NIGERIAN AGRICULTURAL JOURNAL
ISSN: 0300-368X
Volume 52 Number 2, August 2021 Pg. 229-235
Available online at: http://www.ajol.info/index.php/naj
https://www.naj.asn.org.ng

PLANT GROWTH PROMOTING MICROBES IN PLANT TISSUE CULTURE

[1]Nsofor, G. C. and [2]Isaiah, T.

[1]National Root Crops Research Institute, Umudike, Abia State, Nigeria
[2]Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria
Corresponding Authors' email: nsofor.gladys@yahoo.com

Abstract
Plant Growth Promoting Microbes (PGPMs) are key players in major ecological processes like atmospheric nitrogen fixation, water uptake, solubilization, and transport of minerals from the soil to the plant. A broad spectrum of PGPMs has been proposed as biofertilizers, biocontrol agents and biostimulants to enhance plant growth, agricultural sustainability and food security. However, little information exists with regard to the application of PGPMs in plant tissue culture. This review therefore presents an insight into the importance of PGPMs in plant tissue culture from relevant available articles. In addition, exploiting the potential benefits of PGPMs will lead to a significant reduction in the cost production of *in vitro* plantlets during plant tissue culture.

Keywords: Microbial plant, Enhancers, Tissue culture, Micropropagation

Plant Growth Promoting Microbes (PGPMs) are selected microorganisms usually found in the soil and roots of plants (rhizosphere) which have positive impact on plant growth, development and survival (Jitendra *et al.* 2017). Plant Growth Promoting Microbes include Plant Growth-Promoting Rhizobacteria (PGPR) and Plant Growth-Promoting Fungi (PGPF) (Spaepen *et al.* 2009), but some Protists like Amoabae, Ciliates and Flagellates, and Archaea (like Natrinema, Halobacterium, Haoferaxa, etc.) have been reported as PGPMs (Koller *et al.* 2013, Ajar *et al.,* 2017). Plant Growth Promoting Microbes are involved in the degradation, mineralization of organic compounds, solubilization of inorganic compounds and release of biologically active compounds like chelators, phytohormones, and antibiotics, among others, which are necessary for the general well-being of plants (Kapulnik and Okon 2002). Usually, plant tissue culture systems are carried out in sterile conditions (Diedhiou *et al.,* 2016). The explant is surface sterilized to eliminate all microbial contaminants. Since the role of PGPMs in plant growth and protection has been established, more attention has been paid to beneficial effects of these microorganisms in *in vitro* plant tissue cultures. In this respect, the use of competent PGPMs in tissue culture under *in vitro* and *ex vitro* conditions has been examined and called "biotization" (Trigiano and Gray, 2016). Microplant biotization is a biotechnological practice aimed at reducing chemical input in plant production (Kanani *et al.,* 2020). The biotization can be done at all stages of *in vitro* propagation namely; Stage 0 (Plant stock immobilization and pre-treatments, selection of the explants), Stage I (Culture establishment), Stage II (Elongation and multiplication), Stage III (Rooting), Stage IV (Weaning, hardening, and acclimatization) and Stage V (Transfer under natural conditions-to the field) (Diedhiou *et al.,* 2016). In stages II and III of micropropagation by micro-cutting, PGPMs act generally as bio-stimulants by promoting elongation and increasing rooting, while in stage IV, they act as biocontrol agents and help to deal with biotic and abiotic stress factors (Diedhiou *et al.,* 2016). It is at this stage of acclimatization that biotization of microplants seems to be most important (Orlikowska *et al.,* 2017). In addition to their three main growth promoting mechanisms namely; direct, indirect/direct and indirect, certain PGPMs such as *Rhizobium*, *Frankia*, *Bradyrhizobium*, and mycorrhizal fungi have been recognized to be able to improve the physical properties of the soil by making it more conducive for plant growth and development (Egamberdieva *et al.,* 2019).

Beneficial effects of Plant Growth Promoting Fungi in *in vitro* Plant Culture

Tolerance to water and nutrient up take: Plantlets obtained from micropropagation through tissue culture are often adversely affected by water stress; because of low water absorption capacity of their roots (Diedhiou *et al.,* 2016). Inoculation with Arbuscular Mycorrhizal Fungi (AMF) *in vitro* is an important tool to deal with this problem (Rai, 2011). Through biosynthesis of phytohormones or Plant Growth Regulators (PGRs), AMF impact on post-transplant performance of *in vitro* grown plants by increasing nutrients availability and inducing resistance to pathogens (Akin-Idowu *et al.,* 2009). According to Chanclud *et al.* (2016) and Streletskii *et al.* (2019), fungi produce phytohormones such as auxins, cytokinins (CKs), abscisic acid (ABA), gibberellic acids (GAs), ethylene (ET), salicylic acid (SA), and jasmonic acid (JA). These hormones control plant development and activate signaling pathways during biotic and abiotic stress. Meixner *et al.* (2005) showed that plants inoculated with AMF had a higher level of auxins than non-inoculated plants. It has been shown that a large diversity of fungal species can produce CKs for hyphal development and nutrient uptake during mycorrhizal symbiosis. Auxin and cytokinin act as messengers to regulate various cellular processes in plants such as bud activity, branching, cell cycle, synchronization of fruit setting and dropping (Muller and Leyser, 2011), plant defense responses (Naseem and Dandekar, 2012), grain size, and biomass production (Osugi and Sakakibara, 2015). Fungi, especially AMF, play important role in water uptake and availability (Puschel *et al.,* 2020), thereby increasing the rate of photosynthesis and osmotic adjustment under environmental stress (Soumare *et al.,* 2017). Arbuscular Mycorrhizal Fungi also increase the uptake of micronutrients such as P, Zn, Cu, Fe, etc. Arbuscular Mycorrhizal Fungi contribution is especially important during the acclimatization phase, because the adventitious and weak root system, without root hair, of vitroplants do not allow optimal absorption of nutrients from the soil during the early stage of the weaning step. Arbuscular Mycorrhizal Fungi can help to overcome this problem, through their arbuscules and hyphae which transfer nutrients, especially phosphate from the soil to the plant (Chen *et al.,* 2018). Beneficial endophytic fungi promotes plant growth by improving uptake of phosphorus, potassium, and zinc and/or production of phytohormones such as cytokinins, indole acetic acids, and gibberrellic acids (Rana *et al.,* 2019). The lower survival rate and poor establishment of vitroplants in field conditions may be because the transferred vitroplants did not find their natural microsymbiont partner (Harish *et al.,* 2008). Also, certain endophytic fungi can be plant pathogens and limit the micropropagation process. It is the case with *Fusarium equiseti* which was suspected to cause bamboo blight and culm rot disease (Tyagi *et al.,* 2018). Although, the mycorrhization technique is important for the growth and development of the micropropagated plantlets, some problems need to be solved to optimize the technology efficiency. The main problem to be solved is how to produce pure fungal inoculum without contaminants for micropropagation. Currently, the disinfection and germination of spores in the agar medium are difficult. On the other hand, Murashige and Skoog medium (MS) systematically used in micropropagation does not seem to be favorable for the germination and growth of spores (Rana *et al.,* 2019). This suggests that the methodology of propagation needs to be adapted by modifying the nutrient medium to overcome the problem (Srinivasan *et al.,* 2014).

Beneficial effects of Plant Growth Promoting Bacteria in *in vitro* Plant Culture

Improved photosynthetic efficiency and biomass production: Elmeskaoui *et al.* (2015) have shown that biotized plant tissue cultures benefit from microbial presence through an improvement in photosynthetic efficiency and biomass production. Generally, PGPB improves growth by releasing PGRs required for vitropropagation (Quambusch and Winkelmann, 2018). Auxins and cytokinins biosynthesis are widespread among rhizobacteria, and different biosynthetic pathways have been identified (Amara *et al.,* 2015). For instance, it is assumed that many bacteria can produce cytokinins in pure culture and more than 80% of soil bacteria in the rhizosphere can produce auxins especially indole-3-acetic acid (IAA), which is the major auxin active form in plants (Souza *et al.,* 2015). Plant Growth Regulators from microorganisms play a compensatory role, especially when micropropagated plants are under sub-optimal environment with insufficient endogenous production. For different strains belonging to *Bacillus*, *Pseudomonas*, *Rhizobium*, *Bradyrhizobium*, *Enterobacter*, *Methylobacterium*, *Microbacterium*, *Rhodococcus*, and *Acinetobacter*, these PGRs have been quantified, characterized, and tested in plant tissue culture (Spaepen and Vanderleyden, 2011). Their application *in vivo* and *in vitro* on the plant leads to galls, stem fasciation, and brooms. The study from Erturk *et al.* (2010) demonstrated that different PGPB strains belonging to genus *Bacillus*, *Paenibacillus*, and *Comamonas* promoted root formation in kiwifruit cuttings in mass clonal propagation through IAA production. More recently, Lim *et al.* (2016) reported that the diazotroph; *Herbaspirillum seropedicae* induced the proliferation and differentiation of calli and embryogenic calli of oil palm through nitrogen fixation and IAA production. Similar findings were previously reported by Rodríguez- Romero *et al.* (2008) on micropropagated banana plants with *P. fluorescens*, with a consistent increase of plant development. Kargapolova *et al.* (2020) demonstrated the efficacy of the inoculation with *Ochrobactrum cytisi* on potato microplants. A 50% increase of mitotic index of root meristem cells and 34% increase of shoot length were reported under *ex vitro* conditions.

***Production of phytohormones*:** some plant growth promoting rhizobacteria (PGPR) can induce the production of phytohormones by the plant. Analyzing plant molecular responses to *Burkholderia phytofirmans* colonization, Poupin *et al.* (2013) showed that genes involved in auxin and gibberellin pathways were induced in *Arabidopsis thaliana*. Moreover, bacterial phytohormones such as gibberellins can interact with other hormones to support elongation (Bottini *et al.,* 2014). Other phytoregulators such as abscisic acid and salicylic acid are produced by PGPB, but they are less studied. The phytohormones regulate both growth and senescence by modulating ethylene levels in the plant tissue which plays an essential role in the plant defense mechanisms against infections and external aggressions (Iqbal *et al.,* 2017). Decreasing ethylene levels allows the plant to be more resistant to different environmental stresses (Glick, 2015). The growth of pathogens is suppressed by producing toxins, antibiotics, HCN, and/or hydrolytic enzymes such as proteases, chitinases, and lipases. These compounds degrade the cell wall, virulent, or pathogenic factors (Compant *et al.,* 2015).

Enhanced stress tolerance and plant growth**:** It has been shown that inoculation with some PGPB produces aminocyclopropane carboxylate (ACC) deaminase, which help to relief stress and enhance plant growth (Ruzzi and Aroca, 2015). Through a reduction of ethylene production, Gupta and Pandey (2019) and Souza *et al.* (2015) reported that ethylene acts as stress phytohormone which adversely affects the growth of the roots under abiotic and biotic stress. Similar results were previously reported on *Camelina sativa* by Heydarian *et al.* (2016), which have shown that PGPB can enhance growth and salt tolerance in camelina by the production of ACC deaminase. *In vitro* co-culture of explants with PGPB induces developmental and metabolic changes, which enhance their tolerance to abiotic and biotic stress. In this regard, Sgroy *et al.* (2009) demonstrated that *Bacillus*, *Lysinibacillus*, *Pseudomonas*, *Achromobacter*, and *Brevibacterium* associated with the halophyte *Prosopis strombulifera* act as stress homeostasis-regulating bacteria through IAA, zeatin, and GA production.

Production of volatile metabolites**:** Bacteria can produce volatile metabolites which can induce organogenesis (Gopinath *et al.,* 2015), improve the efficiency of photosynthesis (Xie *et al.,* 2009), and provide protection against abiotic stressors (Orlikowska *et al.,* 2017). Plant Growth Promoting bacteria and fungi living in the rhizosphere induce systemic resistance (ISR) and enhance defense against a broad range of pathogens and insects. Some PGPB (e.g., *Pseudomonas* and *Bacillus*) and some PGPF (e.g., *Trichoderma*) can sensitize the plant immune system for enhanced defense without directly activating costly defenses (Pieterse *et al.,* 2014; Al-Ani and Mohammed, 2020).

Beneficial effects of Plant Growth Promoting Archaea in *in vitro* Plant Culture

Archaea are well distributed in extreme environments like hot springs and salt lakes, but due to their metagenomic traits, they can be found in a wide range of habitats such as soils, oceans, and marshlands. The beneficial effects of Archaea as PGPMs in *in vitro* cultures have being scarcely studied, among which includes; nitrogen fixation by methanogens (Leigh, 2000), siderophore production (Dave, 2006), phosphorus solubilization by haloarchaea (Yadav *et al.,* 2015) and Indole Acatic Acid production (White, 2007). Phosphate-solubilizing halophilic archaea identified as Natrialba, Natrinema, Halolamina, Halosarcina, Halostagnicola, Haloarcula, Natronoarchaeum, Halobacterium, Halococcus, Haloferax and Haloterrigena have been isolated from halophilic plants namely; *Abutilon, Cenchrus, Dicanthium,* and *Sporobolous* (Yadav *et al.,* 2015).

Beneficial effects of Plant Growth Promoting Protists in *in vitro* Plant Culture

Most Protists are heterotrophs, feeding on bacteria and fungi though a few are parasitic such as apicomplexan or oomycetes, and phototrophic algae (Bonkowski 2004, Pawlowski, 2013). The beneficial effects of Protists to plants growth as documented include:

Nutrient release**:** They feed on bacteria and fungi releasing excess of nutrients like nitrogen, phosphorus, or other micronutrients. These nutrients are often limited in soil and kept locked in microbial cells if not released by the activity of protists (Kreuzer *et al.,* 2006).

Selection of specific bacteria**:** Protists are selective in choosing their prey on the basis of several characteristics including surface properties or size (Swallow *et al.,* 2013). Predator activity of Protists plays a major role in promoting the growth and survival of toxic microorganisms in the rhizosphere. For instance, the productions of broad-spectrum bioactive secondary metabolites like alkaloids (Klapper *et al.,* 2016), lipopeptides (Mazzola *et al.,* 2009) or polyketides and inoculation of amoebae in rice rhizosphere multiplied the fitness of Pseudomonas by a factor of three, due to the elimination of the nontoxic competitors of the introduced bacteria (Jousset *et al.,* 2009).

Improved Plant-Microbe Symbioses**:** A research conducted by Koller *et al.* (2013) showed that mycorrhiza function is largely dependent on protists. It was noted that Amoebae increased nitrogen turnover around the hyphae and stimulated its transfer to the plant. Similarly, mycorrhiza fungi themselves failed to produce the required enzymes needed for mineralizing soil organic material. Instead they secreted plant-derived carbon in their surroundings, makes nutrients available for the associated microbial communities. Without predation, these communities would die due to nutrient limitation (Jousset *et al.* 2009).

Stimulate Beneficial Trait Expression**:** Some bacterial traits linked to plant growth promotion such as production of siderophores or toxic secondary metabolites, are strongly affected by the presence of bacterivorous protists (Alexandre, 2017). Cyclic lipopeptides (Mazzola *et al.* 2009) or 2,4-DAPG production increased after confronting bacteria with amoebae or their supernatant (Jousset *et al.,* 2010).

Plant Growth Promoting Microbes in Plant Tissue Culture and Future Prospects

The application of PGPMs in tissues cultures has not been studied enough to advance its application on a commercial basis. Our present knowledge in PGPM behavior at the root, leaf, or whole plant level and their function in the natural environment is still limited (Sunita *et al.,* 2020). In the future, detailed research is needed to select efficient, multifunctional, stress tolerant PGRs-producing microbes that combine ecological tolerance for application in plant tissue culture. Indeed, the wide diversity of possible uses of beneficial microbes in plant tissue culture opens new doors to identify appropriate PGPMs to be used at the different stages of plant tissue culture (Soumare *et al.,* 2020). On the other hand, more efforts should be channeled towards bio-formulation of these microbes for suitable application in plant tissue culture. Currently, there are some challenges for the delivery of PGPMs, especially during explant cultivation, elongation, multiplication, and rooting. To implement their application in plant tissue culture, researchers need to develop strategies to improve microbial inoculants and inoculation technologies. The use of nanoformulations may improve the stability of biofertilizers (Malusá *et al.* 2012; Arora *et al.,* 2020) with respect to heat, desiccation, and ultra violet inactivation. Recently, some studies have shown huge interest in bionanotechnological inputs using PGPMs *in vitro* tissue culture, especially in the application of biotization in the pharmaceutical industry-targeted *in vitro* plants approach. The other challenge is improving the quality of microbial inoculants for *in vitro* plants and developing adequate inoculation protocols. With respect to this, the utilization of genetically modified inoculants may offer opportunities in order to achieve a specific purpose in the agricultural and food sector. Recent advances in biotechnological tools, such as functional genomics, signaling in rhizosphere, etc., could be useful in the engineering of micro-organisms to confer improved benefits to plants, especially in plant tissue culture field. A salient question is; can endophytic beneficial PGPMs be bio-engineered as an effective vector through which gene constructs can be transferred to vitroplants for crop improvement? If and when this ability is acquired, it is believed that the huge negative sentiments and resentment that are still held against genetically modified organisms will subside. If this happens, such innovation will revolutionize the agricultural seed sector, enhance the use of micro-organisms to produce pharma-metabolites *in-vitro*, and take unconventional crop improvement to a new level.

Perceived or Possible Demerits of the use of PGPMs

Though a number of success have been achieved in the quest to improve plant growth and development through PGPMs in *in vitro* and *in vivo* cultures, certain draw backs have been observed, among which include: foremost, difficulty of host plant to adapt to a particular soil, climatic conditions or pathogen which pose a serious challenge during the selection and characterization of the organism (Anjali *et al.,* 2019); achieving consistent environmental conditions in the field is not feasible, because variability of abiotic and biotic factors is higher and competition with indigenous organisms is more stressful compared to the green house conditions (Paulitz and Bélanger, 2001). Furthermore, scaling up of fermentation conditions for commercial production, while, maintaining quality, stability, and efficacy of the product is usually difficult (Mathre *et al.,* 2009). Lastly, toxigenicity, allergenicity and pathogenicity studies, and persistence in environment and horizontal gene transfer potential studies are required to improvise the health and safety measures of the products (McSpadden-Gardener and Fravel, 2002, Anjali *et al.,* 2019).

Ways to Enhance the Adoption of PGPMs

Possible ways to ensure that PGPMs is well acknowledged in the society as documented, may include the following approach: developing high precision and accuracy bioassay systems to select superior strains which would ensure maximum productivity (McSpadden-Gardener, 2002, Mathre *et al.,* 2009), developing better formulations or composite formulations to enhance the survival, proliferation and activity of the organisms when planted in the field (Bowen and Rovira, 2009, Mansouri *et al.,* 2002). Furthermore, for easy identification, proper categorization of PGPMs is important, either as biofertilizer or a biological control agent. Lastly capital costs and potential markets should be studied in order to commercialize them (Anjali *et al.,* 2019).

Conclusion

Plant Growth Promoting Microbes (PGPMs) remain the major stake holders in ecological processes responsible for nutrient recycling and availability for plant use. They have been proposed as biofertilizers, biostimulants and biocontrol agents to enhance plant growth and development for agricultural sustainability and food security. The application of PGPMs in plant tissue culture remains a viable option in the quest to improve plant growth and development in in *vitro* cultures. Plant Growth Promoting Microbes have the innate potential to produce Plant Growth Regulators and can be considered as potential biofactories. The development and use of inoculants based on PGPMs will help to reduce the cost of production of *in vitro* plants by partially or totally replacing some commercial synthetic products with microbial phytohormones and increasing the survival rate of *in vitro* plants. In addition, much remains to be studied about PGPMs in order to identify appropriate species and to develop bioformulations for suitable application in plant tissue culture.

References

Al-Ani, L.K.T. and Mohammed, A.M. (2020). "Versatility of *Trichoderma* in plant disease management" in *Molecular Aspects of Plant Beneficial Microbes in Agriculture.* Cambridge: Elsevier Science, Pp. 159–168.

Alexandre, J. (2017). Application of Protists to Improve Plant Growth in Sustainable Agriculture. Availaible at https://www.researchgate.net/publication/318840423.

Amara, U., Khalid, R. and Hayat, R. (2015). Soil bacteria and phytohormones for sustainable crop production. In Bacterial metabolites in sustainable agroecosystem. Sustainable development and biodiversity. *Springer*, 12: 87-103.

Anjali, J., Kavan, A. Priyank, P. and Rohit, K. (2019). Plant Growth Promoting Rhizobacteria: Mechanism, Application, Advantages and Disadvantages. Astral International Pvt. Ltd.New Delhi, Pp. 13-40.

Akin-Idowu, P.E., Ibitoye, D.O. and Ademoyegun, O. T. (2009). Tissue culture as a plant production technique for horticultural crops. *African Journal of Biotechnology*, 8: 3782–3788.

Ajar, N.Y., Rajeev, K. and Anil, S. (2017). Endowed with Plant Growth Promoting Attributes. Available at https://www.researchgate.net/publication/318227054.

Arora, N.K., Fatima, T., Mishra, J., Verma, S., Verma, R. and Verma, M. (2020). Halo-tolerant plant growth promoting rhizobacteria for improving productivity and remediation of saline soils. *Journal of Advanced Research,* 26: 69–82.

Bowen, G. D. and Rovira, A. D. (2009). The rhizosphere and its management to improve plant growth. *Advances in Agronomy,* 66: 1-102.

Bonkowski, M. (2004). Protozoa and plant growth: the microbial loop in soil revisited. *New Phytology*, 162:617–631

Bottini, R., Cassan, F. and Piccoli, P. (2014). Gibberellin production by bacteria and its involvement in plant growth promotion and yield increase. *Applied Microbiology and Biotechnology*, 65: 497–503.

Chanclud, E., Kisiala, A., Emery, N. R. J., Chalvon, V., Ducasse, A. and Romiti-Michel, C. (2016). Cytokinin production by the rice blast fungus is a pivotal requirement for full virulence. *Plant Science Pathogens*, 12:10-15.

Chen, M., Arato, M., Borghi, L., Nouri, E. and Reinhardt, D. (2018). Beneficial services of arbuscular mycorrhizal fungi: From ecology to application. *Frontiers Plant Science*, 9:1270.

Compant, S., Dufy, B., Nowak, J., Clement, C. and Barka, E. A. (2015). Use of plant growth-promoting bacteria for biocontrol of plant diseases: Principles, mechanisms of action and future prospects. *Applied Environmental Microbiology,* 71: 4951-4959.

Dave, B. (2006). Siderophores of halophilic Archaea and their chemical characterization. *Indian Journal of Experimental Biology,* 4(4): 340-344.

Diedhiou, A. G., Mbaye, F. K., Mbodj, D., Faye, M. N., Pignoly, S. and Ndoye, I. (2016). A field trial reveals ecotype-specific responses to mycorrhizal inoculation in rice. *Plant Science Journal,* 11:1-5.

Egamberdieva, D., Wirth, S., Bellingrath-Kimura, S.D., Mishra, J. and Arora, N.K. (2019). Salt-tolerant plant growth promoting rhizobacteria for enhancing crop productivity of saline soils. *Frontiers in Microbiology*, 10:2791.

Elmeskaoui, A., Damont, J., Poulin, M., Piche, Y. and Desjardins, Y. (2015). A tripartite culture system for endomycorrhizal inoculation of micropropagated strawberry plantlets *in vitro*. *Mycorrhiza*, 5: 313–319.

Erturk, Y., Ercisli, S., Haznedar, A. and Cakmakci, R. (2010). Effects of plant growth promoting rhizobacteria (PGPR) on rooting and root growth of kiwifruit (*Actinidia deliciosa*) stem cuttings. *Biological Research*, 43: 91–98.

Gupta, S. and Pandey, S. (2019). ACC deaminase-producing bacteria with multifarious plant growth promoting traits alleviates salinity stress in french bean (*Phaseolus vulgaris*) plants. *Frontiers in Microbiology*, 10:15-21.

Glick, B. R. (2015). Modulation of plant ethylene levels by the bacterial enzyme ACC deaminase. *FEMS Microbiology Letters*, 251: 1-7.

Gopinath, S., Kumaran, K. S. and Sundararaman, M. (2015). A new initiative in micropropagation: airborne bacterial volatiles modulate organogenesis and antioxidant activity in tobacco (*Nicotiana tabacum* L.) callus: *Invitro* Cell Development. *Biology of Plants*, 51: 514-523.

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

Heydarian, Z., Yu, M., Gruber, M., Glick, B. R., Zhou, R. and Hegedus, D. D. (2016). Inoculation of soil with plant growth promoting bacteria producing 1-aminocyclopropane-1-carboxylate deaminase or expression of the corresponding *acdS* gene in transgenic plants increases salinity tolerance in *Camelina sativa*. *Frontiers in Microbiology*, 7:19-26.

Iqbal, N., Khan, N. A., Ferrante, A., Trivellini, A., Francini, A. and Khan, M. (2017). Ethylene role in plant growth, development and senescence: interaction with other phytohormones. *Frontiers in Plant Science*, 8:475.

Jitendra, M., Rachna, S. and Naveen, A. (2017). Plant Growth-Promoting Microbes: Diverse Roles in Agriculture and Environmental Sustainability. Available at ttps://www.researchgate.net/publication/318185430.

Jousset, A., Scheu, S. and Bonkowski, M. (2008) Secondary metabolite production facilitates establishment of rhizobacteria by reducing both protozoan predation and the competitive effects of indigenous bacteria. *Functional Ecology,* 22:714–719.

Jousset, A., Rochat, L., Scheu, S., Bonkowski, M. and Keel, C. (2010). Predator-prey chemical warfare determines the expression of antifungal genes by rhizosphere pseudomonads. *Applied Environmental Microbiology,* 76:5263–5268.

Kapulnik, Y., Okon, Y. (2002). Plant growth promotion by rhizosphere bacteria. In: Waisel Y., Eshel A., Kafkafi, U. (eds) Plant roots: the hidden half. Marcel Dekker, New York, Pp. 869–885.

Kreuzer, K., Adamczyk, J. and Iijima, M.I. (2006). Grazing of a common species of soil protozoa (*Acanthamoeba castellanii*) affects rhizosphere bacterial community composition and root architecture of rice (*Oryza sativa* L.). *Soil Biology* *and Biochemistry,* 38:1665–1672.

Koller, R., Rodriguez, A., Robin, C., Scheu, S. and Bonkowski, M. (2013). Protozoa enhance foraging efficiency of arbuscular mycorrhizal fungi for mineral nitrogen from organic matter in soil to the benefit of host plants. *New Phytology*, 199(1):203–211.

Kanani, P., Modi, A. and Kumar, A. (2020). "Biotization of Endophytes in Micropropagation: A helpful enemy" In *Microbial endophytes:Prospects for sustainable agriculture, food science, technology and nutrition*. Woodhead Publishing, Pp. 357–379.

Kargapolova, K.Y., Burygin, G.L., Tkachenko, O.V., Evseeva, N.V., Pukhalskiy, Y.V. and Belimov, A.A. (2020). Effectiveness of inoculation of *in vitro-grown* potato microplants with rhizosphere bacteria of the genus *Azospirillum*. *Plant Cell Tissue and Organ Culture*, 141: 351–359.

Leigh J.A. (2000). Nitrogen fixation in methanogens: the archaeal perspective. *Current Issues in Molecular Biology,* 2(4): 125-131.

Lim, S.L., Subramaniam, S., Zamzuri, I. and Amir, H.G. (2016). Biotization of *in vitro* calli and embryogenic calli of oil palm (*Elaeisguineensis* Jacq.) with diazotrophic bacteria Herbaspirillum seropedicae (Z78). *Plant Cell Tissue and Organ Culture*, 127: 251-262.

Malusa, E., Sas-Paszt, L. and Ciesielska, J. (2012). Technologies for beneficial microorganisms inocula used as biofertilizers. *Science World Journal,* 12: 1–12.

Mansouri, H., Petit, A., Oger, P. and Dessaux, Y. (2002). Engineered rhizosphere: the trophic bias generated by opine-producing plants is independent of the opine type, the soil origin, and the plant species. *Applied and Environmental Microbiology*, 68(5): 2562-2566.

Mazzola, M., DeBruijn, I., Cohen, M.F. and Raaijmakers, J.M. (2009). Protozoan-induced regulation of cyclic lipopeptide biosynthesis is an effective predation defense mechanism for *Pseudomonas fluorescens*. *Applied Environmental Microbiology,* 75:6804–6811.

Mathre, D. E., Cook, R. J. and Callan, N. W. (2009). From discovery to use: traversing the world of commercializing biocontrol agents for plant disease control. *Plant Disease*, 83(11): 972-983.

McSpadden-Gardener, B. B. and Fravel, D. R. (2002). Biological control of plant pathogens: research, commercialization, and application in the USA. *Plant Health Progress*, 10(10):1094.

Meixner, C., Ludwig-Müller, J., Miersch, O., Gresshoff, P., Staehelin, C. and Vierheilig, H. (2005). Lack of mycorrhizal autoregulation and phytohormonal changes in the supernodulating soybean mutants 1007. *Planta*, 222: 709-715.

Muller, D. and Leyser, O. (2011). Auxin, cytokinin and the control of shoot branching. *Annal of Botany*, 107: 1203–1212.

Naseem, M. and Dandekar, T. (2012). The role of auxin-cytokinin antagonism in plant pathogen interactions. *Plant Science Pathogens*, 8:10-16.

Orlikowska, T., Nowak, K. and Reed, B. (2017). Bacteria in the plant tissue culture environment. *Plant Cell Tissue and Organ Culture*, 128: 487-508.

Osugi, A. and Sakakibara, H. (2015). Q&A: how do plants respond to cytokinins and what is their importance? BMC Biology, 13:102-108.

Paulitz, T.C. and Bélanger, R.R. (2001). Biological control in greenhouse systems. *Annual review of phytopathology*, 39(1):103-133.

Pawlowski, J. (2013). The new micro-kingdoms of eukaryotes. *BMC Biology,* 11:1–3

Pieterse, C. M. J., Zamioudis, C., Berendsen, R. L., Weller, D. M., Van Wees, S. C. M. and Bakker, P. A. (2014). Induced systemic resistance by beneficial microbes. *Annual Review on Phytopathology*, 52: 347–375.

Poupin, M.J., Timmermann, T., Vega, A., Zuñiga, A. and González, B. (2013). Effects of the plant growth-promoting bacterium *Burkholderia phytofirmans* PsJN throughout the life cycle of *Arabidopsis thaliana*. *Plant Science*, 8:6-19.

Puschel, D., Bitterlich, M., Rydlová, J. and Jansa, J. (2020). Facilitation of plant water uptake by an arbuscular mycorrhizal fungus: a Gordian knot of roots and hyphae. *Mycorrhiza*, 30, 299–313.

Quambusch, M. and Winkelmann, T. (2018). Bacterial endophytes in plant tissue culture: mode of action, detection, and control. *Methods of Molecular Biology*, 1815: 69-88.

Rai, M.K. (2011). Current advances in mycorrhization in microporopagation. *In Vitro Cell Development Biology of Plants,* 37: 158–167.

Rana, K.L., Kour, D., Sheikh, I., Yadav, N., Yadav, A. N. and Kumar, V. (2019). Biodiversity of endophytic fungi from diverse niches and their biotechnological applications. In Advances in endophytic fungal research. Fungal biology. Cham: Springer, Pp. 105–144.

Rodríguez-Romero, A.S., Badosa, E., Montesinos, E. and Jaizme-Vega, M.C. (2008). Growth promotion and biological control of root-knot nematodes in micropropagated banana during the nursery stage by treatment with specific bacterial strains. *Annals of Applied Biology*, 152: 41–48.

Ruzzi, M. and Aroca, R. (2015). Plant growth-promoting rhizobacteria act as biostimulants in horticulture. *Science of Horticulture,* 96: 124–134.

Swallow, M.J.B., Quideau, S.A. and Norris, C.E. (2013). Ciliate dependent production of microbial anthranilic acid occurring within aspen litter. *Soil Biology and Biochemistry,* 60:113–121.

Souza, R.D., Ambrosini, A. and Passaglia, L.M. (2015). Plant growth-promoting bacteria as inoculants in agricultural soils. *Genetics and Molecular Biology*, 38: 401-419.

Spaepen, S. and Vanderleyden, J. (2011). Auxin and plant-microbe interactions. *Cold Spring Harb Perspective of Biology*, 3: 14-19.

Spaepen, S., Vanderleyden, J. and Okon, Y. (2009). Plant growth-promoting actions of rhizobacteria. *Advances Botanical Research*, 51:283–320.

Srinivasan, M., Kumar, K., Kumutha, K. and

Marimuthu, P. (2014). Establishing monoxenic culture of arbuscular mycorrhizal fungus *Glomus* intraradices through root organ culture. *Journal of Applied Natural Science*, 6: 290–293.

Soumare, A., Diedhiou, A.G. and Kane, A. (2017). Eucalyptus plantations in the Sahel: distribution, socio-economic importance and ecological concern. *International Journal of Biological and Chemical Science*, 11: 3005–3017.

Streletskii, R.A., Kachalkin, A.V., Glushakova, A.M., Yurkov, A.M. and Demin, V.V. (2019). Yeasts producing zeatin. *Peer Journal*, 7:64-74.

Sgroy, V., Cassán, F., Masciarelli, O., Del Papa, M.F., Lagares, A. and Luna, V. (2009). Isolation and characterization of endophytic plant growth-promoting (PGPB) or stress homeostasis-regulating (PSHB) bacteria associated to the halophyte *Prosopis strombulifera*. *Applied Microbiology and Biotechnology,* 85: 371–381.

Soumare, A., Boubekri, K., Lyamlouli, K., Hafidi, M., Ouhdouch, Y. and Kouisni, L. (2020). Efficacy of phosphate solubilizing *Actinobacteria* to improve rock phosphate agronomic effectiveness and plant growth promotion. *Rhizosphere,* 17:100284.

Sunita, K., Mishra, I., Mishra, J., Prakash, J. and Arora, N.K. (2020). Secondary metabolites from halotolerant plant growth promoting rhizobacteria for ameliorating salinity stress in plants. *Frontier of Microbiology,* 11:567-768.

Trigiano, R.N., and Gray, D.J. (2016). *Plant tissue culture, development, and biotechnology.* United States: CRC Press, pp: 608.

Tyagi, B., Tewari, S. and Dubey, A. (2018). Biochemical characterization of fungus isolated during in vitro propagation of *Bambusa balcooa*. *Pharmacogenesis and Management*, 13(4): S775–S779.

White, R.H. (2007). Indole-3-acetic acid and 2-(indol-3-ylmethyl) indol-3-yl acetic acid in the thermophilic archaebacterium Sulfolobus acidocaldarius. *Journal of Bacteriology,* 169(12): 5859-5860.

Xie, X., Zhang, H. and Pare, P. (2009). Sustained growth promotion in *Arabidopsis* with long-term exposure to the beneficial soil bacterium *Bacillus subtilis* (GB03). *Plant Signal and Behaviour*, 4: 948–953.

Yadav A.N., Priyanka, V., Rajeev, K., Harcharan, S.D. and Anil, K.S. (2015). Haloarchaea endowed with phosphorus solubilization attribute implicated in phosphorus cycle. *Scientific Reports,* 5: 12293.