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The Root Endophytic Fungus *Piriformospora indica* as a Bio-Hardening Agent for Tissue Cultured Plantlets

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Abstract

A critical stage of micropropagation is the transfer of *in vitro* raised plantlets to *ex vitro* conditions. Transient transplant shock during the transfer of plantlets is the major reason for the high rate of mortality. Many plants usually will not recover from stunted growth, and they are often attacked by soil-borne microbes. The root endophytic fungus, *Piriformospora indica* has been used as a potential tool for bio-hardening in tissue culture plantlets to cope with these problems. *P. indica* enhance nutrient uptake, facilitate plants to survive under stress conditions and stimulate plant growth. In tissue culture plants, it helps in biological hardening by providing protection against shock of transplantation and root pathogens. Tissue culture plants inoculated with *P. indica* are better in terms of plant biomass and rate of survival. Therefore, *P. indica* with multiple beneficial traits can be used as a bio-protectant in commercial tissue culture.

Introduction

Tissue culture or micropropagation, the technique of producing plantlets from tissues on artificial media under controlled environmental conditions, has been used for the production of about 50% of flori-horticultural plants. The uniformity of plants and greater synchrony of flowering and fruiting in the field make the technique popular for a wide scale commercial use. Moreover, the production of high-quality pathogen-free planting materials on a large scale throughout the seasons increases the acceptability of micropropagation.

A critical stage of micropropagation is the transfer of *in vitro* raised plantlets to *ex vitro* conditions. Acclimatization and hardening in micro propagated plants could be accomplished as a one-step procedure within a short period of time before transplanting. Time of harvesting of the micro shoots, shoot size, soil pH, treatments with plant growth regulators, CO_2 enrichment and light conditions in specially designed hardening chambers have impacts on the success of hardening in micropropagation. Acclimatization phase encounters problems in survival and development of plantlets. About 10-40% of plantlets either die or do not attain market standards thereby causing significant losses at commercial level. The mortality rate is also increased by the transient transplant shock on the transfer of plantlets from culture media to the field. Many plantlets transferred to *ex vitro* conditions have non-functional stomata, weak root systems and poorly developed cuticles and photosynthetic inefficiency. Stunted growth of the plants often leads to non-recovery and they are often attacked by soil-borne plant pathogens.

Bio-Hardening

Research on the control of environmental conditions including both physical and chemical parameters have resulted in reduction in mortality in plantlets and their growth performance at the acclimatization stage. Bio-hardening, a biological approach that reduces the stress of acclimatization which provides faster growth of plantlets is gaining popularity as a powerful tool. Tissue culture plants subjected to biotization/ bio-hardening undergoes physiological and developmental changes, resulting in enhanced biotic and abiotic stress resistance when inoculated with beneficial microbes. Over the past few years, *Glomus aggregatium, Trichoderma harazianum, Piriformspora indica, Azospirillum, Pseudomonas fluorescens* and actinomycetes have been used as bio-hardening agents to cope with these problems.

Association with beneficial fungi, if established during the early stage of acclimatization, can reduce the stress of acclimatization and promote the growth of micro propagated plantlets. Inoculation with arbuscular mycorrhizal fungi (AMF) (Glomeromycota) and *P. indica* (Basidiomycota) has proven to be a promising alternative for the production of plantlets of superior quality. The beneficial effects of these mutualistic symbionts include improved nutrient uptake by the host plants, increased tolerance to biotic and abiotic stress including drought, salt and heavy metals, and higher productivity.

Piriformospora indica

P. indica, one of the most important root endophytic fungi, a member of the Sebacinales, was discovered in the Indian Thar desert of Rajasthan. *P. indica* enhanced nutrient uptake, facilitate plants to survive in extreme drought, temperature and salt conditions, imparts systemic resistance to toxins, acts as bio-fertilizer, bio-protector, bio-herbicide, immune-modulator, phytoremediator, stimulator of growth with increased seed production, and plays a key role in increasing the tolerance to herbivorous insects. It helps in biological hardening to tissue culture plants, provides protection against shock of transplantation and pathogens affecting root system. Effect of *P. indica* has been studied in more than 150 plants. Promising outputs of laboratory experiments and small field trials indicated the need for its mass cultivation and usage. Hence, it provides a good model organism for the investigations of beneficial plant-microbe interactions.

Unlike AMF, which is an obligate symbiont, the root endophyte *P. indica* can be axenically cultivated under laboratory conditions using routine microbiological media (Figure 1A and 1B). Therefore, mass multiplication, application and commercial exploitation of the fungus become easy and feasible.

P. indica as a Bio-Hardening Agent

P. indica has been used as a potential tool for bio-hardening in tissue culture plants. Sahay and Varma (1999) reported that tissue culture raised tobacco plantlets inoculated with *P. indica* are better in terms of plant biomass, percent root colonization and rate of survival. It was grown in a culture bottle containing minimal medium for 7 days in the dark and the regenerated shoots and the regenerated plantlets were co-cultured for 10-15 days. During the acclimatization process, *P. indica*-inoculated plants showed more than 90% survival rate and control plantlets had a moderately low survival rate of 62%. The interaction between AMF and *P. indica* improved the growth and nutrient uptake in micropropagation-derived pineapple plantlets and this bio-hardening proved to be a promising method that can be employed to produce high quality propagative material for the market (Moreira *et al*., 2015).

Black pepper (*Piper nigrum*), one of the most important spice crops of India is a host to the beneficial fungus *P. indica* (Anith *et al*., 2011). It was reported by our group that bio-inoculation with *P. indica* improves growth, yield and piperine content in black pepper (Anith *et al*., 2018). It has also been used as a bio-hardening agent in black pepper (Figure 1).

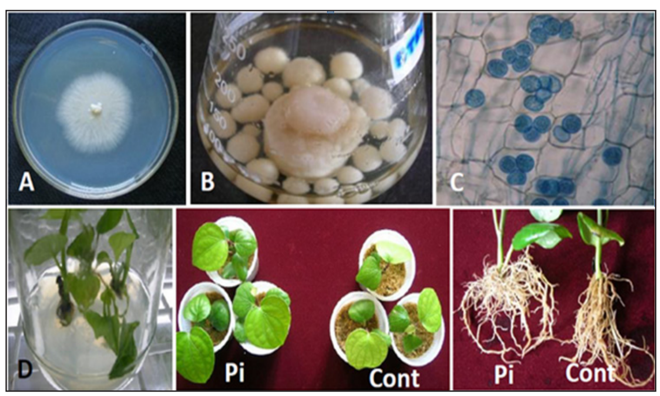

Figure 1: Bio-hardening of tissue cultured plantlets of black pepper [A) *P. indica* grown on PDA; B) *P. indica* in PDB; C) Chlamydospores of *P. indica* within cortex region of black pepper roots; D) Tissue cultured plantlets of black pepper; Pi - TC plantlets of black pepper inoculated with *P. indica*; Cont - Uninoculated TC plantlets]

P. indica treated tissue culture plantlets of black pepper showed a higher percentage survival of 76.6% when compared to untreated control where it was only 58.3%. Plant growth parameters including number of leaves and fresh weight of plants, observed 30 days after transplanting was significantly higher in *P. indica* treated plants (Table 1 and 2).

Table 1: Hardening of black pepper TC plants using different bio and chemical agents

Treatment	% Survival	
	After 15 days	After 45 days
P. indica	78.3	76.6
Bacillus pumilus	70.0	58.3
Pseudomonas fluorescens PN 026	76.6	76.6
Bavistin	91.6	91.6
Control	70.0	58.3

Table 2: Growth characters of black pepper TC plants during hardening (30 days after planting)

Treatment	No. of leaves	Plant height (cm)	Fresh weight of Plants (g)
P. indica	4.90	5.04	1.68
Bacillus pumilus	3.57	4.84	1.37
Pseudomonas fluorescens PN 026	3.80	4.09	1.36
Bavistin	4.00	3.79	1.59
Control	3.30	7.00	1.14

Conclusion

Research findings support the assumption that bio-inoculation with mutualistic symbionts is useful in micropropagation techniques for better survival and development of healthy plants during the acclimatization stage. It is achievable by treatment with several beneficial fungi at an early stage of acclimatization by carrying out fungal treatment immediately after transfer from the closed culture vessel to the hardening chamber. The plant growth promoting beneficial root endophytic fungus *P. indica* appears to be a suitable bio-protectant during the hardening stages of many of the micro propagated plantlets. An added advantage with *P. indica* colonization would be improved growth performance of the plants in the main field as well.

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