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Review Article
Review on Problems and its Remedy in Plant Tissue Culture

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Abstract

Plant tissue culture is the finest method to propagate in large scale and to protect the rare, endangered and important plant species. It is in urgent to concentrate on conservation of rare, endangered medicinal and commercially important plant species. But the success rate of propagation in this method is attenuate in particular with a few medicinal plants. Also the researchers are facing lot of troubles with propagation of plant tissues and acclimatization of *in vitro* raised plants in the natural habitat. There are many reasons behind these problems. This review covered all that difficulties right from laboratory construction up to field adaptation of tissue cultured plants along with remedies to elucidate all the complications in this technique.

Key words: Plant tissue culture, propagation of plant tissues, *in vitro* raised plants, rare, endangered and important plant species

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

To satisfy the global demand in the field of agriculture for producing more food crops and plant based medicine, it is in urgent to conserve the agricultural, economical, rare and endangered plants. They had high medicinal and ecological impact and should be propagated widely, even it was a failure in the conventional methods. Plant tissue culture is an important tool to propagate the plants in large scale through the eminent way in the short[1]. Culture of plant and various parts in the aseptic condition with the concept of totipotency[2-4]. A special media fortified with inorganic nutrients, vitamins, carbohydrates and environmental factors are added *in vitro* condition[5]. Cell totipotentiality and cellular plasticity is the major physiological principle behind the plant tissue culture. Cell plasticity responses for the division and differentiation capacity of the culture cells[6]. The ability of the single cell to transform into a whole plant alike as the mother plant[7]. The propagation method should be under the controlled environment, hence there is absence of seasonal effect. Though the plants are cultivating in the large scale, plants will be genetically uniform, true to type[8]. In plant tissue culture, plants are grown in the nutrient media, where the nutrients required for the plants are added and also had a space for microbes, because of having rich nutrients. Microbial growth in the media is due to improper sterilization and ill procedures. Contamination in tissue culture is not universal to find the exact reason but implies perfection in every step leads to reduce the contamination menace in plant tissue[9]. Contamination may be physiological and pathological and the contaminants in the culture are bacteria, fungi and yeast[10]. For a successful culture of plant tissue needs to provide aseptic condition, selection of plant tissue free from microbes, proper sterilization, appropriate nutrition in tissue culture media, taking right measures in the browning and somaclonal variation. Hardening in the green house and acclimatization to the field conditions is the final problem in the *in vitro* raised plants.

PROBLEMS AND ITS REMEDY

Selection of explants: Explant selection is the initial step in the plant tissue culture, any part of the plant can be selected as explants. It should be from the healthy mother plant, possibility for better growth was determined by position and age of the explants[11]. If the explants are collected from the green house condition is considered as the better one and the rate of survivable capacity will be higher. Survivability of the culture was increased, where the explants were collected in the dry spell of the year[12]. Shoot tip and internodes were found as the best source of explants for sterilization and better growth[13].

Sterilization of media and culture vessel: The most important step in tissue and organ culture techniques is sterilization[14]. Contamination occurs through improper sterilization of glassware and vessels. It is controlled by chemical and physical methods, dry heat, wet heat, chemical and ultra filtration. Media should be autoclaved for 15 min at 121°C, which kills the microbes on the culture vessel[15,16]. Glassware's which are not able to autoclave properly are sterilized with the help of sodium hypochlorite with 0.002% concentration and heat sterilized with hot air oven is better for the glassware sterilization[17].

Explant sterilization: Surface sterilization of the explants is the essential step in the plant tissue culture[18]. If explants taken from the external environment was exposed to microbial contamination will leads to the mortality of the plant tissue[19]. Microbial contamination in the external part of the explants can be sterilized with the running water and chemical substances, includes ethanol, sodium hypochlorite, mercuric chloride and plant preservative mixtures etc. Ethanol is the strong sterilizing agent but it was phytotoxic to the plant cells. High concentration of ethanol and mercuric chloride affects the growth of the plant in the culture[12]. There is the evidence for plant preservative mixture is a best sterilizing agent for the culture of *Cestrum nouturnum* L.[20]. For the successful propagation of *Andrographis paniculata,* fungicide, bleaching powder, amoxicillin antibiotic, carbendazim and mercuric chloride were used in the surface sterilization. Mercuric chloride at the concentration of 0.1% solution treated for 2 min showed the best result in number of plant species. Calcium hypochlorite in the concentration of 5% for 20 min shows a better result in the propagation of Ziziphus *Spina-christi* (L.) Desf[21]. Mercuric chloride provides better result in the explants sterilization of *Pinellia ternate* (Thunb.) Breit[22]. Effective sterilization method may vary with the nature of the explants. Even if the explants taken from the different part of the same plant, their effective sterilizing agent was varied in the *Salacia chinensis* L.[23].

Effect of ultra violet-c radiation in explants sterilization: The explants are allowed to ultraviolet-C radiation for 5 min

and surface sterilization for about 10 min, acts as the better way to reduce the external contamination in *Solanum tuberosum*. The intensity in the UV-C radiation can deactivate the DNA in the pathogen[24]. But there may be a chance to damage the plant molecules especially with plant DNA.

Role of endophytic organism in contamination: Epiphytic microbes in the explants were removed by surface sterilization but the occurrence of the endophytic microbes in the explants leads to contamination in the culture[25,26]. The concentration of sterilizing the agents depends upon the plant explants may vary from one to another.

Medium preparation, nutrients and hormonal imbalance: Media is the place where the explants are inoculated to grow and the success of the tissue culture depends upon the selection of medium. Most commonly used medium is MS by Murashige and Skoog[5]. The right nutritional medium consists of inorganic salts, organic supplements, vitamins, growth regulators and carbon source[27]. Agar is used as a gelling agent in the culture medium[28]. Right pH is required for the medium and optimum pH of the nutrient medium is maintained properly (5.6-5.8). There may be a lack of uptake of nutrient from the medium when it is highly alkaline or acidic[29]. Plant growth hormones are naturally synthesized by the plant but there is need to add some external growth hormones for the better growth and to enrich the metabolite synthesis, particularly plant bioactive molecules[30]. Plant growth hormones are auxins, cytokinins and gibberellins. Some of the major auxins includes indole-3-acetic acid, indole-3-butyric acid, 2, 4 dichlorophenoxyacetic acid, naphthalene acetic acid, 2, 4, 5-trichlorophenoxyacetic acid and naphthoxyacetic acid, which helps in the cell division, elongation and root differentiation. Cytokinins like benzyl amino purine, isopentenyl adenine, kinetin, 4-hydroxyl-3-methyl-trans-2-butenylaminopurine plays a vital role in the shoot induction, development and proliferation. Gibberellins help in flowering and elongation[29]. Thiamine is considered as the essential vitamin in the medium. Glucose or sucrose is considered as the carbon source at the concentration of 2-4%. Liquid and semi solid medium is better for the successful propagation of plant species. There is a need of relative humidity in the medium[31] about 70-75%. Humidity in the culture vessel also plays a vital role in the growth of the plant[32].

Contamination: Contamination is the common problem in the *in vitro* culture. To determine the source of contamination is tedious. Endophytic microbial contamination was not possible to stop with the surface sterilization; still it is a big problem for number of plant species. It may cause severe problem to the culture especially the growing plant tissue[33]. Microbes which are resistant to surface sterilization and the endophytic microbes cause drastic problem to the culture[34]. Laboratory must be maintained in sterile condition with good ambience which is the root cause of causing contamination[35]. The data were presented and documented in the Table 1 and 2 for future reference which includes the common bacteria and fungi are involving in contaminating the culture of different plant tissues.

The occurrence of microbes in the culture varies with the plant species and the zone of geographical distribution[49]. Aseptic condition in the *in vitro* culture is the best way to eradicate contamination.

Construction of tissue culture laboratory: Even the construction and structural deformities of the laboratory can cause problems in the cultures with possible contamination. The well designed and planned laboratory with washing room, sterile room, growth culture room, store room and hardening area is quite mandatory for successful cultures. There should be a well-designed and proper construction of various rooms, which may have less chance of contamination. The wash room and sterile room are distant from each other, sterile and growth room has to be kept close to each other to reduce the various sources of contamination.

Role of antibiotics: Usage of antibiotics plays an essential role in the elimination and inhibition of microbial contamination[50,51]. By adding appropriate amount of antibiotics like Streptomycin, Tetracycline, Vancomycin, Rifampicin, Gentamicin, Cefotaxime in the medium may eradicate the contamination in the culture[17,3,52,53]. Benomyl and Streptomycin were added in the medium provides better culture of nodal explants[54]. In the *in vitro* cultivation of potato, Streptomycin is considered as the better antibiotic to reduce contamination and to enhance the growth of the plant tissue[42]. Use of nano-particles in the culture medium can reduce the contamination, like nano-silver and titanium dioxide were having anti-microbial activity[55].

Culture types in plant tissue culture: Propagation in the culture media had different forms of callus cultures like cell

Table 1: Bacterial contamination in plant tissue culture

Plants	Bacterial contaminants	References
Manihot esculenta	*Pseudomonas flourescens*, *Staphylococcus aureus*, *Corynebacterium* sp., *Bacillus subtilis*	Odutayo *et al.*[9]
Hibiscus cannabinus	*Pseudomonas flourescens*, *Staphylococcus aureus*, *Corynebacterium* sp., *Bacillus subtilis*	Odutayo *et al.*[9]
Vigna unguiclata	*Pseudomonas flourescens*, *Staphylococcus aureus*, *Corynebacterium* sp., *Bacillus subtilis*	Odutayo *et al.*[9]
Musa paradisiacal	*Pseudomonas flourescens*, *Staphylococcus aureus*, *Corynebacterium* sp., *Bacillus subtilis*	Odutayo *et al.*[9]
Musa spp.	*Proteus*, *Erwinia*, *Klebsiella*, *Staphylococcus*	Msogoyo *et al.*[36]
Ipomoea batatas	*Corynebacterium* sp., *Klebsiella* sp., *Pseudomonas* sp.	Jena and Samal[37]
Phoenix dactylifera L.	*Bacillus subtillus*, *Staphylococcus aureus*, *Proteus* sp.	Abass[8]
Elaeis guineensis Jacq.	*Proteus vulgarius*, *Bacillus subtilis*, *Pseudomonas flourescens*, *Erwinia* sp., *Staphylococcus aureus*, *Corynebacterium* sp.	Eziashi *et al.*[38]
Malus spp.	*Bacillus* spp., *Bacillus subtilis*, *Coryneform bacteria*, *Actinomyces* spp.	Hennerty *et al.*[39]
Gerbera spp.	*Bacillus* spp., *Coryneform bacteria*, *Bacillus polymyxa*, *Acinetobacter calcoaceticus*, *Agrobacterium radiobacter*, *Erwinia* spp.	Podwyszynska and Hempel[40]
Begonia spp.	*Bacillus circulans*, *Bacillus cereus*	Podwyszynska and Hempel[40]
Hevea spp.	*Bacillus* spp., *Propionibacterium* spp., *Bordetella branchiseptica*, *Acinetobacter calcoaceticus*, *Agrobacterium radiobacter*, *Erwinia* spp., *Enterobacter cloacae*	Enjalric *et al.*[41]
Nephrolepsis spp.	*Bacillus* spp.	Leifert *et al.*[10]
Nauclea spp.	*Bacillus* spp.	Leifert *et al.*[10]
Pteris spp.	*Bacillus* spp., *Erwinia carotovora*	Leifert *et al.*[10]
Saxifraga spp.	*Bacillus* spp., *Erwinia carotovora*	Leifert *et al.*[10]
Viola spp.	*Bacillus* spp., *Bacillus subtilis*, *Agrobacterium radiobacter*, *Erwinia* spp.	Leifert *et al.*[10]
Fragaria spp.	*Bacillus circulans*, *Bacillus cereus*, *Coryneform bacteria*, *Acinetobacter calcoaceticus*, *Erwinia* spp.	Podwyszynska and Hempel[40]
Primula spp.	*Bacillus circulans*, *Bacillus subtilis*	Leifert *et al.*[42]
Phoenix spp.	*Bacillus circulans*	Leifert *et al.*[10]
Astilbe spp.	*Bacillus subtilis*, *Bacillus pumilus*, *Acinetobacter calcoaceticus*	Leifert *et al.*[42]
Arunchus spp.	*Bacillus pumilus*	Leifert *et al.*[42]
Cotinus spp.	*Bacillus subtilis*, *Bacillus pumilus*	Leifert *et al.*[42]
Pulmunaria spp.	*Bacillus pumilus*	Leifert *et al.*[10]
Delphinium spp.	*Bacillus subtilis*, *Micrococcus* spp., *Staphylococcus* spp., *Staphylococcus warneri*, *Lactobacillus acidophilus*, *Acinetobacter calcoaceticus*, *Klebsiella oxytoca*	Leifert *et al.*[42]
Hemerocallis spp.	*Bacillus subtilis*, *Lactobacillus plantarum*, *Staphylococcus epidermidis*, *Coryneform bacteria*, *Staphylococcus* spp., *Micrococcus kristinae*, *Enterobacter cloacae*, *Rhanella aquatilis*	Leifert *et al.*[10]
Thalictrum spp.	*Bacillus subtilis*	Leifert *et al.*[42]
Fremontodendron spp.	*Coryneform bacteria*	Boxus and Terzi[43]
Geranium spp.	*Coryneform bacteria*	Leifert *et al.*[10]
Prunus spp.	*Coryneform bacteria*, *Micrococcus* spp., *Erwinia* spp.	Cornu and Michel[44]
Solanum spp.	*Coryneform bacteria*	Leifert *et al.*[10]
Sungonium spp.	*Mycobacterium scrofulaceum*	Taber *et al.*[45]
Spathiphyllum spp.	*Mycobacterium scrofulaceum*	Leifert *et al.*[10]
Choysia spp.	*Staphylococcus* spp., *Staphylococcus epidermidis*, *Staphylococcus saprophyticus*, *Micrococcus* spp., *Agrobacterium radiobacter*, *Achromobacter* spp., *Erwinia* spp.	Leggatt *et al.*[46]
Hosta spp.	*Staphylococcus epidermidis*, *Acinetobacter calcoaceticus*, *Staphylococcus warneri*, *Micrococcus kristinae*, *Erwinia* spp.	Leifert *et al.*[42]
Paeony spp.	*Staphylococcus warneri*, *Micrococcus* spp., *Agrobacterium radiobacter*	Gunson and Spencer-Phillips[47]
Ficus spp.	*Streptomyces* spp., *Agrobacterium radiobacter*, *Erwinia* spp.	Leifert *et al.*[10]
Syngonium spp.	*Streptomyces* spp., *Agrobacterium radiobacter*, *Erwinia* spp.	Leifert *et al.*[10]
Dieffenbachia spp.	*Streptomyces* spp., *Agrobacterium radiobacter*, *Erwinia* spp.	Leifert *et al.*[10]
Zantedeschia spp.	*Streptomyces* spp., *Agrobacterium radiobacter*, *Erwinia* spp.	Leifert *et al.*[10]
Iris spp.	*Alcaligenes denitrificans*, *Erwinia carotovora*, *Enterobacter cloacae*, *Rhanella aquatilis*	Leifert *et al.*[10]
Coffea spp.	*Erwinia* spp., *Enterobacter cloacae*	Leifert *et al.*[10]

Table 2: Fungal contamination in plant tissue culture

Plants	Fungal contamination	References
Manihot esculenta	*Fusarium oxysporium*, *Fusarium culmorum*, *Aspergillus niger*, *Mucor racemosus*	Odutayo *et al.*[9]
Hibiscus cannabinus	*Fusarium oxysporium*, *Fusarium culmorum*, *Aspergillus niger*, *Mucor racemosus*	Odutayo *et al.*[9]
Vigna unguiclata	*Fusarium oxysporium*, *Fusarium culmorum*, *Aspergillus niger*, *Mucor racemosus*	Odutayo *et al.*[9]
Musa paradisiacal	*Fusarium oxysporium*, *Fusarium culmorum*, *Aspergillus niger*, *Mucor racemosus*	Odutayo *et al.*[9]
Musa spp.	*Aspergillus*, *Fusarium*, *Penicillium*, *Candida*	Msogoyo *et al.*[36]
Phoenix dactylifera L.	*Alternaria alternate*, *Aspergillus niger*, *Aspergillus clavatus*, *Scytalidium lignicola*, *Alternaria citri*, *Aspergillus terreus*, *Cladosporium* sp., *Epicoccum* sp., *Penicillium* spp., *Chaetomium atrobrunneum*, *Eurotium amstelodami*, *Fusarium* sp.	Abass[8]
Musa textiles	*Aspergillus* sp., *Chrysosporium* sp.	Cobrado and Fernandez[48]

suspension culture, protoplast culture, embryo culture, anther culture and pollen culture. Each type is preferred for various aspects in the culture of plants. Cell suspension culture is mainly used to enhance the quantity of plant secondary metabolites[56]. And the embryo culture is succeeded by efficient germplasm hybrids[57].

Browning of explant: Oxidation of phenols within the tissue leads to browning of explants. It may found in the *in vitro* culture of woody plants. Browning reduces the cell division and explant regeneration capacity, may leads to the failure in the plant tissue culture. Use of absorbent and antioxidants is the finest way to reduce the browning in the *in vitro* propagation. There are some evidences recorded, ascorbic acid reduces the oxidized substrates and hence it prevents the browning efficiently[58]. Browning leads to darken the medium and incompatible for cell cultures. Potassium citrate is variably better antioxidant in the culture of *Musa pardisiaca*[59]. Rate of browning may vary and depends upon the season and also upon the presence of plant secondary metabolites. If the explants collected during the months December and March, the low level of browning was observed due to poor production of secondary metabolites[60]. The best way of removal of secondary products from the explants is to collect explants at earlier morning before sunrise (the plant do not start the photosynthesis before sunrise) and immerse the explants in the water immediately after collection as it is a best solvent to elucidate the secondary compound[61].

As per my experience, one of the major problems associated with tissue culture is browning of explants due to oxidation of phenolic compounds released from the cut end of the explants by poly-phenoloxidases, peroxidases, as this led to cell death. Propensity to browning was anti-oxidant specific and the presence of an anti-oxidant greatly minimized the browning rate. To overcome phenolic exudation, CA, AC and PVP were investigated and activated charcoal found to be the best[62]. Standardization of surface sterilization is need for prevention of phenolic browning and microbial contaminations of the explants[63].

Somaclonal variation: The genetic change may occur in the *in vitro* raised plants is termed as somaclonal variation[64]. The genotypic nature of the plant, growth regulators, frequency of sub culturing, proliferation rate of tissues are the reasons for somaclonal variations[65]. Factors that influence the genetic variability are mutations, natural selection and migration has rich genetic variations in *in vitro* cultures[1]. But for commercially producing plants like Casuarinas, it is not necessary to concentrate on somaclonal variations.

Contamination in sub-culture: Sub-culture is the process to multiply the plantlets *in vitro*. Aseptic conditions are essential to maintain and regulate the sub-culturing of plant species. After sub-culturing the cultured vessels are stored in better environment with proper identification and much needed aseptic conditions[17].

Hardening and acclimatization: Hardening refers to transfer of the explants from *in vitro* to the green house. During primary hardening soil fumigants are used for sterility. Dimethyl disulfide, methyl bromide, chloropicrin are frequently used as fumigants[17]. Use of farm yard manure in the ratio of 1:4 provides, better results in the hardening[66]. Successful propagation of plants depends upon the field survival. So the process of plant tissue culture is based upon the varied plant species[31,67]. But the mangrove plant somewhat difficult to propagate by *in vitro* techniques due to its high content of phenolics[68].

CONCLUSION

Widespread of medicinal and economically important plants have been recorded in India as well as in the World, though some plant species were still in critical condition. Conservation of plants is the main aim in the plant tissue culture. This paper is widely focused on the problems that occur during plant tissue culture. Also an amicable solution was made by enabling the previous records along with my fifteen years' of exposure and experience in the same field have documented. Utmost care in every step may lead to success in the field of plant tissue culture and conservation of valuable and rare plant population.

REFERENCES

1. Tazeb, A., 2017. Plant tissue culture technique as a novel tool in plant breeding: A review article. Am.-Eurasian J. Agric. Environ. Sci., 17: 111-118.
2. Haberlandt, G., 1902. Kulturversuche mit isolierten pflanzenzellen. [Culture experiments with isolated plant cells]. Situngsber. Mat. Nat. Kl. Kais Akad. Wiss. Wien, 111: 69-92.
3. Sharma, H.C., J.H. Crouch, K.K. Sharma, N. Seetharama and C.T. Hash, 2002. Applications of biotechnology for crop improvement: Prospects and constraints. Plant Sci., 163: 381-395.
4. Neelakandan, A.K. and K. Wang, 2012. Recent progress in the understanding of tissue culture-induced genome level changes in plants and potential applications. Plant Cell Rep., 31: 597-620.

5. Murashige, T. and F. Skoog, 1962. A revised medium for rapid growth and bio assays with tobacco tissue cultures. Physiol. Plantarum, 15: 473-497.
6. Garcia-Gonzales, R., K. Quiroz, B. Carrasco and P. Caligari, 2010. Plant tissue culture: Current status, opportunities and challenges. Ciencia Investigacion Agraria, 37: 5-30.
7. Bhoite, H.A. and G.S. Palshikar, 2014. Plant tissue culture: A review. World J. Pharm. Sci., 2: 565-572.
8. Abass, M.H., 2013. Microbial contaminants of date palm (*Phoenix dactylifera* L.) in Iraqi tissue culture laboratories. Emirates J. Food Agric., 25: 875-882.
9. Odutayo, O.I., N.A. Amusa, O.O. Okutade and Y.R. Ogunsanwo, 2007. Sources of microbial contamination in tissue culture laboratories in southwestern Nigeria. Afr. J. Agric. Res., 2: 67-72.
10. Leifert, C., C.E. Morris and W.M. Waites, 1994. Ecology of microbial saprophytes and pathogens in tissue culture and field-grown plants: Reasons for contamination problems *in vitro*. Crit. Rev. Plant Sci., 13: 139-183.
11. Gamborg, O.L. and G.C. Phillips, 1995. Plant Cell, Tissue and Organ Culture: Fundamental Methods. Springer, New York, ISBN-13: 9783540580683.
12. Kataky, A. and P.J. Handique, 2010. Standardization of sterilization techniques prior to *in vitro* propagation of *Andrographis paniculata* (Burm.f) Nees. Asian J. Sci. Technol., 10: 119-122.
13. Dar, C.T., J.O. Abdullah, P. Namasivayam and S.H. Roowi, 2012. Sterilization of *Hibiscus rosa-sinensis* L. vegetative explants sourced from plants grown in open environment and influences of organic ingredients on *in vitro* direct regeneration. Am. J. Plant Sci., 3: 791-798.
14. Singh, C.R. and K. Kathiresan, 2015. *In-vitro* callus induction from *Ceriops decandra*-A true mangrove viviparous. Int. J. Adv. Multidiscip. Res., 2: 01-05.
15. Ikenganyia, E.E., M.A.N. Anikwe, T.E. Omeje and J.O. Adinde, 2017. Plant tissue culture regeneration and aseptic techniques. Asian J. Biotechnol. Bioresour. Technol., 1: 1-6.
16. Kyesmu, P.M., C.P.E. Omaliko and A. Maduekwe, 2004. Basic Facts on Plant Tissue Culture: A Guide to Plant Tissue Culture Practice. Dolio-B Press, Abuja, Nigeria.
17. Leelavathy, S. and P.D. Sankar, 2016. Curbing the menace of contamination in plant tissue culture. J. Pure Applied Microbiol., 10: 2145-2152.
18. Singh, V., A. Tyagi, P.K. Chauhan, P. Kumarai and S. Kaushal, 2011. Identification and prevention of bacterial contimination on explant used in plant tissue culture labs. Int. J. Pharm. Pharm. Sci., 3: 160-163.
19. Oyebanji, O.B., O. Nweke, O. Odebunmi, N.B. Galadima and M.S. Idris *et al*., 2009. Simple, effective and economical explant-surface sterilization protocol for cowpea, rice and sorghum seeds. Afr. J. Biotechnol., 8: 5395-5399.
20. Mahmoud, S.N. and N.K. Al-Ani, 2016. Effect of different sterilization methods on contamination and viability of nodal segments of *Cestrum nocturnum* L. Int. J. Res. Stud. Biosci., 4: 4-9.
21. Assareh, M.H. and H. Sardabi, 2005. Macropropagation and micropropagation of *Ziziphus spina-christi*. Pesq. Agropec. Bras, 5: 459-465.
22. Xu, T., L. Zhang, X. Sun and K. Tang, 2005. Efficient *in vitro* plant regeneration of *Pinellia ternata* (Thunb.) Breit. Acta Biol. Cracov. Ser. Bot., 47: 27-32.
23. Majid, B.N., G. Roopa, K.K.S. Kumara, K.R. Kini and H.S. Prakash *et al*., 2014. Establishment of an efficient explant surface sterilization protocol for *in vitro* micropropagation of *Salacia chinensis* L., an endangered anti-diabetic medicinal plant. World J. Pharm. Pharm. Sci., 3: 1266-1274.
24. Gangopadhyay, M., S. Nandi and S.K.B. Roy, 2017. An efficient ex plant sterilization protocol for reducing microbial contamination of *Solanum tuberosum* CV. 'Kufri jyoti for establishing micropropagation in rainy season. J. Basic Applied Plant Sci., Vol. 1.
25. Van den Houwe, I. and R. Swennen, 2000. Characterization and control of bacterial contaminants in *in vitro* cultures of banana (*Musa* spp.). Acta Hortic., 530: 69-79.
26. Sinha, S.K. and A.C. Deka, 2016. Effect of osmotic stress on *in vitro* propagation of *Musa* sp. (Malbhog variety). Afr. J. Biotechnol., 15: 490-496.
27. Gaikwad, A.V., S.K. Singh and R. Gilhotra, 2017. Plant tissue culture-A review. J. Pharm. Res. Educ., 2: 217-220.
28. Bhojwani, S.S. and M.K. Razdan, 1996. Plant Tissue Culture: Theory and Practice: Developments in Crop Science, Volume 5. 1st Edn., Elsevier, Amsterdam, ISBN: 9780080539096, Pages: 766.
29. Srivastava, L.M., 2002. Plant Growth and Development: Hormones and Environment. 1st Edn., Academic Press, New York, ISBN: 978-0126605709, pp: 140-143.
30. Chang, W.D., W.W. Huang, C.C Chen, Y.S. Chang and H.S. Tsay, 1994. The production of secondary metabolites from Chinese medicinal herbs by suspension cell and tissue culture. Proceedings of the 7th International Congress of SABRAO and WASS, November 16-19, 1994, Taipei, Taiwan, pp: 535-540.
31. Chougule, A.S., C.S. Thosar and S.S. Bagewadi, 2015. Article on "Tissue culture of the plants". Unique J. Ayurvedic Herbal Med., 3: 46-49.
32. Chen, C., 2004. Humidity in plant tissue culture vessels. Biosyst. Eng., 88: 231-241.
33. Kneifel, W. and W. Leonhardt, 1992. Testing of different antibiotics against Gram-positive and Gram-negative bacteria isolated from plant tissue culture. Plant Cell Tissue Organ Cult., 29: 139-144.

34. Rayaprolu, S., G. Mathian and G. Ranjitha, 2015. Can *in vitro* contaminated culture be revived-A case study with contaminated cultures of tuberose (*Polianthes tuberosa*). Curr. Biotica, 9: 285-288.
35. Orlikowska, T. and M. Zawadzka, 2006. *In vitro* Bacteria in plant tissue cultures. Biotechnologia, 4: 64-77.
36. Msogoyo, T., H. Kanyagha, J. Mutigitu, M. Kulebelwa and D. Mamiro, 2012. Identification and management of microbial contaminants of banana *in vitro* cultures. J. Applied Biosci., 55: 3987-3994.
37. Jena, R.C. and K.C. Samal, 2011. Endogenous microbial contamination during *in vitro* culture of sweet potato [*Ipomoea batatas* (L.) Lam]: Identification and prevention. J. Agric. Technol., 7: 1725-1731.
38. Eziashi, E.I., O. Asemota, C.O. Okwuagwu, C.R. Eke, N.I. Chidi and E.A. Oruade-Dimaro, 2014. Screening sterilizing agents and antibiotics for the elimination of bacterial contaminants from oil palm explants for plant tissue culture. Eur. J. Exp. Biol., 4: 111-115.
39. Hennerty, M.J., M.E. Upton, P.A. Furlong, D.J. James, D.P. Harris and R.A. Eaton, 1988. Microbial contamination of *in vitro* cultures of apple rootstocks M26 and M9. Acta Hortic., 225: 129-138.
40. Podwyszynska, M. and M. Hempel, 1987. Identification and elimination of "Slowly growing" bacteria from a micropropagated gerbera. Acta Hortic., 212: 112-112.
41. Enjalric, F., M.P. Carron and L. Lardet, 1988. Contamination of primary cultures in tropical areas: The case of hevea brasiliensis. Acta Hortic., 225: 57-66.
42. Leifert, C., W.M. Waites and J.R. Nicholas, 1989. Bacterial contaminants of micropropagated plant cultures. J. Applied Microbiol., 67: 353-361.
43. Boxus, P.H. and J.M. Terzi, 1988. Control of accidental contaminations during mass propagation. Acta Hortic., 225: 189-192.
44. Cornu, D. and M.F. Michel, 1987. Bacteria contaminants in shoot cultures of *Prunus avium* L. choice and phytotoxicity of antibiotics. Acta Hortic., 212: 83-86.
45. Taber, R.A., M.A. Thielen, J.O. Falkinham III and R.H. Smith, 1991. *Mycobacterium scrofulaceum*: A bacterial contaminant in plant tissue culture. Plant Sci., 78: 231-236.
46. Leggatt, I.V., W.M. Waites, C. Leifert and J. Nicholas, 1988. Characterisation of micro-organisms isolated from plants during micropropagation. Acta Hortic., 225: 93-102.
47. Gunson, H.E. and P.T.N. Spencer-Phillips, 1993. Latent Bacterial Infections: Epiphytes and Endophytes as Contaminants of Micropropagated Plants. In: Physiology, Growth and Development of Plants in Culture, Lumsden, P.J., J.R. Nicholas, B.J. Davies (Eds.)., Springer, Dordrecht, ISBN: 978-94-010-4339-7, pp: 379-396.
48. Cobrado, J.S. and A.M. Fernandez, 2016. Common fungi contamination affecting tissue-cultured abaca (*Musa textiles* Nee) during initial stage of micropropagation. Asian Res. J. Agric., 1: 1-7.
49. Kaluzna, M., A. Mikicinski, P. Sobiczewski, M. Zawadzka, E. Zenkteler and T. Orlikowska, 2013. Detection, isolation and preliminary characterization of bacteria contaminating plant tissue cultures. Acta Agrobotanica, 66: 81-92.
50. Cole, M., 1996. Microbial Contaminations and Aseptic Technique in Plant Tissue Culture. In: Tissue Culture of Australian Plants, Taji, A. and R. Williams (Eds.)., University of New England, Armidale, Australia, pp: 204-228.
51. Reed, B.M., P.M. Buckley and T.N. DeWilde, 1995. Detection and eradication of endophytic bacteria from micropropagated mint plants. *In Vitro* Cell. Dev. Biol., 31: 53-57.
52. Habiba, U., S. Reza, M.L. Sata, M.R. Khan and S. Hadiuzzaman, 2002. Endogenous bacterial contamination during *in vitro* culture of table banana: Identification and prevention. Plant Tissue Cult., 12: 117-124.
53. Eed, A.M., S.A. Reddy, K.M. R eddy, J.A.T. da Silva, P.V. Reddy, H. Beghum and P.Y. Venkatsubbiah, 2010. Effect of antibiotics and fungicides on the *in vitro* production of *Citrus limonia* osbeck nodal segment and shoot tip explants. Asian Aust. J. Plant Sci. Biotechnol., 4: 66-70.
54. Buckseth, T., R.K. Singh, A.K. Sharma, S. Sharma, V. Modgil and A. Saraswati, 2017. Effect of streptomycin and gentamycin on *in vitro* growth and cultural contaminants of potato cultivars. Int. J. Curr. Microbiol. Applied Sci., 6: 4038-4043.
55. Safavi, K., 2012. Evaluation of using nonmaterial in tissue culture media and biological activity. Proceedings of the 2nd International Conference on Ecological, Environmental and Biological Sciences, October 13-14, 2012, Bali, Indonesia, pp: 5-8.
56. Stafford, A., P. Morris and M.W. Fowler, 1986. Plant cell biotechnology: A perspective. Enzyme Microbial. Technol., 8: 578-587.
57. Cox, E.A., G. Stotzky and R.D. Goos, 1960. *In vitro* culture of *Musa balbisiana* colla embryos. Nature, 185: 403-404.
58. Ahmad, I., T. Hussian, I. Ashraf, M. Nafees, Maryam, M. Rafay and M. Iqbal, 2013. Lethal effects of secondary metabolites on plant tissue culture. Am.-Eurasian J. Agric. Environ. Sci., 13: 539-547.
59. Onuoha, I.C., C.J. Eze and C.I.N. Unamba, 2011. *In vitro* prevention of browning in plantain culture. Online J. Biol. Sci., 11: 13-17.
60. Prasad, K.N. and H.C. Chaturvedi, 1998. Effect of season of collection of explants on micropropagation of *Chrysanthemum morifolium*. Biol. Planta., 30: 20-24.

61. Singh, C.R., R. Nelson, N.S. Boopathy, K. Kathiresan and C.S. Kumar, 2012. *In vitro* conservation and protective effect of *Premna serratifolia* L.-An important medicinal tree. Int. J. Pharm. Applic., 3: 332-343.
62. Chinnappan, R.S., N. Ruthar and S.S. Sethu, 2011. Rapid *in vitro* propagation of *Premna serratifolia*, a medicinally important declining shrub, India. Conserv. Evidence, 8: 66-73.
63. Kathiresan, K. and S.C. Ravinder, 2013. Preliminary conservation effort on *Rhizophora annamalayana* Kathir., the only endemic mangrove to India, through *in vitro* method. J. Plant Dev., 20: 57-61.
64. George, E.F., 1993. Plant Propagation by Tissue Culture. Eastern Press, Eversley.
65. Alkhateeb, A.A., 2008. A review the problems facing the use of tissue culture technique in date palm (*Phoenix dactylifera* L.). Sci. J. King Faisal Univ. (Basic Applied Sci.)., 9: 85-104.
66. Jagadeesh, B., M. Kumar, M. Shekhar and P. Udhakar, 2011. Amenability of the sugarcane variety 2005 T 16 to shoot tip culture. J. Sugarcane Res., 1: 75-77.
67. Singh, C.R., R. Nelson and S. Sivasubramanian, 2011. Successful plant regeneration from callus culture through organogenesis in *Premna serratifolia* L.: An important medicinal tree. Open Access J. Med. Aromat. Plants, 2: 1-5.
68. Singh, C.R., K. Kathiresan, S. Anandhan and K. Suganthi, 2014. Antioxidant and antibacterial activity of field grown and tissue cultured root callus of mangrove species. Eur. J. Med. Plants, 4: 723-742.