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TECHNOLOGICAL ADVANCEMENT IN POTATO TISSUE CULTURE

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Abstract

Potato (*Solanum tuberosum* L.) is ranked after wheat, rice and maize, a food crop having the potential of meeting food requirements of the world. Potatoes are economically used for human food and animal feed, also for seed tuber production and industrial uses. But the national average yield of potato is very low. This is attributed to a number of factors, mainly susceptibility of the existing varieties particularly to late blight, acute storage of good quality seed tuber free of disease complex, use of degenerated seed tuber and local varieties susceptible to disease and poor yielding capacity. One of the methods for obtaining virus free potato is viral eradication through tissue culture technique. This methodology allows quick propagation of plant material and production health plants regardless of location and season of the year. In this review paper, potato micropropagation techniques, common problem in micropropagation of potato, production of disease free potato and factor affecting potato tissue culture are discussed. Also, Ethiopian's experience in potato tissue culture is discussed. In this line, Ethiopia is advancing in potato micropropagation techniques by investing in potato tissue culture R&D in south and Amhara regional agricultural research institution, Melikassa agricultural research center and Holeta agricultural research center. In Holeta agricultural research center, majority of the work is practicing on the diagnosis and elimination of potato virus from potato varieties of *Menagesha, Zengena* and *Jalenie* and in vitro conservation of potato varieties such as *Guassa, Jalenie* and *Awash*. Generally, it is recommended that in Ethiopia the potato plantlets produced in the agricultural research center should be provided in large number and makes them easily available to the consumer.

Keywords: potato, tissue culture, micro propagation, virus free potato, plant material.

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INTRODUCTION

Potato comes from the highlands of Peru and Bolivia probably in the high plateaus in the neighborhood of Lake Titicaca 3512m above the sea level. Potatoes were grown by the Incas together with maize provided their stable food. Ever since this tuber are dominated and still continues to dominate life in the Andean region of South America. The importance of potato in the lives of early Andean people is evident from its representation in designs on early pottery. Almost every important character of potato cultivation is represented on the pottery of Incas, now housed on the museum of Europe [1]**.** Potato is one of the world's most economically important tuber crops, belonging to the family Solanaceae. In Sri Lanka, potato is grown over an area of 7000 ha and the annual seed potato requirement is nearly 15,000 t0nnes.l The conventional method of' potato propagation is by seed tubers, which has the disadvantage of being contaminated with disease-causing pathogens. In vitro rapid multiplication of' disease-free planting material provides a solution to this problem. Such material facilitates germplasm movement in quarantine aspects. The parental stocks can be maintained in vitro to ensure a clean source of plants from which tubers can be produced [2]. Potato (*Solanum tuberosum* L) is a tuber bearing herbaceous perennial but is treated as an annual under cultivation. The aerial part of the stem is erect in the early stages of growth but later it becomes more spreading. Potato is specially a crop of cool moist regions and grows best in a climate where cool nights alternate with warm days during the period of tuber formation. Such conditions are met with in large parts of Europe which accounts for roughly 70% of the world's production. Potato now occupies a prominent position in the world's food economy. As a fresh vegetable potatoes are consumed in a great varieties of ways, boiled steamed, fried, baked or roasted. They are also processed in to many products such as potato chips or crisps. Potato is a good substrate for the growth of microorganisms. The liquor from boiled potatoes (potato broth) has long been used as a nutrient medium in experimental microbiological work. Potato can be used as human or animal feed but also seed tuber production and industrial and non industrial aspects. In the non industrial aspects, potatoes are used to produce starch, alcohols etc. [1]. Potato is one of the most important crops in the world and therefore the subject of constant interest and numerous studies including those classified as plant biotechnology [3]**.** Potatoes are an economical food; they provide a source of low cost energy to the human diet. It is a rich source of starch, vitamins specially vitamin C and vitamin B1 and minerals. It contains 20.6% carbohydrates, 2.1% protein, 0.3%fat, 1.1% crude

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fiber and 0.9%ash. It also contains a good amount of essential amino acids like leucine, tryptophan and isoleucine [4]. The potential value of plant tissue culture technology is being commercially exploited by various organizations all over the world. The horticulture industry responds very quickly to the micropropagation research. At present more than 400 millions plants are produced through tissue culture in different parts of the world. There is a potential market for billions of US dollars per year worldwide for tissue culture products. There are more than 65 laboratories all over the world producing more than a million plants per year. The total production capacity of North America laboratories probably exceeds 150 million plants per year [5]. The vegetative propagation of stem cuttings or other growing plant parts to produce genetic clone is common for some agricultural crops like potato which is cultivated by vegetative propagation. Techniques exist to propagate and regenerate whole plants from tissue or isolated plant cells in culture. This set of techniques is complete for some agricultural species like potatoes. Cell culture techniques allow scientists to regenerate numerous copies (clones) of the manipulated variety, which is easier, more efficient and more convenient, especially for producing significant quantities of stock plants. Therefore this technique is important to increase the productivity and versatility of agriculture. In general way tissue culture may not be the most practical option because it can be an expensive and labor intensive process. For every year excessive amounts of grower, time, labor and space are spent on unproductive seeds and cuttings. Significant numbers of young plants are lost due to a susceptibility of the existing varieties particularly to late blight (*Phytophtora infestance*), acute shortage of good quality seed tuber free of disease complex, use of degenerated seed tuber and local varieties susceptible to disease and poor yielding capacity. But tissue culture techniques are less subjected to such attacks and disasters because in the sterile environment of the laboratory they are not exposed to pathogens or extreme conditions that affect many plants grown in the field or greenhouse [6].

POTATO MICROPROPAGATION TECHNIQUES

Potato micropropagation is called in vitro clonal propagation. The significant advantage offered by the aseptic methods of clonal propagation (micropropagation) over the conventional method is that in a relatively short span of time and space, a large number of plants can be produced starting from a single individual [7]. Potato can be propagated sexually (by botanical seed also called true potato seed) and asexually (vegitatively) by means of tubers. Tubers are shortened and thickened underground stems with auxiliary buds. The physiological status and health of seed tubers are among the important factors influencing potato yield. It is possible to use tissue culture

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technology to propagate conserved potato germplasm. Potato is normally vegetatively propagated. In this way most contamination of seed material is caused by pathogens (bacteria, virus, and fungi) cause several reductions in yield that is why, despite tremendous efforts little success has been achieved in conventional seed potato production scheme. In this event plant biotechnology offers a great potential to complement conventional breeding methodology for potato improvement and production via plant tissue culture technique (mass production of pathogen free potato material through micropropagation). But lack of budget, limited resource allocation and relatively high recurrent cost (chemical expenses) of this technology has been as a major obstacle in benefiting from this technology in developing country [8]. Advantage of micro propagation over traditional method includes: (i) it is possible to produce clones of some kinds of plants that are otherwise slow and difficult to propagate vegitatively; (ii) it certifies virus free plants can be produced in large number; (iii) easily limited space is required to maintain and produce large number of plants; (iv) a plant material requires less attention between subcultures and there is no labor or material requirement for watering, weeding, spraying etc. Micropropagation is most advantageous when cost is less than traditional methods of multiplication; (v) production can be continued all the year round and independent of seasonal changes; (vi) propagation is ideally carried out under aseptic conditions, free from pathogens. Once culture has been stated there should be no loss through disease and the plantlets finally produced should be free from bacteria, fungi and other microorganisms and (vii) the rate of propagation is much greater in micropropagation and many more plants can be produced in a given time. This will enable selected varieties to be made available quickly and widely. Disadvantages of micropropagations include: (i) the chances of producing genetically aberrant plants (ii) it needs advanced skills, expensive production facilities, availability of specific systems for each species and variety, labor intensive and high cost of propaguels; (iii) the plant derived from the cultures are not initially able to produce their own requirements of organic matter by photosynthesis and have to go a transitional period before they are capable of independent growth and (iv) the young plants produced through micropropagation are more susceptible to water loss in an external environment. Therefore it should be hardened in an atmosphere by slowly decreasing the humidity and increasing the light [6].

TYPES OF MICROPROPAGATION TECHNIQUES

Meristem Culture: The active growing point of the plant shoot is the meristem. This is a small organ composed of rapidly dividing (meristematic) cells. For propagating potato shoot cultures , it

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is the ideal starting material having favorable characteristics. The dome of a shoot apical meristem contains the truly meristematic cell and is surrounded by leaf primordia and primary leaves. Since the more differentiated vascular tissue occur away from the meristem or towards to older tissue, the vascular elements of the leaf primordia are still very developing and these elements have not made contact with the main standard of the vascular system in the stem [9]. Shoot of all angiosperms and gymnosperms grow by the virtue of their apical meristems. The apical meristem is usually a dome of tissue located at the extreme tip of a shoot. The meristem has well known structure comprising an epidermal part of two or three layers and an internal part in a cross section. One can observe an apical part when the cell makes reduced mitotic activity after which there is an initial ring composed of actively dividing cells that are responsible for stem growth [7].Meristem tip refers to the region of shoot apex lying distal to the youngest leaf primordia, where as shoot apex includes meristem tips plus a few adjacent leaf primordia. Meristem tip culture is a method in which shoot meristem or meristem tip is cultured in vitro with a few leaf primordia. The meristem when cultured in vitro on a suitable media, differentiates in to whole plants identical to the parent plant [6].

Nodal Cutting

A number of different approaches have been successfully used in the regeneration of potato plants from in vitro cultures. Roset and Bekleman [10] obtained plantlet regeneration from potato stem segments. Espinoza [9] have reported on the micropropagation of potato through nodal section cultures. Randlli [11] reported a typical method for potato micropropagation namely single node propagation or micro grafting which contains single node cuttings that will generate shoots and finally one plant. At CIP, micropropagation of potato is performed by taking nodal cuttings. Single node with leaves is excited from small in vitro plantlets and when a suitable number of small plantlets have been produced. They need to be rooted and transferred to non sterile conditions.Therfore; this segment obtained from micropropagated material can be transferred for further multiplication [9].

Bud is isolated along with a piece of stem (node) with the purpose of forming a shoot by allowing the bud to develop. The bud in the axial of the newly formed leaves can then also be subcultured and allowed to develop into plantlets. When enough shoots have been obtained these must be rooted and then transferred to the soil [7].

Microtuberization: Tubers are the most common source of planting material in potato production and approximately 15% of the total area under potato cultivation around the world is

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used for the production of seed tuber [12]. Micro tuber is one of the strategies of plant tissue culture that can eliminate virus in seed production programs. Because of their small size and weight, micro tubers have tremendous advantages in terms of storage, transportation and mechanization .They can be directly sown in to the soil and can be produced in bulk in any season. They have the smaller morphological biochemical characteristics to field produced tubers. Therefore, mass production of potato micro tuber is likely to revolutionize the world potato production. A number of research groups all over the world are trying to bring about this revolution [13-15]. Microtuberization is a very useful method to propagate and store variable potato stocks and may be adoptable for commercial propagation. Usually one mirotuber fully develops on each shoot. This may be due to the fact that during the tuberization process, there is apparently strong competition between auxillary meristem along the shoot such that the first meristem to tuberize inhibits tuberization of the other meristem [16]. Depending on cultivar, Potato microtuber development may be promoted under short day photoperiod and following addition of cytokine and high level sucrose to the medium [17,18].

Photoautotrophic and Photomixotrophic Micropropagation of Potato

Propagation of plants through tissue culture is heterotrophic in nature. Tissue culture systems have relied on the use of exogenous sugar as a source of carbon for growth. However new culture protocols and innovative design of growth containers providing aeration, high CO_2 or forced ventilation to the culture vessel. Newly developed photoautotrophic micropropagation systems (PAM) were adapted to produce quality plant propaguels at minimum usage resources to maximize production and minimize cost. It was observed that plants can grow in the absence of sugar, if provided with proper ventilation system coupled with high photosynthetic photon and lower percentage of relative humidity inside the culture vessel [19]. Photoautotrophic micropropagation systems that uses a sugar free culture medium has many advantages over the conventional photomixotrophic micropropagation systems(PMM) that uses sugar containing medium [20].The advantages include minimum risk of contamination in the culture media, enhancement of plantlet growth and high CO_2 concentration inside the vessel [21,22].

COMMON PROBLEMS IN MICROPROPAGATION OF POTATO

Most microorganisms such as bacteria (*pseudomonas spewing sp* and *Bacillus sp*) are the major contaminants during large scale micro propagation of potato. These slow growing bacteria may persist even after initial surface sterilization of explants. Such contaminants may persist for many generation with out being noticed and cause reduction in vigor of plants. Addition of antibiotics

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or fungicides to the culture medium may control the infection [6]. One of the most encountered problems with micro propagation is the production of exudates by explants. These exudates are usually considered to be various phenol molecules oxidize to form brown material in the medium. This substance tends to be inhibitory to development. This problem can be minimized by treating the explants with antioxidant (citric or ascorbic acid) including an adsorbent material in the medium (poly vinyl pyrroly doidone or activated charcoal), and frequent transfer to a new media [23]. Some explants like root tip are hard to isolate and contaminated with soil micro flora that become problematic during tissue culture process. Certain micro flora can form tight associations with the root system or even grow with in the root. Soil particles bound to roots are difficult to remove without injury to the roots that allows microbial attack. These associated micro flora with generally over grow the tissue culture medium before there is a significant growth of plant tissue. Aerial explants are also rich in undesirable micro flora. However, they are more easily removed from the explants by gentle renising and the rest usually can be killed by surface sterilization. Most of the surface micro flora do not form tight association with the plant tissue and such association can usually be found by visual inspection as microorganisms or localized necrosis on the surface of the explants [5].

PRODUCTION OF DISEASE FREE POTATO

One of the most viable methods for obtaining virus free potato from propagation materials that comes from infected plants is viral eradication by using tissue culture techniques, aided or not by thermo and chemotherapies. These methodologies allow quick propagation of plant materials producing healthy plants from a single individual in a short time, regardless of location on season of the year. Meristem culture is possible to develop virus free potato. But it is not yet possible to develop fungal resistance cultivates through in vitro culture techniques. Now a day, genetic transformation techniques are being used to develop disease resistance varieties including potato [24].

TECHNIQUES TO DEVELOP DISEASE FREE POTATO

Meristem Isolation

Isolation of the apical part called the meristem tip, under aseptic conditions and its culture on an adequate aseptic medium leads to the development of plantlets. This development in principle follows a pattern similar to that the entire plant, the cells of the meristem divide and the differentiation of tissue continues. The nutrition of the excised portion of the plant is supplied by the artificial medium. This technique is called meristem culture [6].

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The aseptic dissection of the meristem is delicate process and requires many hours of practice. Cut stems in to segment each containing one node with its auxiliary bud carefully removes the leaves. Disinfect pieces for 30seconds in 70% alcohol, followed by calcium hypochlorite for 15minutes and wash four times with sterile distilled water to remove excess hypochlorite. Under a binuclear dissecting microscope cut and remove the leaflets surrounding the growing point until only the apical dome and a few leaf primordia remain. Cut off the dome and two leaf primordia and transfer to medium [9].

Meristem Culture

Culturing is done in the medium under standardized and suitable environmental conditions for the species and variety. Meristem culture may not lead to culture of virus free plants and the virus free plant is not virus resistance. Virus free plants can serve as a source of propagation of other virus free plants [5]. Meristem culture was to obtain virus free plants. The technique of meristem culture has been greatly required and used for obtaining plants that are free from virus. For example; in India some valuable clones of potato have been free from virus infections through meristem culture. The technique involved by taking the meristem with little surrounding tissue as possible to minimize the chance of virus particles being present in the explants. The larger the meristem the greater the chance of its survival and shoot development but the risk of infection by the virus also increase with explant size. Therefore; a compromise has to be reached between this two opposing forces in deciding about the explant size . Meristem culture is mainly employed to obtain virus free plants since in infected plants the apical meristem are generally either free or carry a low concentration of viruses. The question need to be answered is why the meristem escapes the viruses and how meristem culture leads to the production of virus free plants. The first reason is a competition in meristem between cell production and virus multiplication. Another reason is there is a higher activity of virus silencing mechanism in the meristem than any other region. Absence of vascular system and plasmodermata in the meristem greatly hinders the transport of virus particles [6].

POTATO TISSUE CULTURE FOR AGROBACTERIUM MEDIATED GENE TRANSFER

The potato is highly amenable to genetic engineering through the use of *Agrobacterium tumefaciens*. Potato was the first food crop to be genetically transformed and it has long maintained its position as a leader among transgenic crops. Gene transfer in to potato via agro infection is efficient, easy and less expensive. The agro infection protocol is therefore well

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adopted for use in developing country laboratories, which do not always have the capacity to purchase sophisticated and costly equipment's [6]. The control of many plant virus diseases is heavily depending on the use of pesticides to kill the biological vector that disseminate the viruses. Public concern about the human health and environmental impacts of pesticides used control pests and disease is a worldwide problem and to develop alternative pesticide free method for virus disease control is underway. Development of disease resistance is one of the several classical approaches to virus disease control. There are two types of resistance, genetic and induced. Genetic resistance which are long been considered an ideal means of controlling disease. It is easy and less expensive to apply and generally has no undesirable environmental or human impacts [25]. Although potato is being considered as one of the food crops and its productivity is hampered due to the attack of virus, fungi and bacteria disease. For instance in Bangladish, the total loss caused by these diseases is 30-100% during cultivation and 2-6 months of storage [26].It has been estimated that as high as 57.2% loss of yield occurs in Bangladesh due to late blight alone [27]. In recent years genetic transformation techniques are being used to develop disease resistant varieties in many crop plants. Although there are reports on the genetic transformation of potato [24,28,29] no work has been carried out on locally available Bangladesh varieties. Therefore attempts have been made to optimize various factors influencing reproducible transformation protocols in two indigenous potato varieties using two strains of agrobacterium containing different vectors. Maximum shoot regeneration in two indigenous potatoes (*Solanum tuberosum* **L.)** was observed on MS medium supplemented with 1.0 mg/l BAP and 0.1 mg/l GA_3. Among the two varieties, namely Laopakri and Jam alu the former showed best response interms of number of shoots (explant, nodes) and shoot length. Half strength of MS containing 0.1mg/l IAA was found to be best for root induction from the excited shoots. Two strains of agrobacterium namely, LBA4404 containing PBI124 and EHA105 having plasmid PCAMBIA 1301 were used for transformation. Among two strains, LBA4404 with the vector PBI showed better response. Regeneration from the transformed tissue was obtained on MS supplemented with 1.0mg/l BAP and 0.1mg/l GA_3 [30].

FACTORS AFFECTING POTATO TISSUE CULTURE

In vitro response and its relationship with different varieties, explants and media were investigated in potato (*Solanum Tuberosum* L.). Direct invitro regeneration protocol from diverse explant source is a prerequisite. Statistically the explant source had significant effect on direct regeneration and the nodal explant had maximum regeneration. Regeneration response in vitro is

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generally species and genotype specific [31]. Therefore, Regeneration conditions and characteristic may vary among genotype and need to be determined prior to regeneration [32].

Living plant materials from the environment are naturally contaminated on their surfaces (and sometimes interiors) with microorganisms. The compositions of the medium, particularly the plant hormones and the nitrogen source (nitrate versus ammonium salts or amino acids) have profound effect on the morphology of the tissues that grow from the initial explant (unpublished). In general during potato tissue culture the following factors should be considered: (i) examine the effect of media combination on invitro response; (ii) regeneration ability of the explant (iii) morphogenetic potential and genotypic character of the explants on regeneration [32].

Effect of Media

In invitro condition, plant and microbes have basically some requirements. When the culture medium contains sugar (as a carbon source), it attracts a variety of microorganisms, which grow faster in a medium and they ultimately kill the plant cells. It is therefore, necessary to have complete aseptic condition around the culture equipments, which prevent contamination of the culture medium. The main sources of contamination of the medium includes: (i) the microorganisms which are presents in the nutrient medium at the time of its preparation; (ii) during the transfer of the plant materials to the medium or from one medium to another; (iii) the acidity and alkalinity property (P^H) of the media and (iv) a high concentration of the gelling agent which make the media very hard and decreases the nutrient uptake of the tissues. The four classes of the growth regulators are commonly used in tissue culture media are auxin, cytokinin, giberellins and abscisic acid. The type of growth regulators and concentration used will vary according to the cell culture purpose. An auxin (IAA, NAA, 2,4-D and IBA) is required for the induction of cell division and leaf initiation in culture tissues. The auxins are mostly used in combination with cytokinins. The 2, 4-D is used for callus induction where as IAA, IBA and NAA are used for root induction. Though the auxins are thermo stable, however the IAA is destroyed by low P^H, light, oxygen and peroxides. The NAA and 2, 4-D are most stable form of auxin. The cytokinins (KN, BAP, 2IP and zeatin) are adenine derivatives, promote cell division, and shoot proliferation, organogenesis and somatic embryogenesis. They have essential role in differentiation and micropropagation of most plant species. Cytokinins are thermo stable during autoclaving in media. The abscisic acid is heat stable but light sensitive. The gibberellins are infrequently used in plant tissue cultures can inhibit callus growth but for meristem culture after shoot primordia formation are used in plant regeneration and elongation [6]. Plant hormones have

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a profound effect on cell differentiation and development. There is no simple dose effect relationship between the amount of hormone added and the concentration effective at the point of action. Therefore experimental results must be interpreted with great caution concerning the hormones and their concentrations. Thus a single hormone may induce different effects on the same tissue in different species or developmental stages. Particular effects are not limited to particular groups of hormones. For example auxin stimulates organogenesis but in some species gibberellins do the same. The effects are due to: (i) hormones may be stored, modified or inactivated; (ii) exogenous supplied hormones control endogenous synthesis via feed back mechanism; (iii) hormones may be carried along through several passages i.e. hormone free media do not guarantee absence of hormone effects [33].

Effect of Explant

A piece of plant tissue taken out from original site of plant and transferred to an artificial tissue culture media for the growth or maintenance is known as explants material. It is necessary to identify the explant in the correct way because a mistake at this stage can multiply the problem many times and cause much economic loss. Success in micropropagation is to be large extent a function of the Juvenility status of the explant [23]. The choice of tissue depends upon ultimate goal of the tissue culture project. Any piece of the plant tissue can be used as an explant material. Various factors of an explant tissue source influence the tissue culture: (i) physiological and age of organ or tissue; (ii) quality of source plant; (iii) season in which explant tissue is obtained; (iv) size of the explant and (v) the explant may carry microorganisms within it [6].

Table 1: Effect of Explant on the goal of the culture

Goal	Explant tissue
Bud culture	Apical & auxiliary bud
Meristem culture	Apical meristem
Micropropagation	Shoot apex or lateral bud or embryo
Root culture	Lateral roots from adult or seedling

The regeneration capability of the plant is usually inversely proportional to the age and size of the explant and to the age of the explants source. Choice of the young plant parts with active meristematic tissue is therefore imperative. The risk of contamination is much less when the interior plant parts or aerial plant parts are given priority over exterior or under ground parts. Also, the smaller the explants size the lesser is the risk of contamination. For most micro propagation work, the explant of choice is an apical or auxiliary bud. In some species leaf pieces are used on which adventitious buds are induced. The use of adventitious buds however, involves

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the risk of increasing chances of variation. When the objective is to produce virus-free plants from infected individuals, and it becomes imperative to start with sub millimeter shoot tips [5].

Genotypic Effect

A successful and reproducible plant transformation system requires a responsive in vitro regeneration system. Regeneration response in vitro is generally species and genotypic specific [31]. Therefore, regeneration conditions and characteristics may vary among genotypes and need to be determined prior to transformation. In potato different approaches so far have been adapted to obtain efficient system either from intact leaflets, leaves, tuber discs and stems after passing through callus phase. At this phase, it is difficult to separate genotypic effects on variation from the differences caused by in vitro regeneration response, since both characters are genetically controlled [32]. In uniform or genetically identical crops, plant pathogens can spread rapidly through the field after the cultured plant transfer to acclimatization room if weather conditions are right and if the plants are not genetically resistant to the pathogens [34].

POTATO GERMPLASM CONSERVATION USING TISSUE CULTURE

Germplasm may be conserved either in situ in which the whole ecosystem is preserved under minimal human interference or exsitu in which the seeds are preserved for the medium to long terms under controlled storage conditions. The seeds to low moisture content (5-7%) and sealed in containers (glass bottles, aluminum metal cans, etc), finally stored for either medium storage (5 to 10 years) in which storage temperature range varies from 0 to-10 or long term storage (over 10 years) with a temperature of -192^0C [5]. Tissue culture method gives the opportunity for collecting, multiplication and distribution of important plants that are on the verge of extinction. These two major in vitro storage strategies are slow growth and cryopreservation technique is now successful for many of horticultural species. Some international institutions have very large collections of old and current varieties, available for exchange and introduction in crop improvement programs. The international potato center (CIP) in Peru has a large world potato collection [6]. The International Potato Center (CIP) maintains a potato germplasm with some 600 clones. This collection serves as a rich source of genetic diversity for use by breeders in potato improvement programs. The maintenance of this collection in the field is expensive, and the collection is susceptible to a wide range of risks such as disease infection or adverse weather conditions. The maintenance of the germplasm collection invitro holds a number of advantages over conventional field maintenance. These include: (i) reduction of labor cost; (ii) avoidance of field infections; (iii) avoidance of environmental hazards example hail frost; (iv) easy availability

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of material for micro propagation and (v) easy availability of material for pathogen elimination [6]. Axillary buds on excised stem segments in culture will grow out to form shoots. Shoot cultures of potato obtained in this way can be maintained indefinitely by repeated subculturing. They are a valuable source material for tissue culture and a means of keeping genetic stocks. They also provide the basis for rapid multiplication of potato stocks by micro-propagation. Following surface sterilization. stem segments are cultured on standard agar media, in the absence of growth regulators. or with low levels of cytokinin [35]. Shoots which develop can, in turn. be cut into nodal segments to repeat the process. A multiplication rate of about x l0 per month occurs under continuous light (6000-8000Iux) at 25^0C and, by 18 weeks. Over 500 plants can be obtained from sprouts from one medium- sized tuber [35]. Cultured shoots rapidly develop roots and can be transferred to soil after washing off the agar. Alternatively, stem segments can be cultured, rooted in liquid medium and transferred directly to soil. a method which is more convenient but which results in lower final multiplication rates [35].

STATUS OF POTATO TISSUE CULTURE IN ETHIOPIA

Tissue culture application in Ethiopia dates back to the 1980's when it was first applied at Addis Ababa University (AAU). By then, the focus was on micropropagation of indigenous forest species notable of which included: Podocarpus sp., Cordiaafricana, and Hagenia abyssinica. Characteristically, these tree species are either difficult to regenerate vegetatively or require long time, and thus the justification to adopt tissue culture for their multiplication. To this list, additional Ethiopian plant species like endod, tef (Eragrostis tef) and enset (Enset ventricosum) were added. It suffices to note that a more comprehensive and concerted tissue culture research programme was rolled out by the Ethiopian Institute of Agricultural Research (EIAR) in 2000, with emphasis on protocol optimization for micropropagation and virus elimination in economically important crops and/or plant species including: banana, cardamom, grapevine, citrus, garlic, potato, geranium, enset, coffee, pineapple, black pepper sweet potato cassava and Aframomumcorrorima. In addition EIAR contributed towards the development of double haploid plants of Eragrostistef and Brassica. Following remarkable progress made in tissue culture, regional agricultural research institutes like Amhara Agricultural Research Institute and Southern Agricultural Research Institute and some higher learning institutions adopted and expanded tissue culture application in Ethiopia. In addition, private enterprises such as Mekele Plant Tissue Culture Laboratory also joined the public institutions in undertaking tissue culture activities. Indeed, an association dubbed "Ethiopian Association for Plant Tissue Culture (EAPTC) has been

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formed to further strengthen and/or promote tissue culture for research and development in Ethiopia [36]. Ethiopia is advancing in potato micropropagation techniques by investing in potato tissue culture R&D in south and Amhara regional agricultural research institution, Melikassa and Holetta agricultural research center. In Holetta agricultural research center(HARC) the majority of the work is practicing on the diagnosis and elimination of potato virus. For this purpose sprouted tubers of three released potato varieties(*Menagesha*, *zengena* and *Jalenie)* where collected from diffused light store for HARC. These tubers where planted on pots in screen house at the center. Foliar samples were tested against potato virus Y (PVY), potato virus X (PVX) and potato leaf roll virus (PLRV) a month after planting using the double –antibody sandwich method of enzyme–linked immunosorbent assay (DAS-ELISA) following procedures presented by CIP. Samples showing positive reaction to the three viruses were submitted to in vitro culture in the laboratory and propagated to have excess materials for the experiments [37]. However, this practice also tested in single node explants of three potato varieties (*Jalenie,Guassa* and *Awash)* obtained in vitro from CIP-Kenya and one variety (*Menagesha)* from HARC tissue culture laboratory were used. Nodal explants were cultured on MS medium plus three levels of sorbitol for in vitro conservation. Two explants were cultured per test tube containing 15ml media and treatments were replicated 5 times. Then the cultures were kept in culture room at a temperature range of 13-15 ^{0}c controlled by air conditioner. For *Awash* and *Menagesha* sorbitol levels best retarded the growth and hence can be used alternatively for conservation similarly [38].

FUTURE PERSPECTIVES OF POTATO TISSUE CULTURE

When we look in to the distant future, it seems likely that plants will continue to provide the three basic necessities of life as well as other use full items. Nevertheless, the problem of food production is a case and will become more critical with each passing year. Increasing the world's food supply will involve many different approaches; one of this is the introduction of modern agricultural technology to all underdeveloped countries of the world [3]. Modern biotechnology owes much to its roots derived from potato tissue culture and micropropagation indeed, the land mark publication by Gottlieb Haberlandt who is the "father of tissue culture", is often cited as the origin and emergence of plant tissue culture and its subsequent applications. Uses of potato tissue culture especially practical applications of micropropagation are presented, which enables science to transition in to an evaluation of what lies tissue culture and biotechnology to further our understanding of potato plant physiology [39].

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CONCLUSION AND RECOMMENDATIONS

Potato (*Solanum tuberosum* L.) is important cash crop widely cultivated through out the world. The leading countries which produce potato on large scale are Germany, U.S.S.R, U.S.A, Poland, France, Spain, Czechoslovakia, China and India. Even if it has wide applications like as human food and animal feed and in industries for starch and alcohol production. But it is not giving the value that is expected. There are different reasons that affect in the yield of potato includes, it is prone to several fungal, virus, and bacterial pathogen and cause heavy economic losses every year. In this event, plant biotechnology gives great potential to complement conventional breeding methodology for potato improvement and production through plant tissue culture technique or mass production of pathogen free potato through micropropagation. In developing countries like Ethiopia due to lack of budget, limited resource allocation and relatively high recurrent cost (chemical expenses) of this technology has been an obstacle in benefiting from this technology.

Based on this review, the countries should consider the following points:

- producing skilled man power in the area
- Give emphasis for agricultural institutions who are engaged in agricultural biotechnology.
- Intensive work in using diagnostic methods for potato infection.
- Identify the best hormone composition of the media, appropriate
- explant and variety combination in the countries condition.

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