

International Journal of Scientific Research and Reviews

Enhancing Acclimatization of Tissue Cultured Plants By Biotization- A Review

G.Indravathi^{1,2*} and P.SureshBabu³

¹Dept. of Biotechnology, KVR Govt. College for Women(A), Kurnool, Andhra Pradesh, India.

²Dept. of Biotechnology, Jawaharlal Nehru Technological University, Anantapur,
Andhra Pradesh, India.

³ Department of Biology, Indian Institute of Science Education and Research, Tirupati, Andhra Pradesh, India.

ABSTRACT

The plants raised from tissue culture experience high mortality following laboratory to land transfer. Apart from the abiotic causes, one major cause of high mortality of such aseptically raised plants is their sudden exposure particularly the root system to microbial communities, including soil pathogens. Efficient antagonistic plant growth-promoting microbes have been used for hardening to increase the survival and to augment overall plant growth. Biotization is the metabolic response of *inviter* grown plant material to microbial no Colum. My corrhization of tissue cultured plants is believed to provide an advantage to the transplanted propagates in terms of nutrient availability, soil pH, aeration and protection from water stress. The tissue culture raised plants when treated with bacterial inoculants produce plant growth promoting substances and some secondary metabolites which enhance nutrient uptake and provide resistance against pathogens. multimicrobial biotization is inoculation of more than one microbial species to micro plants. Plantlet survival rate was maximum in dual inoculation, this mustbedue to the positive interaction between two species and their ability to enhance stress tolerance by protecting them from subsequent 'trans plantation shock'. Thus biological hardening envisages physical, chemical and environmental conditioning of the micro propagated plantlets. Differentparameters were reported for control and biotized plantlets to know the effect of microbes on plant performance. This review is focused on the effect of biotization on enhancing acclimatization of tissue culture raised plants.

KEY WORDS: Biotization, acclimatization, micro propagation, biotic factors.

***Corresponding author:**

G. Indravathi

Dept. of Biotechnology,

KVR Govt. College for Women(A),

Kurnool - 518002

Andhra Pradesh, India.

E-mail: gindravathi@gmail.com, Mobile : 9989067747

INTRODUCTION:

Tissue culture raised plants experience high mortality following laboratory to land transfer. One major reason of high mortality of aseptically raised plants apart from the abiotic factors is their sudden exposure of their root system to microbial communities, present in the soil. The primary target of several research groups is to induce stress resistance by utilizing microbial inoculants during micropropagation¹.

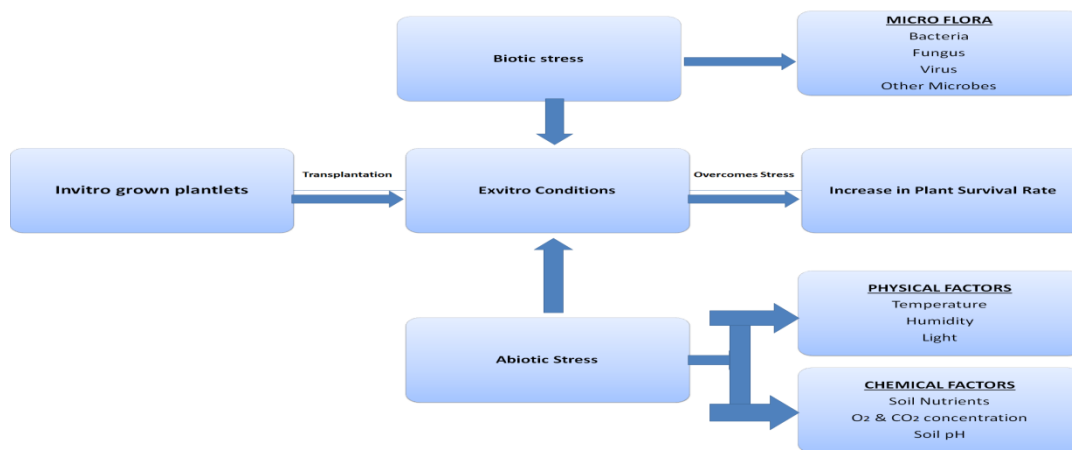
Biotization is the metabolic response of in vitro grown plant material to microbial inoculums, leading to the morphological and physiological development and providing biotic and abiotic stress resistance. Biotization is an emerging field of micro propagation technique where the growth of the host plant is promoted by the formation of secondary metabolites related to plant defense. These systems allow for mutual adaptation between the host plant and the introduced bacteria. Bacterized plantlets not only grow faster than control plantlets but they are sturdier and have a well developed root system². The inoculation of seeds with useful microorganisms has been practiced for several years, but the inoculation of tissue culture raised plantlets is an innovative aspect. Plant tissue culture relies on aseptic conditions, hence microorganisms including beneficial endophytes are treated as the problem causing contaminants. But nowadays microbial inoculants, primarily bacteria, and mycorrhizae are being evaluated as biopriming agents for successful transplanting. Biotization may be achieved during in vitro rooting or under ex vitro conditions. Thus efficient antagonistic plant growth-promoting bacteria used for biological hardening envisages physical, chemical and environmental conditioning of the micro propagated plantlets. All beneficial microorganisms helped in the increased uptake of nutrients, nitrogen fixation, resistance to soil-borne diseases and improved plant water relations. Although the soils are not deficient in phosphorous, its inaccessibility is found to be a major constraint in acidic soils.

Effective microbes aids with in the uptake of mineral nutrients in the available form where the normal

plant roots fail. The microbial association helps in the enhanced hydraulic conductivity of the roots which contributes to the higher uptake of water thereby resulting in the reduction of desiccation and wilting in immature plantlets. These favorable conditions facilitate to increase the number of lateral roots and root length. Also, there was a rise in the activity of all the defense-related enzymes like phenylalanine ammonia lyase, peroxidase, and β -1,3-glucanase with in the leaves of the plants under treatment³. Lower incidence of rotting and wilting diseases was detected in bioinoculant-treated plantlets. This suggests that bioinoculants were capable of eliciting systemic resistance in plants. Apart from bacteria and fungi, different endophytes like algae, amoebae, virus, archae,

oomycetes also show a symbiotic association with plants⁴. This mutual association was reported in Green algae *Coccomyxa* species with *Ginkgo biloba*⁵ and amoebal cysts with *Eleutherococcus sieboldianus*⁶

Effect of abiotic and biotic factors on acclimatization of tissue culture raised plants.



MYCORRHIZATION:

Tissue culture raised plants experience high mortality following laboratory to land transfer. One major reason of high mortality of aseptically raised plants apart from the abiotic factors is their sudden exposure of their root system to microbial communities, present in the soil. The primary target of several research groups is to induce stress resistance by utilizing microbial inoculants during micropropagation¹. Biotization is the metabolic response of in vitro grown plant material to microbial inoculum, leading to the morphological and physiological development and providing biotic and abiotic stress resistance. Biotization is an emerging field of micropropagation technique where the growth of the host plant is promoted by the formation of secondary metabolites related to plant defense. This systems allow for mutual adaptation between the host plant and the introduced bacteria. Bacterized plantlets not only grow faster than control plantlets but they are sturdier and have a well developed root system². The inoculation of seeds with useful microorganisms has been practiced for several years, but the inoculation of tissue culture raised plantlets is an innovative aspect. Plant tissue culture relies on aseptic conditions, hence microorganisms including beneficial endophytes are treated as the problem causing contaminants. But nowadays microbial inoculants, primarily bacteria, and mycorrhizae are being evaluated as bioprimer agents for successful transplanting. Biotization may be achieved during in vitro rooting or under ex vitro conditions. Thus efficient antagonistic plant growth-promoting bacteria used for biological hardening envisages

physical, chemical and environmental conditioning of the micro propagated plantlets.

All beneficial microorganisms helped in the increased uptake of nutrients, nitrogen fixation, resistance to soil-borne diseases and improved plant water relations. Although the soils are not deficient in phosphorous, its inaccessibility is found to be a major constraint in acidic soils. Effective microbes aids with in the uptake of mineral nutrients in the available form where the normal plant roots fail. The microbial association helps in the enhanced hydraulic conductivity of the roots which contributes to the higher uptake of water thereby resulting in the reduction of desiccation and wilting in immature plantlets. These favorable conditions facilitate to increase the number of lateral roots and root length. Also, there was a rise in the activity of all the defense-related enzymes like phenylalanine ammonia lyase, peroxidase, and β -1,3-glucanase with in the leaves of the plants under treatment 3. Lower incidence of rotting and wilting diseases was detected in bioinoculant-treated plantlets. This suggests that bioinoculants were capable of eliciting systemic resistance in plants.

Apart from bacteria and fungi, different endophytes like algae, amoebae, virus, archae, oomycetes also show a symbiotic association with plants⁴. This mutual association was reported in Green algae *Coccomyxa* species with *Ginkgo biloba*⁵ and amoebal cysts with *Eleutherococcus sieboldianus*⁶

BACTERIZATION :

The tissue culture raised plants when treated with bacterial inoculants produce plant growth promoting substances and few secondary metabolites that enhance nutrient supply and provide resistance against pathogens. These useful soil bacteria are named as plant growth promoting rhizobacteria (PGPR), which are preferentially related to the roots. PGPR include many bacterial genera like *Pseudomonas*, *Bacillus*, *Azospirillum*, *Azotobacter*, etc. Bacterial infection occurs through roots and they are translocated to all parts of the plant via the xylem, the aerenchyma and through interconnecting intercellular spaces²⁷. These diazotrophs would have affected plant growth by the synthesis of phytohormones and vitamins, inhibition of plant ethylene synthesis and improved nutrient uptake²⁸. Bacterized plantlets were greener, had elevated levels of cytokinins, phenylalanine ammonia lyase, and free phenolics and contained more lignin²⁹. Both in vitro and ex vitro benefits of bacterization relied on plant species, cultivar, and growth conditions.

Among the PGPR, *Pseudomonas* deserves a special mention because it improves the plant growth directly or indirectly by the production of plant growth substances, increasing the uptake of certain nutrients from the soil and additionally shows antagonistic effects against some important plant pathogenic microorganisms. In addition to this *Pseudomonas* species have been used to enhance tolerance to transplanting stress in potato 62. In vitro co-cultivation of soybean cotyledon

explants with two strains of *Pseudomonas maltophilia*, stimulated the development of nodular callus with high regeneration potential³⁰. It was reported that *Pseudomonas* strain PsJN enhanced the tolerance to transplanting stress in potato and was found the most effective plant growth promoting bacterium under in vitro conditions³¹. The Oregano plantlets co-cultured with *Pseudomonas* spp. prevented vitrification and contained a lot of phenolics and chlorophyll than non bacterized controls³². Greenhouse experiments also demonstrated that plants derived from dual cultures of potato and the pseudomonad bacterium had a larger root system, and gave better tuber yield than control³³. The tea plants inoculated with *Bacillus subtilis* and *Pseudomonas corrugate* acted as biocontrol agents and were able to defend pathogenic attack probably due to their antagonistic properties³⁴. Three plant growth-promoting rhizobacteria viz. *Bacillus megaterium*, *B. subtilis* and *Pseudomonas corrugata* were used for biological hardening of micro-propagated plantlets of *Picrorhiza kurrooa*³⁵. These bacterial isolates antagonized the pathogenic fungal species and positively influenced survival and growth parameters. The in vitro grown Oil palm plantlets inoculated with *Azospirillum brasilense* produced higher root and shoot biomass and more secondary roots³⁶. Endophytic colonization in rice callus induced by using *Azorhizobium caulinodans* improved yield, grain weight and nutritional quality³⁷.

Thus the reports suggest the use of efficient antagonistic plant growth-promoting bacteria for biological hardening increase plant survival and augment overall plant growth of micro propagated plants.

MULTIMICROBIAL INTERACTION:

Multimicrobial biotization is inoculation of more than one microbial species to microplants. Root colonization by mycorrhizal fungi will have an affect the chemical composition of root exudates. The development of mycelium around roots (mycorrhizosphere) modifies their physical atmosphere and provides a new source of carbon to the microbial community. The development of a mycorrhizosp here has both qualitative and quantitative repercussions on microbial populations in either the rhizosphere or rhizoplane³⁸. Mycorrhization increases the dichotomy of roots and creates new compartments (mycorrhizosphere and microsphere), for the growth of symbiotic microbes.

Mycorrhizae-Helping Bacteria (MHB) enhance the growth of the plants. It was reported that rhizosphere strains of *Bacillus mycoides* and *Pseudomonas fluorescens* promoted AMF formation in various crop plants by improving susceptibility of roots to AMF³⁹. Other rhizosphere microorganisms which are known to act as a phytostimulators or which possess antagonistic activities toward plant pathogens may be used in conjunction with AMF for biotizing microplants. They include bacteria like *Pseudomonas* spp. and *Bacillus* spp., and fungi such as *Gliocadium* spp.

and *Trichoderma* spp. Plantlet survival rate was maximum in dual inoculation, this must be due to the positive interaction between two species and their ability to enhance stress tolerance by protecting them from subsequent 'transplantation shock'.

It was found that the combined use of *Glomus mosseae* and *Pseudomonas fluorescens* caused a greater increase in plant growth of tomato as compared to the individual application⁴⁰. Studies showed that plant-mediated interactions among *Pseudomonas fluorescens*, *Rhizobium leguminosarum*, and AMF on Pea enhanced nodulation by four fold⁴¹. *P. fluorescens* and ectomycorrhizal fungus, *Suillus granulatus*, used as dual inoculants for *Pinus halepensis* showed significantly high number of lateral rootlets as compared to single inoculations⁴². Better root system helped in more nutrient uptake, which resulted in healthy plants with more shoot biomass.

Agrobacterium rhizogenes causing the hairy-root syndrome in dicots is being applied successfully for the promotion of growth of roots and their effective mycorrhization¹³. 100% plant survival was observed after biotization of micropropagated raspberry with *Agrobacterium radiobacter* and *Glomus mosseae*⁶⁴. It was reported that dual inoculation of potato microplants with *P. fluorescens* and mycorrhizal fungi enhanced plant growth and protected against soil-borne potato pathogen *Rhizoctonia solani*⁴³. *Trichoderma* sp. DB11 and *Gliocladium catenulatum* were inoculated in the potting substrates of micro propagated strawberry in order to promote growth and protect against root and collar rot induced by *Phytophthora* spp⁴⁴.

Biotization of micropropagated *Chlorophytum* sp. with the fungus, *Piriformospora indica* and the bacterium, *Pseudomonas fluorescens*, improved plantlet survival rate, growth parameters, field performance, micronutrient acquisition, alkaloid and saponin content⁴⁵. Significantly higher root-shoot biomass was reported in different crops like Maize, Bacopa, Poplar with dual inoculation of *P. fluorescens* and *P. indica* inoculation⁶⁵. Maximum colonization of *P. indica* and bacteria in dual inoculated plants attributed the fact that mycorrhizal root tips tend to support slightly higher populations of *Pseudomonas* than non-mycorrhizal root tips, possibly due to the provision of additional colonization sites or altered root exudation in mycorrhizosphere.

It was reported that acclimatization of in vitro rooted tea plantlets in soil amended with bioinoculants like *Pseudomonas fluorescens*, *Azospirillum brasilense*, and *Trichoderma harzianum*, either individually or in various combinations, promoted plantlet survival⁴⁶. Root rot or wilting of tissue culture derived plants was not observed in bioinoculant-treated plants, as they possessed relatively higher activities of defense enzymes, including peroxidase and phenylalanine ammonia lyase. The synergistic effect of *T. viride* with *P. fluorescens* was that it can solubilize more P in the soil by producing organic acids⁴⁷. Similarly, their combined effect on growth improvement was also reported by other workers^{48,49}.

The application of multimicrobial biotization requires knowledge and understanding of the compatibility between different beneficial microorganisms in their interaction within the mycorrhizosphere, rhizosphere, and rhizoplane.

Table: 1 Effect Of Various Bioinoculants On Plant Performance

S.No.	Bioinoculant	Plant	Effect
Monomicrobial Interaction - Mycorrhization			
1	Ectomycorrhizae	Pine	Increase in Phosphorus uptake & net Photosynthetic rate ^{11,12}
2	<i>Glomus fasciculatum</i>	Banana	Increase in plant biomass ¹⁴
3	<i>Glomus message</i>	Papaya	Increase in nutritional quality ⁵⁰
4	<i>Glomus diazotrophicus</i>	Sugarcane	Efficient Nitrogen fixation ¹⁶
5	<i>Glomus intradices</i>	Cassava, Grape, Olive	Increase in Plant survival & nutrient status ^{51,58}
6	AM fungi	Hazlenut	Resistance towards soil pathogens ¹⁷
7	AM fungi	<i>Ranunulus asiaticus</i>	Earlier flowering, improved flower production & Better rhizome yield ¹⁸
8	<i>Piriformosporaindica</i>	<i>C. borivilianum</i>	Invivo rooting ¹⁹
9	<i>Mycorrhizae</i>	Chestnut	Increase protein content and Photosynthetic rate ²⁰
10	<i>Piriformosporaindica</i>	<i>B. serrata</i>	Increase in plant biomass & Phosphorus uptake ^{21,23}
11	<i>Piriformosporaindica</i>	<i>T. bellerica</i>	Increase in Plant survival and Photosynthetic rate ⁵²
12	<i>Piriformosporaindica</i>	Barley	Resistance to pathogens and tolerance to salt stress ²⁴
13	<i>Trichoderma viride</i>	Broccoli	Maximum N and P content in roots and shoots ²⁶
14	<i>Trichoderma viride</i>	Neem	Invitro rooting ²⁵
Monomicrobial Interaction - Bacterization			
15	<i>Pseudomonas</i>	Potato	Enhance tolerance to transplantation stress ³¹
16	<i>Pseudomonas</i>	Potato	Large root system and better tuber yield ³³
16	<i>Pseudomonas</i>	Soyabean	Increase in Plant survival ³⁴
17	<i>Pseudomonas</i>	Oregano	Prevents vitrification, Increase in phenolic & chlorophyll content ³²
18	<i>Bacillus subtilis</i>	Tea	Resistance towards pathogens ³⁴
19	<i>Bacillus megaterium</i>	<i>Picrorhizakurrooa</i>	Induced systemic resistance ³⁵
20	<i>Azospirillum brasilense</i>	Oil Palm	Increase root and shoot biomass ³⁶
21	<i>Azorhizobium caulinodans</i>	Rice	Improved grain weight and nutritional quality ³⁷
Multimicrobial Interaction			
22	<i>Glomus mossae</i> + <i>P. fluorescens</i>	Tomato	Increase in yield ⁴⁰
23	<i>P. fluorescens</i> + AMF + <i>Rhizobium leguminosarum</i>	Pea	Increased nodulation by four fold ⁴¹
24	<i>P. fluorescens</i> + Ectomycorrhizae	Pinus	Increase in root and shoot biomass ⁴²
25	<i>A. radiobacter</i> + <i>Glomus mossae</i>	Raspberry	Improved plant survival ⁵⁵
26	<i>P. fluorescens</i> + AMF	Potato	Protection against soil borne pathogens ⁴³
27	<i>Trichoderma</i> + <i>Gliocladium catenulatum</i>	Strawberry	Resistance against root rot and collar rot ⁴⁴
28	<i>P. fluorescens</i> + <i>P. indica</i>	Chlorophytum	Efficient nutrient uptake and increased secondary metabolite content ^{19,45}
29	<i>P. fluorescens</i> + <i>P. indica</i>	Maize, Bacopa, Poplar	High root -shoot biomass ⁵⁶

30	<i>P.fluorescens</i> + <i>A. brasilense</i> + <i>T. harzianum</i>	Tea	Prevented wilting and root rot ⁴⁶
31	<i>P.fluorescens</i> + <i>T.viride</i>	Tea	Increase in Phosphorus uptake ⁴⁷
32	<i>P.fluorescens</i> + <i>T.viride</i>	Cotton	Increase in yield ⁴⁹
33	<i>P.fluorescens</i> + <i>T.viride</i>	Soyabean	Resistance against root rot and stem rot ⁴⁸

CONCLUSION:

Micro plants represent an ideal material for developing basic research on microbial biotization and for producing results which can be easily applied in technology transfer. Microplants produced in vitro are usually transplanted into an inert substrate, a relatively simple environment, where the development of the introduced microbe and their impact on plant growth can be easily monitored. A drastic increase in our knowledge about microbial and microbial/plant interactions in the rhizosp here, and particularly of those that are considered beneficial to plant development, is necessary to produce the best results in micro propagation.

In the hardening technique, biotization economizes the production process and simplify the micro propagation technique, which could be adopted at village centres to extend the scientific technology from lab to land. Development of new culture methods allowing the establishment of stable associations between plants and beneficial organisms in vitro and ex vitro and understanding of mechanisms of signal recognition and transduction in plant-microbial associations under different environments are probably the most critical elements of this challenge.

REFERENCES:

1. Lazarovits G & Nowak J. Rhizobacteria for improvement of plant growth and establishment. Hort Science, 1997; 32: 188.
2. Nowak J. Review benefits of in vitro "biotization" of plant tissue cultures with microbial inoculants. In vitro Cell Dev Biol Plant, 1998; 34:122.
3. Rammoorthy V, Viswanathan R, Raguchandar J, Prakasham T & Samiyappan R. Induction of systemic resistance by plant growth promoting rhizobacteria in crop plants against pest and diseases. Crop protection, 2001; 20: 1.
4. Hardoim PR, Van Overbeek LS, Berg G, Pirttilä AM, Compant S, Campisano A, Döring M & Sessitsch A, The hidden world within plants: ecological and evolutionary considerations for defining functioning of microbial endophytes. Microbiol. Mol. Biol. Rev. 2015; 79 (3): 293.
5. Trémouillaux-Guiller J, Rohr T, Rohr R & Huss VA, Discovery of an endophytic alga in Ginkgobiloba. Am. J. Bot, 2002; 89 (5): 727.

6. Müller P , and Döring M, Isothermal DNA amplification facilitates the identification of a broad spectrum of bacteria, fungi and protozoa in *Eleutherococcus* sp. plant tissue cultures. *Plant Cell Tissue Organ Cult*, 2009; 98 (1): 35.
7. Vestberg, M.; Estaun, V. Micropropagated plants, an opportunity to positively manage mycorrhizal activities. In: Gianinazzi, S.; Schuepp, H., eds. *Impact of arbuscular mycorrhizas on sustainable agriculture and natural ecosystems*. Basel: Birkhauser Verlag, 1994:217±226.
8. Morandi D, Gianinazzi S & Gianinazzi-Pearson V, Intérêt de l'endo- mycorrhization dans la reprise et la croissance du Framboisier issu de multiplication végétative in vitro. *Ann. Amélior. Plantes*, 1979; 29: 623.
9. Lovato PE, Gianinazzi-Pearson V, Trouvelot A, Gianinazzi S, The state of mycorrhizas and micropropagation. *Adv. Hort. Sci* , 1996; 10: 46.
10. Allen MF, Smith WK, Moore TS and Christensen M , Comparative water relations and photosynthesis of mycorrhizal and nonmycorrhizal *Bouteloua gracilis* (J. B. K.) Lag ex Steud. *New Phytol* , 1981; 88: 683.
11. Bougher NL, Grove TS, Malajczuk N, Growth and phosphorus acquisition of karri (*Eucalyptus diversicolor* F. Muell.) seedlings inoculated with ectomycorrhizal fungi in relation to phosphorus supply. *New Phytol* , 1990; 114: 77.
12. Rousseau JVD & Reid CPP, Effects of phosphorus and ectomycorrhizas on the carbon balance of Loblolly pine seedlings. *Forest Science*, 1990; 36: 101 .
13. DeClerck C, Plenchette & Strullu DG, Mycorrhizal dependency of banana(*Musa acuminata*, AAA group) cultivar. *Plant & Soil*, 1995; 176: 173.
14. Thaker MN & Jasrai YT, Increased growth of micro-propagated banana (*Musa paradisiaca*) with VAM symbiont. *Plant tissue Culture* , 2002; 12: 147.
15. Mandhare, VK, Suryawanshi AV & Jamadagani BM, Occurrence of powdery mildew (*Leveillula taurica*) on chickpea in Maharashtra. *Journal of Maharashtra Agricultural Universities* , 2005; 30: 340.
16. Muthukumarasamy R, Revathi G & Lakshminarasimhan C. Influence of N fertilisation on the isolation of *Acetobacter diazotrophicus* and *Herbaspirillum* spp. from Indian sugarcane varieties. *Biol Fertil Soils* , 1999; 29: 157.
17. Mirabelli C, Tullio M , Pierandrei F& Rea E, Effect of Arbuscular Mycorrhizal Fungi n on micropropagated Hazelnut (*Corylus avellana* L.) plants. *Acta Hort*, 2009; 812: 467.
18. Borriello R, Maccario D, Viglione S, Bianciotto V & Beruto M, Arbuscular mycorrhiza fungi and micropropagation of *Ranunculus asiaticus* L.: a useful alliance. *Acta Hort*, 2017; 1155: 81.

19. Archana Mathur, Ajay Kumar Mathur, Priyanka Verma, Shrawan Yadav, Moti Lal Gupta & Mahendra P. Darokar Biological hardening and genetic fidelity testing of microcloned progeny of *Chlorophytum borivilianum*. *African Journal of Biotechnology* , 2008; 7 (8): 1046.
20. Martins A, Casimiro MS & Pais ,Influence of mycorrhization on physiological parameters of micropropagated *Castanea sativa* Mill. *Plants.Mycorrhiza* , 1997; 7: 161.
21. Suthar RK & Purohith SD, Biopriming of micropropagated *Boswellia serrate* Roxb plantlets role of endophytic root fungus *P. indica*. *Ind J Biotech* , 2012; 11: 304.
22. Suthar RK and Purohith SD, Root Colonization and growth enhancement of micropropagated *Terminalia bellarica* Roxb plantlets inoculated with *P. indica* during ex vitro acclimatization. *Int J Plant Dev Biol* , 2008; 2: 133.
23. Chittora M, Suthar RK & Purohit SD, Root colonization and improved growth performance of micropropagated *Terminalia bellerica roxb.* plantlets inoculated with *Piriformospora indica* during ex vitro acclimatization - *Acta Hort*, 2010 : 865.
24. Waller F, Achatz B, Baltruschat H, Fodor J, Becker K, Fischer M, Heier T, Huckelhoven R, Neumann C, von Wettstein D, Franken P & Kogel KH , *Proc Natl Acad Sci USA*, 2005; 102: 13386.
25. Lavanya M, Venkateshwarlu B, Devi BP Acclimatization of neem microshoots adaptable to semi-sterile conditions. *Ind J Biotechnol*, 2009; 8: 218.
26. John RP, Tyagi RD, Prevost D, Brar SK, Pouleur S & Surampalli RY, Mycoparasitic *Trichoderma viride* as a biocontrol agent against *Fusarium oxysporum*f.sp. *adzuki* and *Pythium arrhenomanes* and as a growth promoter of soybean. *Crop Protection*, 2010; 29 :1452.
27. Gynaeshwar P, Reddy PM, Ladha JK, Nutrient amendments influence endophytic colonization of rice by *Serratia marcescens* IRBG500 and *Herbaspirillum sero- pedicae* Z67. *J Microbiol Biotechnol*, 2000; 10: 694.
28. Dobbelaere S, Vanderlyden J & Okon Y, Plant growth promoting effects of diazotrophs in the rhizosphere. *Crit Rev Plant Sci* , 2003; 22: 107.
29. Richards J, Induced resistance responses in potato inoculated in vitro with a plant growth promoting pseudomonad bacterium, M.Sc. thesis, Dalhousie University, Halifax, NS, Canada;1997.
30. Yang YS, Wada K, Goto M.;et al., Invitro formation of nodular calli in soya bean induced by co cultivated *Pseudomonas maltophilia*. *Japan. J. Breed*, 1991; 41: 595.

31. Frommel MI, Nowak J & Lazarovits G, Treatment of potato tubers with a growth promoting *Pseudomonas* spp- Plant growth responses and bacterium distributing in the rhizosphere. *Plant Soil*, 1993; 150: 51.
32. Shetty K, Curtis OF, Levin RE.; et al. Prevention of vitrification associated with invitro shoot culture of oregano by *pseudomonas* spp. *J. Plant Physiol*, 1995; 147: 447.
33. Dunbar C, Utilization of see weed extract and plant growth promoting rhizobacterium in green house production of potato minitubers. M.Sc. thesis, Dalhousie University, Halifax, NS, Canada,1997.
34. Palni LMS & Beg N. Biological hardening of tissue culture raised tea plants through rhizosphere bacteria. *Biotechnol. Lett.*, 2000; 22: 1087.
35. Trivedi P & Pandey A, Biological hardening of micro propagated *Picrorhiza kurrooa* Royel ex Benth., an endangered species of medical importance. *World J Microbiol Biotechnol* , 2007;23: 877.
36. Azlin CO, Amir HG, Chai Lai Keng & Zamzuri I, Effect of plant growth promoting rhizobacteria on root formation and growth o f tissue cultured Oil palm .*Biotechnology*, 2007; 6(4): 549.
37. Senthilkumar M, Madhaiyan M, Sundaram SP, Sangeetha H & Kannaiyan S, Induction of endophytic colonization in rice (*Oryza sativa* L.) tissue culture plants by *Azorhizobium caulinodans*. *Biotechnol Lett*, 2008; 30: 1477.
38. Barea JM, Rhizosphere and Mycorrhiza of Field Crops. In: *Biological Resource Management Connecting Science and Policy*. (Ed. Balázs E. et al. Springer, Berlin, Heidelberg) 2000, 81.
39. Von A. State of commercial use of AMF-inoculum in Germany. In: *Arbuscular mycorrhizas in sustainable soil plant systems- Report of 1997 activities, Cost Action* (Ed. Gianinazzi S, & Schuepp H , 821, Iceland) 1998;153.
40. Siddiqui ZA & Mahmood I, Effect of a plant growth promoting bacterium, an AM fungus and soil types on the morph metrics and reproduction of *Meloidogyne javanica* on tomato. *Appl. Soil Ecol*, 1998; 8: 77.
41. Andrade G, De Leij FAAM, Lynch JM, Plant mediated interactions between *Pseudomonas fluorescens*, *Rhizobium leguminosarum* and arbuscular mycorrhizae on pea. *Lett. Appl. Microbiol.*, 1998; 26: 311.
42. Rincon A, Diez BR, Fraile GS, Garcia L, Pascual MF et al, Colonization of *Pinus halepensis* roots by *Pseudomonas fluorescens* and interaction with the ectomycorrhizal fungus *Suillus granulatus*. *FEMS Microbiol Ecol*, 2005; 51: 303.

43. Duffy EM, Hurley EM & Cassells AC, Weaning performance of potato microplants following bacterization and mycorrhization .Potato Research , 1999;42: 521.
44. Vestberg M, Kukkonena S, Saaria K, Parikkab P, Huttunenc J, Tainioc L, Devosd N, Weekersd F, Keversd C, Thonartd P, Lemoinee MC, Cordierf C, Alabouvettef C, Gianinazzif S, Microbial inoculation for improving the growth and health of micro propagated strawberry .Applied Soil Ecology, 2004; 27: 243.
45. Gosal SK, Karlupia A, Gosal SS, Khibba IM & Varma A, Biotization with *P. indica* and *P. fluorescens* improves survival rate , nutrient acquisition,field performance and saponin content of micropropagated Chlorophytum species. Ind J Biotechnol , 2010; 9: 289.
46. Jibu Thomas, Ajay D & Raj Kumar R, Influence of beneficial microorganisms during in vivo acclimatization of in vitro- derived tea (*Camellia sinensis*) plants . Plant Cell Tiss Organ Cult , 2010; 101: 365.
47. Avis TJ, Gravel V, Antoun H & Tweddell RS, Multifaceted beneficial effects of rhizosphere microorganisms on plant health and productivity. Soil Biology and Biochemistry, 2008; 40: 1733.
48. Mishra DS, Gupta AK, Prajapati CR and Singh US, Combination of fungal and bacterial antagonists for management of root and stem rot disease of soybean. Pakistan Journal Botany, 2011;43: 2569.
49. Shanmugaiah V, Balasubramanian N, Gomathinayagam S, Manoharan PT & Rajendran A, Effect of single application of *Trichoderma viride* and *Pseudomonas fluorescens* on growth promotion in cotton plants. African Journal of Agricultural Research, 2009; 4: 1220.
50. Kennedy PM, Lowry JB & Conlan LL, Isolation of grass cell walls as neutral detergent fibre increases their fermentability for rumen micro-organisms. J. Sci. Food Agric, 1999; 79 (4): 544.
51. Carlos Luis Carretero , Manuel Cantos , José Luis García , Rosario Azcón & Antonio Troncoso , Growth Responses of Micropropagated Cassava Clones as affected by *Glomus Intraradices* colonization. Journal of Plant Nutrition, 2009; 32: 261.
52. Vyas S, Nagori R & Purohith SD , Root Colonization and growth enhancement of micropropagated *Feronia limonia* (L) swingle by *P. indica*. Int J Plant Dev Biol 2008; 2: 128.
53. Bashan Y, Inoculants of plant growth promoting bacteria for use in agriculture, Biotechnol Adv, 1998; 16: 729.
54. Lifshitz R , Kloepper JW et al. Growth promotion of canola seedlings by a strain of *Pseudomonas putida* under gnotobiotic conditions. Can .J. Microbiol, 1987; 33: 390.

55. Gianinazzi S, Oubaha L, Chahbandar M, Blal B, Lemoine MC, Biotization of microplants for improved performance. *Acta Horticulturae.* , 2003; 625: 165.
56. Verma S, Varma A, Karl-Heinz R, Hasse IA, Kost G et al, *Piriformospora indica* gen. nov. a new root-colonizing fungus, *Mycologia USA*, 1998; 90: 895.
57. Swada S, Igarashi T & Miyachi S, Effects of nutritional levels of phosphate on photosynthesis and growth studied with single, rooted leaf of dwarf bean. *Plant Cell Physiol* , 1982; 23: 27.
58. Bhargava BS, Raghupathi HB, Analysis of plant materials for macro and micronutrients. In: *Methods of analysis of soils, plants, waters and fertilizer.* (Ed. Tandon HLS , Fertilizer Association and Consultation Organisation, New Delhi) 2001,49.