, Athman S, Kapinduand S, Adipala E (2004) Merging biotechnology with biological control: Banana (*Musa* spp) tissue culture plants enhanced by endophytic fungi. *Uganda Journal of Agricultural Science* **9**, 445-451

Dubuis C, Keel C, Haas D (2007) Dialogues of root-colonizing biocontrol pseudomonads. *European Journal of Plant Pathology* **119**, 311-328

Duffy BK, Simon A, Weller DM (1996) Combination of *Trichoderma koningii* with fluorescent *Pseudomonads* for control of take-all on wheat. *Phytopathology* **86**, 188-194

Duffy EM, Hurley EM, Cassells AC (1999) Weaning performance of potato microplants following bacterization and mycorrhization. *Potato Research* **42**, 521-527

Duijff BJ, Gianinazzi-Pearson V, Lemanceau P (1997) Involvement of the outer membrane lipopolysaccharides in the endophytic colonization of tomato roots by biocontrol *Pseudomonas fluorescens* strain WCS417r. *New Phytologist* **135**, 325-334

Duijff BJ, Recorbet G, Bakker PAHM, Loper JE, Lemanceau P (1999) Microbial antagonism at the root level is involved in suppression of fusarium wilt by the combination of nonpathogenic *Fusarium oxysporum* Fo47 and *Pseudomonas putida* WCS358. *Phytopathology* **89**, 1073-1079

Dunne C, Moenne-Loccoz Y, McCarthy J, Higgins P, Powell J, Dowling DN O'Gara F (1998) Combination of proteolytic and phloroglucinol producing bacteria for improved biocontrol of *Pythium*-mediated damping-off of sugarbeet. *Plant Pathology* **47**, 299-307

Edreva A (2005) Pathogenesis-related proteins: Research progress in the last 15 years. *General and Applied Plant Physiology* **31**, 105-124

El Ghaouth LA, Arul J, Benhamou N, Assselin A, Belanger RR (1994) Effect of chitosan on cucumber plants: Suppression of *Pythium aphanidermatum* and induction of defense reactions. *Phytopathology* **84**, 313-320

Ellis SA, Baker L, Ottway CJ (1998) Chitin for control of pests and diseases of sugarbeet seedlings. *Aspects of Applied Biology* **52**, 109-114

Enebak A, Carey WA (2000) Evidence for induced systemic protection to *Fusarium* rust in loblolly pine by PGPR. *Plant Disease* **84**, 306-308

Esitken A, Karlidag H, Ercisli S, Turan M, Shin F (2003) The effect of spraying a growth promoting bacterium on the yield, growth and nutrient element composition of leaves of apricot (*Prunus armeniaca* L. cv. Hacihaliloglu). *Australian Journal of Agricultural Research* **54**, 377-380

Estrada-Luna AA, Davies Jr. FT (2003) Arbuscular mycorrhizal fungi influence water relations, gas exchange, abscisic acid and growth of micropropagated chile ancho pepper (*Capsicum annuum*) plantlets during acclimatization and post acclimatization. *Journal of Plant Physiology* **160**, 1073-1083

Foyer CH (1993) Ascorbic acid. In: Alscher RC, Hess JL (Eds) *Antioxidants in Higher Plants*, CRC Press, Boca Raton, pp 31-58

Foyer CH, Lelandais M, Kunert KJ (1994) Photo oxidative stress in plants. *Physiologia Plantarum* **92**, 696-717

Fredrickson JK, Elliott LF (1987) Crop residues as substrate for host specific *Pseudomonas*. *Soil Biology and Biochemistry* **19**, 127-134

Fridovich I (1986) Biological effects of the superoxide radical. *Archives of Biochemistry and Biophysics* **247**, 1-11

Frommel MI, Nowak J, Lazarovits G (1991) Growth enhancement and developmental modifications of *in vitro* grown potato (*Solanum tuberosum* ssp. *tuberosum*) as affected by a non *fluorescent Pseudomonas* sp. *Plant Physiology* **96**, 928-936

García de Salamone IE, Hynes RK, Nelson LM (2001) Cytokinin production by plant growth promoting rhizobacteria and selected mutants. *Canadian Journal of Microbiology* **47**, 404-411

Getha K, Vikineswary S, Wong WH, Seki T, Ward A, Goodfellow M (2005) Evaluation of *Streptomyces* sp. strain g10 for suppression of *Fusarium* wilt and rhizosphere colonization in pot-grown banana plantlets. *Journal of Indian Microbiology and Biotechnology* **32**, 24-32

Glick BR (1995) The enhancement of plant growth by free-living bacteria. *Canadian Journal of Microbiology* **41**, 109-117

Glick BR, Cheng Z, Czarny J, Duan J (2007) Promotion of plant growth by ACC deaminase-producing soil bacteria. *European Journal of Plant Pathology* **119**, 329-339

Glick BR, Liu C, Ghosh S, Dumbroff EB (1997) The effect of the plant growth promoting rhizobacterium *Pseudomonas putida* GR-2 on the development of canola seedlings subjected to various stresses. *Soil Biology and Biochemistry* **29**, 1233-1239

Glick BR, Patten CL, Holguin G, Penrose DM (1999) *Biochemical and Genetic Mechanisms Used by Plant Growth Promoting Bacteria*, Imperial College Press, London, pp 125-140

Goellner K, Conrath U (2008) Priming: it's all the world to induced disease resistance. *European Journal of Plant Pathology* **21**, 233-242

Gooday GW (1990) Physiology of microbial degradation of chitin and chitosan. *Biodegradation* **1**, 177-190

Govindarajan M, Balandreau J, Muthukumarasamy R, Revathi G, Lakshminarasimhan C (2006) Improved yield of micropropagated sugarcane following inoculation by endophytic *Burkholderia vietnamiensis*. *Plant and Soil* **280**, 239-252

Grichko VP, Filby B, Glick BR (2000) Increased ability of transgenic plants expressing the bacterial enzyme ACC deaminase to accumulate Cd, Co, Cu, Ni, Pb, and Zn. *Journal of Biotechnology* **81**, 45-53

Grichko VP, Glick BR (2001) Amelioration of flooding stress by ACC deaminase-containing plant growth-promoting bacteria. *Plant Physiology and Biochemistry* **39**, 11-17

Gutterson N (1990) Microbial fungicides: recent approaches to elucidating the mechanisms. *Critical Reviews in Biotechnology* **10**, 69-91

Hadwiger LA, Kendra DF, Fristensky BW, Wagoner N (1986) Chitosan both activates genes in plants and inhibits RNA synthesis in fungi. In: Muzzarelli RA, Jeuniaux C, Gooday GW (Eds) *Chitin in Nature and Technology*, Plenum Press, New York, pp 209-214

Hammerschmidt R, Kuc J (1995) *Induced Resistance to Disease in Plants*, Kluwer Academic Publishers, Dordrecht, The Netherlands, 182 pp

Harish S, Kavino M, Kumar N, Balasubramanian P, Samiyappan R (2009) Induction of defense-related proteins by mixtures of plant growth promoting endophytic bacteria against *banana bunchy top virus*. *Biological Control* **51**, 16-25

Harish S, Kavino M, Kumar N, Samiyappan R (2009a) Biopriming banana with plant-growth promoting endophytic bacteria induces systemic resistance against *Banana bunchy top virus*. *Acta Horticulturae* **828**, 295-302

Harish S, Kavino M, Kumar N, Samiyappan R (2009b) Differential expression of pathogenesis-related proteins and defense enzymes in banana: Interaction between endophytic bacteria, *Banana bunchy top virus* and *Pentalonia nigronervosa*. *Biocontrol Science and Technology* **19 (8)**, 843-857

Harish S, Kavino M, Kumar N, Saravanakumar D, Soorianathasundaram K, Samiyappan R (2008) Biohardening with plant growth promoting rhizosphere and endophytic bacteria induces systemic resistance against *banana bunchy top virus*. *Applied Soil Ecology* **39**, 187-200

Howie WWJ, Echandi E (1983) Rhizobacteria: Influence of cultivar and soil type on plant growth and yield of potato. *Soil Biology and Biochemistry* **15**, 127-132

Iturbe-Ormaetxe I, Escuredo PR, Arrese-Igor C, Bacana M (1998) Oxidative damage in pea plants exposed to water deficit or paraquat. *Plant Physiology* **116**, 73-181

Jacobson CB, Pasternak JJ, Glick BR (1994) Partial purification and characterization of ACC deaminase from the plant growth-promoting rhizobacterium *Pseudomonas putida* GR12-2. *Canadian Journal of Microbiology* **40**, 1019-1025

Jansa J, Vosátka M (2000) *In vitro* and post vitro inoculation of micropropagated Rhododendrons with ericoid mycorrhizal fungi. *Applied Soil Ecology* **15**, 125-136

Jeon YJ, Kim SK (2000) Production of chitooligosaccharides using an ultrafiltration membrane reactor and their antibacterial activity. *Carbohydrate Polymers* **41**, 133-141

Jetiyanon K, Kloepper JW (2002) Mixtures of plant growth-promoting rhizobacteria for induction of systemic resistance against multiple plant diseases. *Biological Control* **24**, 285-291

Ji C, Kuc J (1996) Antifungal activity of cucumber β-1,3-glucanase and chitinase. *Physiological and Molecular Plant Pathology* **49**, 257-265

Jutidamrongphan W, Anderson JB, Mackinnon G, Manners JM, Simpson RS, Scott KJ (1991) Induction of β-1,3-glucanase in barley in response to infection by fungal pathogens. *Molecular Plant-Microbe Interactions* **4 (3)**, 234-238

Kandan A, Ramiah M, Vasanthi VJ, Radjacommare R, Nandakumar R, Ramanathan A, Samiyappan R (2005) Use of *Pseudomonas fluorescens*-based formulations for management of *Tomato spotted wilt virus* (TSWV) and enhanced yield in tomato. *Biocontrol Science and Technology* **15 (6)**, 553-569

Kandan A, Ramiah R, Radja Commare R, Nandakumar A, Raguchander T, Samiyappan R (2002) Induction of phenyl propanoid metabolism by *Pseudomonas fluorescens* against *Tomato spotted wilt virus* in tomato. *Folia Microbiologica* **47 (2)**, 121-129

Kauffmann S, Legrand M, Geoffroy P, Fritig B (1987) Biological function of pathogenesis-related proteins: four PR proteins of tobacco have β-1, 3-glucanase activity. *European Molecular Biology Organization Journal* **6**, 209-3212

Kavino M (2005) Molecular approaches for the management of banana bunchy top virus through induced systemic resistance in banana. PhD thesis, Tamil Nadu Agricultural University, Coimbatore, India, 335 pp

Kavino M, Harish S, Kumar N, Samiyappan R (2009) Rhizobacteria-mediated growth promotion of banana leads to protection against *Banana bunchy top virus* under field conditions. *Acta Horticulturae* **828**, 69-75

Kavino M, Harish S, Kumar N, Saravanakumar D, Damodaran T, Samiyappan R (2007) Rhizosphere and endophytic bacteria for induction of systemic resistance of banana plantlets against bunchy top virus. *Soil Biology and Biochemistry* **39**, 1087-1098

Kavino M, Harish S, Kumar N, Saravanakumar D, Samiyappan R (2008) Induction of systemic resistance in banana (*Musa* spp.) against *Banana bunchy top virus* (BBTV) by combining chitin with root-colonizing *Pseudomonas fluorescens* strain CHA0. *European Journal of Plant Pathology* **120**, 353-362

Keen NT, Yoshikawa M (1983) β-1,3-endoglucanase from soybean releases elicitor-active carbohydrates from fungus cell wall. *Plant Physiol*ogy **71**, 460-465

Klessig DF, Malamy J (1994) The salicylic acid signal in plants. *Plant Molecular Biology* **26**, 1439-1458

Kloepper JW (1996) Host specificity in microbe–microbe interactions. *Bioscience* **46**, 14-18

Kloepper JW, Schroth MN (1981) Plant growth promoting rhizobacteria and plant growth under gnotobiotic conditions. *Phytopathology* **71**, 642-646

Kloepper JW, Zablotoowicz RM, Tipping EM, Lifshitz R (1991) Plant growth promotion mediated by bacterial rhizosphere colonizers. In: Keister DL, Cregan PB (Eds) *The Rhizosphere and Plant Growth*, Kluwer, Dordrecht, pp 315-326

Koch E, Meier BM, Eiben HG, Slusarenko A (1992) A lipoxygenase from leaves of tomato (*Lycopersicon esculentum* Mill.) is induced in response to plant pathogenic pseudomonads. *Plant Physiology* **99**, 571-576

Kokalis-Burelle N (2003) Effects of transplant type, plant growth-promoting rhizobacteria, and soil treatment on growth and yield of strawberry in Florida. *Plant and Soil* **256**, 273-280

Kokalis-Burelle N, Backman PA, Rodríguez-Kabana R, Ploper LO (1992) Potential for biological control of early leaf spot of peanut using *Bacillus cereus* and chitin foliar amendments. *Biological Control* **2**, 321-352

Kokalis-Burelle N, Kloepper JW, Reddy MS (2006) Plant growth-promoting rhizobacteria as transplant amendment and their effects on indigenous rhizosphere microorganisms. *Applied Soil Ecology* **31**, 91-100

Kokalis-Burelle N, Vavrina CS, Reddy MS, Kloepper JW (2003) Amendment of muskmelon and watermelon transplant media with plant growth-promoting rhizobacteria: effects on seedling quality, disease and nematode resistance. *HortTechnology* **13**, 476-482

Kokalis-Burelle N, Vavrina CS, Rosskopf EN, Shelby RA (2002) Field evaluation of plant growth-promoting rhizobacteria amended transplant mixes and soil solarization for tomato and pepper production in Florida. *Plant and Soil* **238**, 257-266

Kurze S, Bahl H, Dahl R, Berg G (2001) Biological control of fungal strawberry diseases by *Serratia plymuthica* HRO-C48. *Plant Disease* **85**, 529-534

Lafontaine PJ, Benhamou N (1996) Chitosan treatment. An emerging strategy for enhancing resistance of greenhouse tomato plants to infection by *Fusarium oxysporum* f.sp. *radicis-lycopersici*. *Biocontrol Science and Technology* **6**, 111-124

Lazarovits G, Nowak J (1997) Rhizobacteria for improvement of plant growth and establishment. *HortScience* **32**, 188-192

Leeman M, Den Ouden FM, van Pelt JA, Dirkx FPM, Steijl H, Bakker PAHM, Schippers B (1996) Iron availability affects induction of systemic resistance to *Fusarium* wilt of raddish by *Pseudomonas fluorescens*. *Phytopathology* **86**, 149-155

Leeman M, Van Pelt JA, Den Ouden FM, Heinsbroek M, Bakker PAHM, Schippers B (1995) Induction of systemic resistance against fusarium wilt of radish by lipopolysaccharides of *Pseudomonas fluorescens*. *Phytopathology* **85**, 1021-1027

Leuba JL, Stossel P (1986) Chitosan and their polymers: antifungal activity and interaction with biological membranes. In: Muzzarelli R, Jeauniaux C, Gooday GW (Eds) *Chitin in Nature and Technology*, Plenum Press, New York, pp 215-222

Lian J, Wang Z-F, Cao L-X, Tan H-M, Inderbitzin P, Jiang Z, Zhou S-N (2009) Artificial inoculation of banana tissue culture plantlets with indigenous endophytes originally derived from native banana plants. *Biological Control* **51**, 427-434

Liddycoat SM, Greenberg BM, Wolyn DJ (2009) The effect of plant growth-promoting rhizobacteria on asparagus seedlings and germinating seeds subjected to water stress under greenhouse conditions. *Canadian Journal of Microbiology* **55 (4)**, 388-394

Liu L, Kloepper JW, Tuzun S (1995a) Induction of systemic resistance in cucumber against *Fusarium* wilt by plant growth promoting rhizobacteria. *Phytopathology* **85**, 695-698

Liu L, Kloepper JW, Tuzun S (1995b) Induction of systemic resistance in cucumber against bacterial leaf spot by plant growth promoting rhizhobacteria. *Phytopathology* **85**, 843-847

Liu Z, Pillay V, Nowak J (1995) *In vitro* culture of watermelon and cantaloupe with and without beneficial bacterium. *Acta Horticulturae* **402**, 58-60

Lucy M, Reed E, Glick BR (2004) Applications of free living plant growth-promoting rhizobacteria. *Antonie Van Leeuwenhoek* **86 (1)**, 1-25

Lugtenberg B, Kamilova F (2009) Plant-growth-promoting rhizobacteria. *Annual Review of Microbiology* **63**, 541-56

M'Piga P, Bélanger RR, Paulitz TC, Benhamou N (1997) Increased resistance to *Fusarium oxysporum* f. sp. r*adicis-lycopersici* in tomato plants treated with the endophytic bacterium *Pseudomonas fluorescens* strain 63–28. *Physiological and Molecular Plant Pathology* **50**, 301-320

Martinez-Esteso MJ, Sellés-Marchart S, Vera-Urbina JC, Pedreño MA, Bru-Martinez R (2009) Changes of defense proteins in the extracellular proteome of grapevine (*Vitis vinifera* cv. Gamay) cell cultures in response to elicitors. *Journal of Proteomics* **73 (2)**, 31-41

Mauch F, Staehelin LA (1989) Functional implications of the subcellular localization of ethylene-induced chitinase and β-1,3-glucanase in bean leaves. *Plant Cell* **1**, 447-457

Maurhofer M, Hase C, Meuwly P, Métraux JP, Défago G (1994) Induction of systemic resistance of tobacco to *Tobacco necrosis virus* by the root-colonizing *Pseudomonas flourescens* strain CHA0: Influence of the *gac*A gene and of pyoverdine production. *Phytopathology* **84**, 139-146

Mayak S, Tirosh T, Glick BR (2004a) Plant growth-promoting bacteria that confer resistance to water stress in tomato and pepper. *Plant Science* **166**, 525-530

Mayak S, Tirosh T, Glick BR (2004b) Plant growth-promoting bacteria that confer resistance in tomato and pepper to salt stress. *Plant Physiology and Biochemistry* **167**, 650-656

Maziah M, Zuraida AR, Halimi MS, Zulkifli HS, Sreeramanan S (2010) Influence of boron on the growth and biochemical changes in plant growth promoting rhizobacteria (PGPR) inoculated banana plantlets. *World Journal of Microbiology and Biotechnology* **26 (5)**, 933-944

Mehnaz S, Weselowski B, Aftab F, Zahid S, Lazarovits G, Iqbal J (2009) Isolation, characterization, and effect of fluorescent pseudomonads on micropropagated sugarcane. *Canadian Journal Microbiology* **55 (8)**, 1007-1011

Mello MRF, Assis SMP, Mariano RLR, Camara TR, Menezes M (2000)

Screening of bacteria and bacterization methods for growth promotion of micropropagated pineapple plantlets. Available online: http://www.ag.auburn.edu/argentina/pdfmanuscripts/mello.pdf

Mendoza AR, Sikora RA (2009) Biological control of *Radopholus similis* in banana by combined application of the mutualistic endophyte *F. oxysporum* strain 162, the egg pathogen *Paecilomyces lilacinus* strain 251 and the antagonistic bacteria *Bacillus firmus*. *BioControl* **54 (2)**, 263-272

Mirza MS, Ahmad W, Latif F, Haurat J, Bally R, Normand P, Malik KA (2001) Isolation, partial characterization, and the effect of plant growth-promoting bacteria (PGPB) on micro propagated sugarcane *in vitro*. *Plant and Soil* **237**, 47-54

Mucciarelli M, Scannerini S, Bertea C, Maffei M (2003) *In vitro* and *in vivo* peppermint (*Mentha piperita*) growth promotion by nonmycorrhizal fungal colonization. *New Phytologist* **158**, 579-591

Müller H, Berg G (2008) Impact of formulation procedures on the effect of the biocontrol agent *Serratia plymuthica* HRO-C48 on *Verticillium* wilt in oilseed rape. *BioControl* **53**, 905-916

Nakkeeran S, Fernando WGD, Siddiqui Z (2005) Plant growth promoting rhizobacteria formulations and its scope in commercialization for the management of pests and diseases. In: Siddique ZA (Ed) *PGPR: Biocontrol and Biofertilization*, Springer Science, Dordrecht, The Netherlands, pp 257-296

Nandakumar R (1998) Induction of systemic resistance in rice with fluorescent pseudomonads for the management of sheath blight disease. MSc thesis, Tamil Nadu Agricultural University, Coimbatore, India, 105 pp

Nowak J, Asiadu SK, Lazarovits G, Pillay V, Stewart A, Smith C, Liu Z (1995) Enhancement of *in vitro* growth and transplant stress tolerance of potato and vegetable plantlets co-cultured with a plant growth promoting pseudomonad bacterium. In: Carré F, Chagvardieff P (Eds) *Ecophysiology and Photosynthetic in Vitro Cultures*, Commissariat l'Energie Atomique, France, pp 173-179

Nowak J, Asiedu SK, Bensalim S, Richards J, Stewart A, Smith C, Stevens D, Sturz AV (1997) From laboratory to applications: challenges and progress with *in vitro* dual cultures of potato and beneficial bacteria. In: Cassells AC (Ed) *Pathogen and Microbial Contamination Management in Micropropagation*, Kluwer Academic Publications, Dordrecht, pp 321-329

Nowak J (1998) Benefits of *in vitro* "biotization" of plant tissue cultures with microbial inoculants. *In Vitro Cellular and Developmental Biology – Plant* **34**, 122-130

Nowak J, Bensalim S, Smith CD, Dunbar C, Asiedu SK, Madani A, Lazarovits G, Northcott D, Sturz AV (1999) Behaviour of plant material issued from *in vitro* bacterization. *Potato Research* **42**, 505-519

Nowak J, Pruski K (2002) Priming tissue cultured propagules. In: Low cost options for tissue culture technology in developing countries. *Proceedings of a Technical meeting organized by the Joint FAO/IAEA Division of nuclear Techniques in food and agriculture*, 26-30 August, Vienna, pp 69-81

Nowak J, Shulaev V (2003) Priming for transplant stress resistance in *in vitro* propagation. *In Vitro Cellular and Developmental Biology – Plant* **39 (2)**, 107-124

Nowak J, Sharma VK, A'Hearn E (2004) Endophyte enhancement of transplant performance in tomato, cucumber and sweet pepper. *Acta Horticulturae* **631**, 253-263

Ortíz-Castro R, Contreras-Cornejo HA, Macías-Rodríguez L, López-Bucio J (2009) The role of microbial signals in plant growth and development. *Plant Signaling and Behavior* **4 (8)**, 701-712

Pandey A, Palni LMS, Bag N (2000) Biological hardening of tissue culture raised tea plants through rhizosphere bacteria. *Biotechnology Letters* **22**, 1087-1091

Paparu P, Dubois T, Coyne D, Viljoen A (2009) Dual inoculation of *Fusarium oxysporum* endophytes in banana: effect on plant colonization, growth and control of the root burrowingnematode and the banana weevil. *Biocontrol Science and Technology* **19 (6)**, 639-655

Paparu P, Dubois T, Gold CS, Niere B, Adipala E, Coyne D (2007) Screen house and field persistence of non pathogenic endophytic *Fusarium oxysporum* in *Musa* tissue culture plants. *Microbial Ecology* **55**, 561-568

Pierson EA, Weller DM (1994) Use of mixtures of fluorescent pseudomonads to suppress take-all and improve the growth of wheat. *Phytopathology* **84**, 940-947

Pieterse CMI, van Wees SCM, Hoffland E, van Pelt JA, van Loon LC (1996) Systemic resistance in *Arabidopsis* induced by biocontrol bacteria is independent of salicylic acid accumulation and pathogenesis-related gene expression. *Plant Cell* **8**, 1225-1237

Pillay VK, Nowak J (1997) Inoculum density, temperature and genotype effects on epiphytic and endophytic colonization and *in vitro* growth promotion of tomato (*Lycopersicon esculentum* L.) by a pseudomonad bacterium. *Canadian Journal of Microbiology* **43,** 354-361

Powell KA, Rhodes DJ (1994) Strategies for the progression of biological fungicides into field evaluation. *British Crop Protection Council Monograph Number* **59**, 307-315

Raaijmakers JM, Paulitz TC, Steinberg C, Yvan CA, Moënne-Loccoz (2009) The rhizosphere: a playground and battlefield for soilborne pathogens and beneficial microorganisms. *Plant and Soil* **321**, 341-361

Radjacommare R, Nandakumar R, Kandan A, Suresh S, Bharathi M, Raguchander T, Samiyappan R (2002) *Pseudomonas fluorescens* based bioformulation for the management of sheath blight and leaffolder in rice. *Crop Protection* **21**, 671-677

Ramamoorthy V, Raguchander T, Samiyappan R (2002a) Induction of defense-related proteins in tomato roots treated with *Pseudomonas fluorescens* Pf1 and *Fusarium oxysporum* f.sp. *lycopersici*. *Plant and Soil* **239**, 55-68

Ramamoorthy V, Raguchander T, Samiyappan R (2002b) Enhancing resistance of tomato and hot pepper to *Pythium* diseases by seed treatment with fluorescent pseudomonads. *European Journal of Plant Pathology* **108**, 429-441

Ramamoorthy V, Samiyappan R (2001) Induction of defense-related genes in *Pseudomonas fluorescens* treated chilli plants in response to infection by *Colletotrichum capsici*. *Journal of Mycology and Plant Pathology* **31**, 146-155

Ramamoorthy V, Viswanathan R, Raguchander T, Prakasam V, Samiyappan R (2001) Induction of systemic resistance by plant growth promoting rhizobacteria in crop plants against pest and diseases. *Crop Protection* **20**, 1-11

Raupach GS, Liu L, Murphy JF, Tuzun S, Kloepper JW (1996) Induced systemic resistance in cucumber and tomato against *Cucumber mosaic cucumovirus* using plant growth-promoting rhizobacteria (PGPR). *Plant Dis*ease **80**, 91-94

Reis VM, Olivares FL, Martinez de Oliveira AL, dos Reis Jr. FB, Baldani JI, Döbereiner J (1999) Technical approaches to inoculate micropropagated sugarcane plants with *Acetobacater diazotrophicus*. *Plant and Soil* **206**, 205-211

Remans R, Beebe S, Blair M, Manrique G, Tovar E, Rao I, Croonenborghs A, Torres-Gutierrez R, El-Howeity M, Michiels J, Vanderleyden J (2008) Physiological and genetic analysis of root responsiveness to auxin-producing plant growth-promoting bacteria in common bean (*Phaseolus vulgaris* L.). *Plant and Soil* **302**, 149-161

Rodríguez-Romero AS, Pinero Guerra MS, Vega MCJ (2005) Effect of arbuscular mycorrhizal fungi and rhizobacteria on banana growth and nutrition. *Agronomy for Sustainable Development* **25**, 395-399

Ryu CM, Hu CH, Reddy MS, Kloepper JW (2003) Different signaling pathways of induced resistance by rhizobacteria in *Arabidopsis thaliana* against two pathovars of *Pseudomonas syringae*. *New Phytologist* **160**, 413-420

Ryu CM, Murphy JF, Mysore KS, Kloepper JW (2004) Plant growth-promoting rhizobacteria systemically protect *Arabidopsis thaliana* against *Cucumber mosaic virus* by a salicylic acid and NPR1-independent and jasmonic acid-dependent signaling pathway. *The Plant Journal* **39**, 381-392

Saravanakumar D, Lavanya N, Muthumeena K, Raguchander T, Samiyappan R (2009) Fluorescent pseudomonad mixtures mediate disease resistance in rice plants against sheath rot (*Sarocladium oryzae*) disease. *Biocontrol* **54 (2)**, 273-286

Saravanakumar D, Samiyappan R (2007) ACC deaminase from *Pseudomonas fluorescens* mediated saline resistance in groundnut (*Arachis hypogea*) plants. *Journal of Applied Microbiology* **102**, 1283-1292

Saravanakumar D, Vijayakumar C, Kumar N, Samiyappan R (2007) PGPR induced defense responses in tea plants against blister blight disease. *Crop Protection* **26**, 556-565

Schlumbaum A, Mauch F, Vogeli WR, Boller T (1986) Plant chitinases are potent inhibitors of fungal growth. *Nature* **324**, 365-367

Schneider S, Ullrich WR (1994) Differential induction of resistance and enhanced enzyme activities in cucumber and tobacco caused by treatment with various abiotic and biotic inducers. *Physiological and Molecular Plant Pathology* **45**, 291-304

Sheehy RE, Honma M, Yamada M, Sasaki T, Martineau B, Hiatt WR (1991) Isolation, sequence, and expression in *Escherichia coli* of the *Pseudomonas* sp. strain ACP gene encoding 1-aminocyclopropane-1-carboxylate deaminase. *Journal of Bacteriology* **173**, 5260-5265

Smith L, Keefe DO, Smith M, Hamill S (2003) The benefits of applying rhizobacteria to tissue cultured bananas. *Banana Topics Newsletter* **33**, 1-4

Solano BR, Maicas JB, Pereyra de la Iglesia MT, Domenech J, Gutiérrez Mañero FJ (2008) Systemic disease protection elicited by Plant Growth Promoting Rhizobacteria strains: relationship between metabolic responses, systemic disease protection, and biotic elicitors. *Phytopathology* **98**, 451-457

Srinath J, Bagyaraj DJ, Satyanarayana BN (2003) Enhanced growth and nutrition of micropropagated *Ficus benjamina* to *Glomus mosseae* co-inoculated with *Trichoderma harzianum* and *Bacillus coagulans*. *World Journal of Microbiology and Biotechnology* **19**, 69-72

Subba Rao NS (Ed) (1982) *Biofertilizers.* In: *Advances in Agricultural Microbiology*, Oxford and IBM Publishing Co. New Delhi, pp 219-242

Sudhakar C, Lakshmi A, Giridarakumar S (2001) Changes in the antioxidant enzyme efficacy in two high yielding genotypes of mulberry (*Morus alba* L.) under NaCl salinity. *Plant Science* **161**, 613-619

Thaler JS, Stout MJ, Karban R, Duffey SS (1996) Exogenous jasmonates simulate insect wounding in tomato plants, *Lycopersicon esculentum*, in the laboratory and field. *Journal of Chemical Ecology* **22**, 1767-1781

Thomashow LS, Weller DM (1998) Role of a phenazine antibiotic from *Pseudomonas fluorescens* in biological control of *Geumannomyces graminis* var. *tritici*. *Journal of Bacteriology* **170**, 3499-3508

Thompson JE, Legge RL, Barber RL (1987) The role of free radicals in senescence and wounding. *New Phytol*ogist **105**, 317-334

Ting ASY, Meon S, Kadir J, Radu S, Singh G (2008) Endophytic microorganisms as potential growth promoters of banana. *Biocontrol* **53**, 541-553

Trivedi P, Pandey A (2007) Biological hardening of micropropagated *Picrorhiza kurrooa* Royal ex Benth., an endangered species of medical importance. *World Journal of Microbiology and Biotechnology* **23**, 877-878

Troxler J, Berling CH, Moënne-Loccoz Y, Keel C, Défago G (1997) Interactions between the biocontrol agent *Pseudomonas fluorescens* CHA0 and *Thielaviopsis basicola* in tobacco roots observed by immunofluorescence microscopy. *Plant Pathology* **46**, 62-71

Udaya Shankar AC, Chandra Nayaka S, Niranjan-Raj S, Bhuvanendra Kumar H, Reddy MS, Niranjana SR, Prakash HS (2009) Rhizobacteria-mediated resistance against the blackeye cowpea mosaic strain of bean common mosaic virus in cowpea (*Vigna unguiculata*). *Pest Management Science* **65 (10)**, 1059-1064

Utkhede RS, Smith EM (1992) Promotion of apple tree growth and fruit production by the EBW-4 strain of *Bacillus subtilis* in apple replant disease soil. *Canadian Journal of Microbiology* **38**, 1270-1273

van Loon LC (1997) Induced resistance in plants and the role of pathogenesis-related proteins. *European Journal of Plant Pathology* **103**, 753-765

van Loon LC (2007) Plant responses to plant growth-promoting rhizobacteria. *European Journal of Plant Pathology* **119**, 243-254

van Loon LC, Pierpoint WS, Boller T, Conejero V (1994) Recommendations for naming plant pathogenesis-related proteins. *Plant Molecular Biology Reporter* **12**, 245-264

van Overbeek LS, van Elsas JD (1995) Root exudates-induced promoter activity in *Pseudomonas fluorescens* mutants in the wheat rhizosphere. *Applied and Environmental Microbiology* **61**, 890-898

van Peer R, Niemann GJ, Schippers B (1991) Induced resistance and phytoalexin accumulation in biological control of *Fusarium* wilt of carnation by *Pseudomonas* sp strain WCS 417r. *Phytopathology* **81**, 728-734

Vavrina CS (2004) Plant growth promoting rhizobacteria via a transplant plug delivery system in the production of drip irrigated pepper. Available online: http://www.imok.ufl.edu/veghort/docs/sta_rpt_veg 996.pdf

Vega MDC, Romero ASR, Guerra MSP (2004) Potential use of rhizobacteria from the *Bacillus* genus to stimulate the plant growth of micro propagated bananas. *Fruits* **59**, 83-90

Verhagen BW, Trotel-Aziz P, Couderchet M, Höfte M, Aziz A (2010) *Pseudomonas* spp.-induced systemic resistance to *Botrytis cinerea* is associated with induction and priming of defense responses in grapevine. *Journal of Experimental Botany* **61 (1)**, 249-260

Vestberg M, Kukkonen S, Saari K, Parikka P, Huttunen J, Tainio L, Devos N, Weekers F, Kevers C, Thonart P, Lemoine MC, Cordier C, Alabouvette C, Gianinazzi S (2004) Microbial inoculation for improving the growth and health of micropropagated strawberry. *Applied Soil Ecology* **27**, 243-258

Vicedo B, Flors V, de la O Leyva M, Finiti I, Kravchuk Z, Real MD, García-Agustín P, González-Bosch C (2009) Hexanoic acid-induced resistance against *Botrytis cinerea* in tomato plants. *Molecular Plant Microbe Interactions* **22 (11)**, 1455-1465

Vidal MT, Azcón-Aguilar C, Barea JM (1992) Mycorrhizal inoculation enhances growth and development of micropropagated plants of Avocado. *HortScience* **27 (7)**, 785-787

Vidhyasekaran P, Rabindran R, Muthamilan M, Nayar K, Rajappan K, Subramanian N, Vasumathi K (1997) Development of powder formulation of *Pseudomonas fluorescens* for control of rice blast. *Plant Pathol*ogy **46**, 291-297

Viswanathan R (1999) Induction of systemic resistance against red rot disease in sugarcane by plant growth promoting rhizobacteria. PhD thesis, Tamil Nadu Agricultural University, Coimbatore, India, 175 pp

Viswanathan R, Samiyappan R (1999) Induction of systemic resistance by plant growth promoting rhizobacteria against red rot disease caused by *Colletotrichum falcatum* Went. in sugarcane. *Proceedings of the Sugar Technologists' Association of India* **61**, 24-39

Viswanathan R, Samiyappan R (2001) Antifungal activity of chitinase produced by some fluorescent pseudomonads against *Colletotrichum falcatum* Went causing red rot disease in sugarcane. *Microbiological Research* **155**, 309-314

Vivekananthan R, Ravi M, Ramanathan A, Samiyappan R (2004) Lytic enzymes induced by *Pseudomonas fluorescens* and other biocontrol organisms mediate defence against the anthracnose pathogen in mango. *World Journal of Microbiology and Biotechnology* **20**, 235-244

Vosatka M, Gryndler M, Jansa J, Vohnik M (2000) Post *vitro* mycorrhization and bacterization of micropropagated strawberry, potato and azalea. *Acta Horticulturae* **530**, 313-324

Vosatka M, Gryndler M, Prikryl Z (1992) Effect of the rhizosphere bacterium *Pseudomonas putida*, VAM fungi and substratum composition on the growth of strawberry. *Agronomie* **12**, 859-863

Walters D, Walsh D, Newton A, Lyon G (2005) Induced resistance for plant disease control: Maximizing the efficacy of resistance elicitors. *Phytopathology* **95**, 1368-1373

Walters DR, Fountaine JM (2009) Practical application of induced resistance to plant diseases: an appraisal of effectiveness under field conditions. *Journal of Agricultural Science* **147**, 523-535

Wang C, Knill E, Glick BR, De'fago G (2000) Effect of transferring 1-aminocyclopropane-1-carboxylic acid (ACC) deaminase genes into *Pseudomonas fluorescens* strain CHA0 and its gacA derivative CHA96 on their growth-promoting and disease-suppressive capacities. *Canadian Journal of Microbiology* **46**, 898-907

Wang S, Wu H, Qiao J, Ma L, Liu J, Xia Y, Gao X (2009) Molecular mechanism of plant growth promotion and induced systemic resistance to *Tobacco mosaic virus* by *Bacillus* spp. *Journal of Microbiology and Biotechnology* **19 (10)**, 1250-1258

Weber OB, Muniz CR, Vitor AO, Freire FCO, Oliveira VM (2007) Interaction of endophytic diazotrophic bacteria and *Fusarium oxysporum* f. sp. *cubense* on plantlets of banana cv. Maça. *European Journal of Plant Pathology* **298**, 47-56

Wei G, Kloepper JW, Tuzun S (1991) Induction of systemic resistance of cucumber to *Colletotrichum orbiculare* by select strains of plant growth promoting rhizobacteria. *Phytopathology* **81**, 1508-1512

Wei G, Kloepper JW, Tuzun S (1996) Induced systemic resistance to cucumber diseases and increased plant growth by plant growth-promoting rhizobacteria under field conditions. *Phytopathology* **86**, 221-224

Whipps JM (2001) Microbial interactions and biocontrol in the rhizosphere. *Journal of Experimental Botany* **52**, 487-511

Xue L, Charest PM, Jabaji-Hare SH (1998) Systemic induction of peroxidases, β-1, 3-glucanases, chitinases and resistance in bean plants by binucleate *Rhizoctonia* species. *Phytopathology* **88**, 359-365

Yang J, Kloepper JW, Ryu CM (2009) Rhizosphere bacteria help plants tolerate abiotic stress. *Trends in Plant Science* **14 (1)**, 1-4

Ye XS, Pan SQ, Kuc J (1990) Association of pathogenesis-related proteins and activities of peroxidase, β-1,3-glucanase and chitinase with systemic induced resistance to blue mould of tobacco but not to systemic tobacco mosaic virus. *Physiological and Molecular Plant Pathology* **36**, 523-531

Zdor RE, Anderson AJ (1992) Influence of root colonizing bacteria on the defense responses in bean. *Plant and Soil* **140**, 99-107