

# Role of Tissue Culture as Applied in Seed Potato Multiplication to Improve Food Security: A Review

Shemshadin Mohammed Umara<sup>1,2,\*</sup>

<sup>1</sup>Crop production and protection, Metta District Agriculture Office, Metta District, East Hararge Zone, Oromia Regional State, Ethiopia.

<sup>2</sup>Plant Breeding program, School of Plant Sciences, Haramaya University, Haramaya, Oromia, Ethiopia.

**How to cite this paper:** Shemshadin Mohammed Umara. (2022) Role of Tissue Culture as Applied in Seed Potato Multiplication to Improve Food Security: A Review. *International Journal of Food Science and Agriculture*, 6(2), 169-174. DOI: 10.26855/ijfsa.2022.06.006

**Received:** March 24, 2022

**Accepted:** April 20, 2022

**Published:** May 17, 2022

\***Corresponding author:** Shemshadin Mohammed Umara, Crop production and protection, Metta District Agriculture Office, Metta District, East Hararge Zone, Oromia Regional State, Ethiopia; Plant Breeding program, School of Plant Sciences, Haramaya University, Haramaya, Oromia, Ethiopia.

**Email:** shemshadinmohammed@yahoo.com

## Abstract

Rapid population growth, natural and man-made factors (COVID-19 and the lack of a social safety net) have led to an increase in the demand for food, which calls for significant improvements to the food system worldwide to supply food more efficiently with the same or fewer resources. Potatoes have great potential to contribute to food security and incomes for rural smallholder farmers, as well as provide nutritious, affordable food for urban consumers. The availability of disease-free and certified seed potatoes of better-performing varieties remains limited. The use of tissue culture to provide a disease-free seed potato is therefore crucial to ensuring food security. A key goal of this paper is to summarize the work done on various aspects of seed potato multiplication, and how it can improve the food security of smallholder farmers. The systematic review method was applied to summarize how tissue culture application can produce excess disease-free seed potatoes to improve food availability for marginal farmers. The most effective way for farmers in developing countries or areas prone to natural or man-made disasters to increase their incomes and improve nutrition is to use high-quality certified seeds. Tissue cultures are used worldwide to produce pre-basic, virus-free seed potatoes. Early Generation Seed (micro-tubers, cuttings and mini-tuber), multiplication of mother plants and production of apical rooted cutting for seed production for field planting are popular. The activities of disease-free seed production start at the laboratory and end at the field with seed production for planting. In general, three major steps were used in seed potato multiplication: (1) Tissue culture (to produce disease-free tissue culture plantlets); (2) Production of cuttings (involves two important stages: (i) multiplication of mother plants and (ii) production of apical rooted cutting for planting) for further multiplication and (3) production of seeds for field planting.

## Keywords

COVID-19, Early Generate Seed, Food Security, Potato, Smallholder, Tissue Culture

## 1. Introduction

Potato (*Solanum tuberosum* L.), is belonging to the *Solanaceae*, an oversized family with over 3,000 species, is one in every of the richest genetic resources having about 200 wild and primitive species. The species of potato ranged from diploid to hexploid [1]. It can provide more carbohydrates, proteins, minerals, and vitamins per unit area of land as

compared to other potential food crops, and is that the major staple food in many developing countries [2]. It is liable to several biotic and abiotic stresses which limit production, particularly among small and marginal farmers with limited resources [3]. Its flexibility to tolerate low soil fertility and role play in ensuring food security of households pointed it out more important in improving the welfare of poor farms [4]. In 2020, the year of production was 49.7%, 30%, 12.5%, 7.3% and 0.5% for Asia, Europe, America, Africa and Oceania, respectively [5]. From 2014 through 2018, the whole annual consumption of potato per capita was 32.41 kg within the World [6]. Small scale farmers, in low-income countries including Africa, may benefit greatly from higher yields and more nutritious potatoes. However, the provision of certified seed potatoes of better-performing varieties is proscribed. The implications of blight for food security are substantial, as a deadly disease infection has the potential to chop back yields by up to 80%. Better incomes and improved nutrition start with high-quality certified seeds for farmers [7].

Hence, the use of high-quality and disease-free seed potatoes is predicted to increase the availability of potatoes for consumption [8, 9]. Tissue culture is that the science of growing plant cells, tissues or organs isolated from the mother plant, on artificial media [10]. Tissue culture techniques became a highly regarded and alternative means for the vegetative propagation of plants in recent years [11]. It has been an enormous role in producing disease-free planting materials of vegetatively propagated crops within the horticulture industry of the many countries [12]. The techniques are used worldwide to provide pre-basic, virus-free seed potatoes spoken as microtubers [13]. Tissue culture, a biological tool that involves exciting prospects for crop productivity and improvement under aseptic conditions, is characterized by very flexible rapid multiplication, giving a high rate of multiplication during a very short period [8].

Plant structure culture offers remarkable opportunities in vitro propagations, plant quality improvement and production of plants with desirable agronomical quality and quantity [3]. Micropropagation of potato contains a good advantage in cultivation for increased yield and uniformity in germplasm [14], particularly, the applying of in vitro culture of isolated tissue for the assembly of potato plantlets followed by several cycles of multiplication into mini-tubers and seed potatoes has already been determined to be an appropriate large-scale intervention by the National Academy of Agricultural Sciences [15]. To optimize the domestic production of improved types of disease-free seed potatoes, there is a necessity for capacity development along the full seed multiplication chain [15].

Many research reports showed that the *in vitro* propagation of potatoes depends on the biological importance of cultivars and explant types (leaf, node, shoot tip, and the like). The appliance of tissue culture provides disease-free plants for production and multiplication through in-vitro techniques with high stability, which is required for the low-income country. The use of these techniques within the potato production chain also directly benefits producers by providing high-quality propagules. Hence, the aim of this review was to summarize the work done regarding various aspects of tissue culture in seed potato multiplication and its role in improving the food security of small-holder farmers.

## 2. Methods

The systematic review method was applied to summarize how tissue culture application can produce excess disease-free seed potatoes to improve food availability for marginal farmers. Various literature sources were used to gather pertinent data, including journals, published papers, reports, review articles, and online documents.

## 3. The Role of Tissue Culture in Potato Seed Multiplication to Improve Food Security

### 3.1. Potato and food security

According to the Statistics Division of the Food and Agriculture Organization of the world organization (FAO), “Global food availability went up 250 million tonnes (5.1%) between 2014 and 2018, to 5.1 billion tonnes, which is in line with the expansion in population from 7.3 billion people in 2014 to 7.6 billion in 2018” [16]. This rapid increase of worldwide population, natural and man-made phenomenon (COVID-19 and social unrest) will have resulted in increasing demand for food, which needs considerable improvements to the worldwide food system worldwide. The foremost challenge may be a thanks to produce more food with identical or fewer resources and waste less [17]. The FAO assessment on global food security status showed that globally, “44 countries, including 33 in Africa, nine in Asia and two in geographical region and also the Caribbean, require external assistance for food” [6]. Over 800 million people across the world head to bed hungry nightly, most of them smallholder farmers who rely on agriculture to create a living and feed their families [18].

Potato has great potential to contribute to food security and incomes among farmers, furthermore providing nutritious, affordable food for urban consumers [19]. It is one, which is able to help match all the constraints concerning the dimensional food security like food availability, food access, food use and quality, and food stability worldwide because of its highly diverse distribution pattern, and its current cultivation and demand, particularly in developing countries with high levels of poverty, hunger, and malnutrition [17].

Farmers, in developing countries or in the area, which is often subjected to droughts, other natural or human disasters, needed availability and access seeds. Thus, seed security is very important to farmers' livelihoods and food security. It

is defined as “ready access by rural households, particularly farmers and farming communities, to adequate quantities of quality seed and planting materials of crop varieties, adapted to their agro-ecological conditions and socio-economic needs, at planting time, under normal and abnormal weather conditions” [20]. For these reason, FAO (Benefit-Sharing Fund 3 project) funded to increase accessibility to disease-free seeds, especially for vegetatively propagated crops such as potatoes and cassava. For instance, In Bhutan, Nepal and Peru, biofortified clones of true potato seeds were multiplied as tubers and were propagated for a limited number of households in highland areas with acute iron deficiency. The multiplication and release of the potato tubers are anticipated in the future [6]. As potatoes are increasingly used by the food processing sector to meet the increasing demand of the fast food, snack and convenience food industries, it is rapidly becoming a valuable source of cash income. Thus, sufficient quantities of quality seed are essential to meet the needs of potato growers, processors and traders. For farmers to capitalize on the potential gains from using quality seed will require improvements in technology, irrigation, fertilizer use, storage facilities, transport and infrastructure. On the other hand, the structural transformation of rural- based economies into more urbanized societies has opened new market opportunities to participants in the potato value chain. Such opportunities can both increase incomes and create employment in the sector [21].

### 3.2. Constraints of Potato Production

Sustainable potato production faces many challenges that are resulted from various factors including biotic (viruses, bacteria, fungal, insect pests) and abiotic (drought, salinity, temperature, frost and postharvest problems, that is, accumulation of reducing sugars during cold storage) stresses [2]. Out of such factors, the foremost devastating one is diseased. Particularly, the diseases thanks to viruses, bacteria and fungus, the more seriously affect potatoes production. For, Potato virus Y (PVY, genus; potyvirus), Potato leafroll virus (PLRV, genus; polerovirus), and Potato virus X (PVX, genus; potexvirus) is probably the foremost diverse and devastating viruses infecting potatoes worldwide [22-25, 2].

Thus, it is highly needed to increase the accessibility to disease-free seeds for small-scale farmers to combat the impact of food security and malnutrition, especially, in low-income countries. Thus, it is highly needed to extend the accessibility to disease-free seeds for small-scale farmers to combat the impact of food security and malnutrition, especially, in low-income countries. As [2] stated that “With the advances in technology, the main focus of agricultural biotechnology has shifted toward both quantitative and qualitative crop improvement, to house the challenges of food security and nutrition”. Besides those, temperature change (increasing temperatures and more frequent droughts), which is the world’s most major problem due to its direct impacts on food production and security, in reducing the yield of potatoes by inhibiting tuber formation and enlargement [19]. Potato’s support to food availability will be achieved through improved productivity, either by increasing yields or expanding production areas, combined with technologies that reduce post-harvest losses. Research and innovation options must answer a spread of food systems and stages of food system evolution to strengthen potato’s contribution to global food security and income generation and to cut back potato’s environmental footprint mainly in industrial agri-food systems.

### 3.3. Seed Potatoes Production and Multiplication Techniques

Identification of the foremost important constraints and proper selection of target areas for potato research and innovation, along with identification of potential synergies among potato-based agrifood systems and customary development objectives, is predicted to hurry up the innovation process, as will optimized scientific and technical knowledge transfer among potato-based agri-food systems worldwide. Regarding seed potato production, high-quality seed production and distribution is anticipated to boost productivity within the rural-based system but also in industrial-based systems with more local production where local agro-ecological conditions permit quality production. The results of the study conducted by [17] revealed that the systems of potato seed production should support the access to high-quality seed potato tubers of improved varieties by combining rapid multiplication technologies (e.g. aeroponics, sand hydroponics, or apical cuttings). Some of them were summarized as follows.

#### 3.3.1. Early generation seed

Early Generation Seed (EGS) is defined as “The planting material produced in tissue culture laboratories (e.g., in-vitro plantlets, micro tubers) or under protective structures, like screen houses (e.g., cuttings, mini-tubers) by specialized entities”. Seed multipliers use EGS to provide certified or quality declared seeds for farm use [26].

The work of EGS production was started within the laboratory, within which its multiplication is going to be finished tissue culture or micropropagation. Two steps were required: (i) Virus elimination using sprouts from infected tubers through meristem culture; and (ii) Rapid multiplication of virus-free plantlets through nodal culture to supply large stocks of cloth [26].

#### 3.3.2. Seed potato multiplication techniques

In conventional systems, seed potato tubers are utilized for multiplication and production [27], which may be a low

rate of multiplication, inefficient and features a high risk of infection by various diseases caused entities like fungus, viruses and bacteria [27-29].

Many techniques and technologies are developed for the assembly and multiplication of disease-free seed potatoes. As an example, tissue culture: sprouts [29, 14], shoots [31], apical stem cuttings [32], axillary buds, anthers, ovaries, leaf, stem, rachis and tuber [33] were used for the assembly of diseases-free micro-tubers.

Apical rooted cutting (ARC) technology, which involves three major steps: Tissue culture (to produce disease-free TC plantlets); Production of cuttings (involves two important stages: multiplication of mother plants and production of ARCs for planting) for further multiplication and production of seeds for field planting [34]. However, to get expected results, all of those activities were required well-trained manpower and shut follow-up. Tissue culture is the *in vitro* regeneration of plants from disease-free plant components (cells, tissue), which enables the generation of disease-free agricultural planting material [35].

To increase tissue culture technology to large-scale propagation, it is necessary to develop relatively simple methods, have a high multiplication rate with a high degree of reproducibility and provides a high survival rate of micro shoots or plantlets upon transfer to *ex-vitro* conditions [36].

Innovative approaches like the employment of liquid culture systems, replacing agar with other gelling agent's viz. guar-gum, growing cultures under a CO<sub>2</sub>-enriched environment, *in vitro* hardening, priming of tissue culture-raised plants using anti-transpirants or biopriming agents during the weaning period and improvement within the culture vessel environment by ventilation and other means have proved highly beneficial [36]. Its applications and techniques help plant breeders and potato growers to boost the availability and enhancements.

### 3.3.3. Pathogen elimination in seed potatoes

Potato is prone to a spread of pests and diseases that have large negative impacts on yield and tuber quality. Seed-borne diseases of potatoes are numerous, and diseased seed tubers lose quality during storage between growing seasons [27]. Thus, the important attention of potato biotechnology within the mid-20<sup>th</sup> century was on pathogen elimination for the assembly of high-health potato seeds. The age of biotechnology in potatoes began in 1956, where heat treatment in addition to *in vitro* culture of shoot tips successfully eliminated potato virus Y. Many programs and corporations are established worldwide to produce pathogen-free seed potatoes for farm supply [37]. Eradication of viruses will be achieved by culturing excised meristematic buds under appropriate conditions, a way that has been successfully applied in many countries. The techniques are described by [38], who list 136 virus-free potato cultivars produced by meristem tip culture.

The overall procedure involves the pre-treatment of potato shoots by growth at raised temperatures (32-37 °C), surface sterilization and excision of apical and axillary meristems [length (1.3-0.7 mm) and transfer of the excised meristem to a paper bridge in an exceeding tube containing the liquid substance]. After culture at 20-25 °C, shoots emerge and might be rooted and potted out. The treated plants are then tested for the presence of viruses [39].

### 3.3.4. Preservation of seed potato and maintenance of germplasm

Micropropagation may be a useful means of multiplying virus-free potato stocks and new or imported cultivars that few tubers could also be initially available, under conditions where reinfection with virus or infection with fungus-borne diseases does not occur. It is used commercially, as an example, by Nickersons (Scotland) as a method of multiplying first-year stock for seed production. The propagation of virus-tested stem cuttings by conventional methods produces 800-900 plants from one clone in 3 years [40].

In contrast, by using *in vitro* micropropagation, many thousands of plants are produced from one clone in an exceedingly single year, although a minimum of one generation within the field is required before use. To keep up genetic resources in potatoes, there's a desire to store primitive potato cultivars and related wild species that cannot be stored as seeds in conventional propagation, there's always a risk of loss [41].

## 4. Conclusion

With over 200 wild and primitive species, the potato (*Solanum tuberosum* L.) is one of every of the richest genetic resources belonging to the Solanaceae family, which has over 3,000 species. As compared to other potential food crops, potatoes can provide more carbohydrates, proteins, minerals, and vitamins per unit area of land, and are the main staple food in many developing countries. It is believed that potatoes could be a vital food security crop and as a substitute for costly cereal imports.

However, its vulnerability to biotic stresses [viruses (Potato virus Y, Potato leaf roll virus and Potato virus X), bacteria, fungus, insect and pests], and abiotic stresses (drought, salinity, temperature and frost) and postharvest problems (that is, accumulation of reducing sugars during cold storage) limits the supply and accessibility of seed potato. To prevail over these constraints, scientists have developed different systems and techniques to spice up the multiplication of disease-free seed potato to increase its production to adequate food availability for low-income farmers in developing

countries.

Nowadays, tissue culture techniques have become a highly regarded and alternative means for the vegetative propagation of crop plants including potatoes. It is played a serious role in producing disease-free seed potatoes. In seed potato multiplication three major steps were employed: Tissue culture (to produce disease-free tissue culture plantlets); Production of cuttings (involves two important stages: multiplication of mother plants and production of apical rooted cutting for planting) for further multiplication and production of seeds for field planting. Briefly stated, this paper summarized the applications and techniques of tissue cultures that help plant breeders and potato growers to spice up the supply of disease-free seed potatoes which is able to increase the yield. Increasing the assembly and productivity of the potato can increase the accessibility and availability of food security for households in low-income countries. It also adheres to the linkage between potato growers; research centers/plant breeders and traders.

## Declarations

### Competing Interests

The author declares NO competing interests.

## Acknowledgements

The author is grateful to Metta District Agricultural Office and Haramaya University for their commitment and Motivation.

## References

- [1] ICAR. (2019). *Indian Horticulture: Role of Biotechnology in Potato Improvement*, vol. 64, no. 6. 2019.
- [2] A. Hameed, S. S. Zaidi, S. Shakir, and S. Mansoor. (2018). "Applications of New Breeding Technologies for Potato Improvement," *Front. Plant Sci.*, vol. 9, no. 925, pp. 1-15, 2018.
- [3] C. Ragavendran and D. Natarajan. (2017). *Role of plant tissue culture for improving the food security in India: A review update*. 2017.
- [4] M. Kaushal and R. Prasad. (2021). *Microbial Biotechnology in Crop Protection*. 2021. P. 453.
- [5] FAOSTAT. (2022). "Production share of Potatoes by region," *Food and Agriculture Organization of the United Nations (FAO)*, 2022. [Online]. Available: [www.fao.org/faostat/en/#data/QCL/visualize](http://www.fao.org/faostat/en/#data/QCL/visualize). [Accessed: 22-Feb-2022].
- [6] FAOc. (2022). "Crop Prospects and Food Situation – Quarterly Global Report No. 1, March 2022," Rome, 2022.
- [7] K. Sharma and E. Sikinyi. (2021). "Development of Seed Potato Certification Protocol for Uganda," *Cip/Cgiar*, no. March, pp. 1-21, 2021.
- [8] P. P. Mohapatra and V. K. Batra. (2017). "Tissue Culture of Potato (*Solanum tuberosum* L.): A Review," vol. 6, no. 4, pp. 489-495, 2017.
- [9] W. and W. FAO, IFAD, UNICEF. (2021). *Food Security and Nutrition In The World: Transforming food systems FOR FOOD Security, Improved Nutrition and Affordable Healthy Diets for All*. Rome, 2021.
- [10] J. Muthoni and J. Kabira. (2014). "Multiplication of seed potatoes in a conventional potato breeding programme: A case of Kenya's national potato programme," *Aust. J. Crop Sci.*, vol. 8, no. 8, pp. 1195-1199, 2014.
- [11] F. Nuwagira, S. Mukasa, W. Wagoire, P. Namugga, I. Kashaija, and A. Barekye. (2016). "Determination of hormonal combination for increased multiplication of tissue culture potato plantlets," *Uganda J. Agric. Sci.*, vol. 16, no. 1, p. 129, 2016.
- [12] H. Tegen and W. Mohammed. (2016). "The Role of Plant Tissue Culture to Supply Disease Free Planting Materials of Major Horticultural Crops in Ethiopia," *J. Biol. Agric. Healthc.*, vol. 6, no. 1, pp. 122-129, 2016.
- [13] E. Dessoky, A. O. Attia, I. A. Ismail, and E. I. El-Hallous. (2016). "In vitro Propagation of Potato under Different Hormonal Combinations," *Int. J. Adv. Res.*, vol. 4, no. 1, pp. 684-689, 2016.
- [14] P. Singh. (2012). "Comparative study of potato cultivation through micropropagation and conventional farming methods," *African J. Biotechnol.*, vol. 11, no. 48, pp. 10882-10887, 2012.
- [15] FAO. "Seed Potato Multiplication to Improve Food Security of the People of Paekam County, Ryanggang Province, and Democratic People's Republic Of Korea," Rome, Italy, 2021.
- [16] FAOSTAT. (2021). "Highlights from 2014-2018 food balances statistics," Rome, 2021.
- [17] A. Devaux, J. Goffart, and P. Kromann. (2021). "The Potato of the Future Opportunities and Challenges in Sustainable Agri-food Systems Introduction : The Current Situation of Global Food Security," *Potato Res.*, vol. 64, pp. 681-720, 2021.
- [18] USAID. (2022). "AGRICULTURE AND FOOD SECURITY." 2022.
- [19] CIP. (2019). "Developing early maturing and stress-tolerant potato varieties for Asia: Project profile," 2019.

- [20] FAO. (2022). "Evaluation of the third project cycle of the Benefit-sharing Fund of the International Treaty on the Plant Genetic Resources for Food and Agriculture," Rome, 02, 2022.
- [21] FAO. (2010). *Strengthening potato value chains: TECHNICAL AND POLICY OPTIONS FOR DEVELOPING COUNTRIES*. 2010.
- [22] S. M. Paul, Khurana. (2006). "Potato Viruses and their Management," in *Diseases of Fruits and Vegetables*, vol. II, S. A. M. H. N. (ed.), Ed. Kluwer Academic Publishers, 2006, pp. 389-440.
- [23] J. D. Fletcher. (2012). "A virus survey of New Zealand fresh, process and seed potato crops during 2010-11," *New Zeal. Plant Prot.*, vol. 65, pp. 197-203, 2012.
- [24] A. Hameed, Z. Iqbal, S. Asad, and S. Mansoor. (2014). "Detection of multiple potato viruses in the field suggests synergistic interactions among potato viruses in Pakistan," *Plant Pathol. J.*, vol. 30, no. 4, pp. 407-415, 2014.
- [25] T. Steinger, H. Gilliland, and T. Hebeisen. (2014). "Epidemiological analysis of risk factors for the spread of potato viruses in Switzerland," *Ann. Appl. Biol.*, vol. 164, no. 2, pp. 200-207, 2014.
- [26] I. Fornkwa, V. Harahagazwe, D. Ngwa, L. Adamu. (2021). "Production of Early Generation Seed (EGS) Potato," 2021.
- [27] S. G. Struik, P.C. Wiersema. (1999). *Seed Potato Technology*. The Netherlands: Wageningen Academic Publishers, 1999.
- [28] H. P. Beukema and D. E. van der Zaag. (1990). *Introduction to potato production*, no. 633.491 B4. Pudoc Wageningen, 1990.
- [29] M. Otrushy. (2006). *Utilization of tissue culture techniques in a seed potato tuber production scheme*. 2006.
- [30] M. M. Al-Taleb, D. S. Hassawi, and S. M. Abu-Romman. (2011). "Production of Virus Free Potato Plants Using Meristem Culture from Cultivars Grown under Jordanian Environment," *J. Agric. Environ. Sci*, vol. 11, no. 4, pp. 467-472, 2011.
- [31] B. P. S. A. K. Srivastava, L. C. Diengdoh, R. Rai, T. K. Bag. (2012). "In Vitro Micropropagation and Micro-tuberization Potential of Selected Potato Varieties," *Indian J. Hill Farming*, vol. 25, no. 2, pp. 14-17, 2012.
- [32] K. Tsoka, O., Demo, P., Nyende, A. B., and Ngamau. (2012). "Potato seed tuber production from in vitro and apical stem cutting under aeroponic system," *African J. Biotechnol.*, vol. 11, no. 63, pp. 12612-12618, 2012.
- [33] A. Karp, et al. (2013). "Potato Protoplasts and Tissue Culture in Crop Improvement Potato Protoplasts and Tissue Culture in Crop Improvement," *Biotechnol. Genet. Eng. Rev.*, vol. 5, no. 1, pp. 1-32, 2013.
- [34] CIP. (2021). "Rapid Seed Potato Multiplication to Strengthen Potato Value Chain in Karnataka," 2021.
- [35] K. Pradhan, S. Rout, B. Tripathy, and U. N. Mishra. (2021). "Role of Biotechnology in Vegetable Breeding," *Turkish Online J. Qual. Inq.*, vol. 12, no. 3, pp. 5092-5102, 2021.
- [36] S. Purohit, J. Teixeira da Silva, and N. Habibi. (2011). "Current Approaches for Cheaper and Better Micropropagation Technologies," *Int. J. Plant Dev. Biol.*, vol. 5, no. 1, pp. 1-36, 2011.
- [37] P. J. Barrell, S. Meiyalaghan, J. M. E. Jacobs, and A. J. Conner. (2013). "Applications of biotechnology and genomics in potato improvement," *Plant Biotechnol. J.*, vol. 11, pp. 907-920, 2013.
- [38] F. C. Mellor and R. Stace-Smith. (1987). "Virus-Free Potatoes Through Meristem Culture," in *Biotechnology in Agriculture and Forestry*, Vol. 3., B. Y. P. S. (eds), Ed. Berlin: Springer, 1987, pp. 30-39.
- [39] A. Karp, M. G. K. Jones, G. Ooms, and S. W. J. Bright. (1987). "Potato protoplasts and tissue-culture in crop improvement," *Biotechnol. & Genet. Eng. Rev.*, vol. 5, pp. 1-32, 1987.
- [40] G. Hussey and N. J. Stacey. (1981). "In Vitro Propagation of Potato (*Solanum tuberosum* L.)," *Ann. Bot.*, vol. 48, no. 6, pp. 787-796, Feb. 1981.
- [41] R. J. Westcott, G. G. Henshaw, and W. M. Roca. (1977). "Tissue culture storage of potato germplasm: Culture initiation and plant regeneration," *Plant Sci. Lett.*, vol. 9, no. 4, pp. 309-315, 1977.