Review Article **CODEN: IJRPJK** **ISSN: 2319 – 9563**

International Journal of Research in Pharmaceutical and Nano Sciences

Journal homepage: www.ijrpns.com

https://doi.org/10.36673/IJRPNS.2021.v10.i04.A26

A REVIEW ON *IN VITRO* GERMINATION AND TISSUE CULTURE PROTOCOLS ESTABLISHMENT FOR NIGERIAN TOMATO (*LYCOPERSICUM ESCULENTUM*)

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ABSTRACT

Tomato is a generally popular crop for its rich nutritional values and constitute an integral part of daily diets for substantial portion of human population of the world. But due to its limitation in conventional breeding methods and increasing demand for this important cropin fast growing population, large scale propagation of tomato through plant tissue culture technique became highly significant. In this work, a protocol has been established for a rapid, high frequency plant regeneration of normal tomato (*Lycopersicum esculentum*). Sterilized seeds were sown on autoclavable bags, the seeds where cultured from zero to five days at room temperature while watered. These cultured seeds were grown and cut into some portions and were cultured into medium with Murashige and Skoog salts. Growth regulators were also added with commercial sucrose as energy source to the plants. After four to six weeks, about 45% of the elongated shoots were excised individually from the explants and sub-cultured into the same medium for rooting. This paper reports, an easy germination of seeds and tissue culture protocols established for tomato in Nigeria.

KEYWORDS

In vitro, Germination, Tissue culture, Protocols, Medium and Seeds.

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INTRODUCTION

Botanically tomato belongs to family Solanaceae and named scientifically as *lycopersicum esculentum*. It is recognized as a highly valuable and nutritious food. It is the next most popular vegetable crop next to potato in the world (Bhatia *et al*, 2004[1], Foolad, 2004[2]). These families also include; chili, peppers, potato, eggplant etc. Like all known species of the genus *Solanum*, tomato is a diploid, it has 2n=24 chromosomes, and a genome

size of 950Mbp, which is composed of 77% heterochromatin and 23% euchromatin (Peterson *et al*, 1996)[3]. Tomato plants are vines typically growing six feet or more above ground if supported. Most tomato plants have compound leaves, the leaves are 10-25 centimeter (4-10 inch) long odd pinnate, with 5-9 leaflets on petioles, each leaflet up to 8 centimeters (3 inch) long, with a serrated margin, both the stem and leaves are densely glandular-hairy. Their flowers appear on the apical meristem and self-fertilizing. The flowers are 1-2 centimeters (0.4-0.8 Inch) across, yellow, with five petiole lobes on the corolla; they are borne in cymes of 3 to 12 together. Tomato fruit is classified as berry. The fruit is consumable, soft, succulent and bright colored. Fruit size is generally 1-2 inches' diameters in wild plants and commonly much larger in cultivated form (Sarker *et al*, 2007)[4]. The tomato is native to South America. Genetic evidence shows that the progenitors of tomatoes were herbaceous green plants with small fruit and in the high land of Peru is the centre of diversity (Cox, 2000[5], Smith and Andrew, 1994[6]). Tomato is growing in tropical, sub-tropical and temperate areas (Atherton and Rudich, 1986)[7]. The wild cherry tomato species was transported to Mexico, where it was grown and consumed (Sink and Reynolds, 1986)[8]. Spanish traveler present tomato to Europe in the 1500s. European took the tomato to China, and South and South East Asia in the 17th century (Siemonsama and Piluek, 1993)[9]. At present tomatoes grow widely in China, USA, Turkey, Russia, Egypt, India, Spain, Mexico, Nigeria and many other countries of the world. Tomato is achieving tremendous popularity as vegetables. It can be used in various ways. Green and ripe tomato used as vegetables, ripe tomato also used as salad, sauce, jam, ketchup, pickle etc. Tomato is very nutritious and beneficial for our health. They are also very low in any fat contents and zero cholesterol levels. None the less they're excellent source of antioxidants, dietary fibers and minerals. Due to their all-round qualities, dieticians and nutritionists often recommended them to be included in cholesterol controlling and weight lost programs. The antioxidants present in the tomatoes are scientifically found to be protective against cancers including colon, prostate, breast, endometrial, lung, and pancreatic tumors etc. (Rudrappa, 2009)[10]. It is an excellent source of vitamins and other minerals (Bose and Som, 1990)[11]. Well ripen tomato contains 94.2gm water, 23cal energy, 1.00gm calcium, 7.00gm magnesium, 1000 IU vitamin A, 22.00 mg ascorbic acid, 0.09 mg thiamine, 0.03 mg riboflavin and 0.80 mg niacin per 100gm fresh weight (MacGillivery, 1961)[12]. Vitamin A deficiency in humans represents a global health problem affecting approximately one third of the countries of the world (Mayer *et al*, 2008)[13]. In 2011, about 150 million tons of tomatoes were produced in the world in 4,751,530 Ha of land with an average yield 335,359Kg/ha (FAOSTAT, 2011)[14]. Tomatoes are the world's 2nd important crop in terms of production. At present, it is growing in more than 120 countries of the world. China is the largest producer followed by United States and India. Tomato cultivation area is increasing day by day; this has led to increased farmers' income. However, complexes of pest and diseases and environmental stress as well as post-harvest loss threaten the stability of the production. There are more than 200 pathogens, like, fungi, bacteria, virus, nematode etc. that cause tomato disease (Watterson, 1986)[15]. Fungal diseases (especially, early blight, late blight and *Fusarium* wilt), bacterial diseases (bacterial wilt, bacterial spot) and viral diseases (tobacco mosaic virus, leaf curl, spotted wilt, etc.) are a serious problem in several countries. Tomato are exposed to a number of environmental stresses, especially maximum temperature, drought, salinity, excessive moisture and environmental pollution. High temperature can cause significant losses in tomato production due to reduced fruit setting, and smaller and lower quality fruits (Stevens and Rudich, 1978)[16]. High temperatures causing fruit set failure in tomato; this includes bud drop, abnormal flower development, poor pollen production, ovule abortion and poor viability, reduced carbohydrate availability, and other reproductive Abnormalities (Hazra *et al*, 2007)[17]. In addition, significant

inhibition of photosynthesis occurs at temperatures above optimum, leading to considerable loss of potential productivity. Salt stress is reflected in loss of turgor, wilting, growth reduction, leaf curling and epinasty, leaf abscission, respiratory changes, loss of cellular integrity, decreased photosynthesis, tissue necrosis, and potentially death of tomato plant (Jones, 1986[18], Cheeseman, 1988[19]). Flooded tomato plants accumulate endogenous ethylene that causes damage to the plants and flooding with rising temperatures cause rapid wilting and death of tomato plants (Drew, 1979[20], Kuo *et al*, 1982[21]). There is a need to grow varieties that can withstand such environmental stress. Apart from environmental stresses, tomato yield is also affected breeding techniques, growth habit, varieties, etc. However, there is immediate necessity to enhance tomato production in other to meet the demand of fast expanding human population and plant tissue techniques may offer a good opportunity fulfilling the requirement by propagating tomato plants in large scale production. There is species of successful tomato regeneration in vitro using different explants (Bhatia *et al*, 2004[1], Mamidala and Nanna, 2011[22], Lima *et al*, 2004[23], Chandra *et al*, 2013[24]). Several protocols have been developed for *invitro* plant germination for *Lycopersicon species*. These methods are previously reported in a general tedious and time consuming, with various efficiencies and high production cost. In all cases regeneration systems containing growth regulators. Significant change in global climate occurred. This is impacting agriculture and thus affecting the world food supply. Climate change is not always harmful in any case, the problem arises from extreme events that are difficult to predict (FAO, 2001)[25]. Fixed rainfall patterns and extreme temperature spells may consequently reduce crop productivity. Latitudinal and altitudinal shifts in ecological and Agro-economic zones, land degradation, high geophysical events, reduce water availability and rise in sea level and salinization are demanded (FAO, 2004)[26].

LITRATURE REVIEW

Tissue culture is the *in vitro* aseptic culture of cells, tissues, organs or whole plant under controlled nutritional and environmental conditions (Thorpe T, 2007)[27] often to produce the clones of plants. The resultant clones are true-to sort of the chosen genotype. The controlled conditions provide the culture an environment suitable for his or her growth and multiplication. These conditions include pH medium, adequate temperature, proper supply of nutrients, proper gaseous and liquid environment. Plant tissue culture technology is being widely used for large scale plant multiplication. Apart from their use as a tool of research, plant tissue culture techniques have in recent years, become of major industrial importance in the area of plant propagation, disease elimination, plant improvement and production of secondary metabolites. Small pieces of explants can be used to produce hundreds and thousands of plants in a continuous process. A single explant can be multiplied into several thousand plants in relatively short time period and space under controlled conditions, irrespective of the season and weather all year-round basis (Akin-Idowu P E, *et al*, 2009)[28]. Endangered, threatened and rare species have successfully been grown and conserved by micro-propagation because of high coefficient of multiplication and small demands on number of initial plants and space. In addition, plant tissue culture is considered to be the most efficient technology for crop improvement by the production of somaclonal and gametoclonal variants. The micro-propagation technology has a vast potential to produce plants of superior quality, isolation of useful variants in well-adapted high yielding genotypes with better disease resistance and stress tolerance capacities (Brown DCW, Thorpe TA (1995)[29]. Established type of callus cultures give rise to clones that have inheritable characteristics different from those of parent plants due to the possibility of occurrence of somaclonal variability (George EF 1993)[30], which results in the event of commercially important improved varieties. Commercial production of plants through micro-

propagation techniques has several advantages over the normal methods of propagation through seed, cutting, grafting and air-layering etc. It is a quick propagation processes that can lead the way to the production of plants virus free (Garcia-Gonzales R *et al*, 2010)[31]. *Coryodalisyanhusuo*, an important medicinal plant was propagated by somatic embryogenesis from tuber-derived callus to produce disease free tubers (Sagare AP *et al*, 2000)[32]. Meristem tip culture of banana plants devoid from banana bunchy top virus (BBTV) and brome mosaic virus (BMV) were produced (El-Dougdoug KA, El-Shamy MM 2011)[33]. Excessive yields have been obtained by culturing pathogen free germplasm *in vitro*. Increase in surrender to 150% of virus-free potatoes was obtained in controlled conditions (Singh RB 1992)[34]. The main objective of writing this chapter is to describe the tissue culture techniques, various developments, present and future trends and its application in various fields.

Somatic embryogenesis

Somatic embryogenesis is the process in which haploid or diploid somatic cells evolved into discriminated plants through characteristic embryological phases without fusion of gametes. It involves two main steps, the process of induction and the resultant embryos expression. When the embryo induces directly from a cell or tissue the process is called direct. The embryos found are genetically identical to the parent tissue and are hence clones. In plant breeding, this technique ignored the rooting face requirements needed in micro-propagation using pre existing axillary buds and organogenesis. Different researches have been conducted to develop somatic embryogenesis systems for legumes and some reviews have examined relevant aspects associated with this topic. Pratap *et al*, (2010)[35], examine appropriate for producing numerous plants in a short term.

Recently, Ochatt and Revilla (2016)[36] studied some of the problems associated to embryogenesis and some possible outcomes to solve them. However, different in vitro cultural conditions have been studied for improvement of the frequency of somatic embryo production. The essential minerals required for growth and development and growth regulator substances (GRS) such as auxins, cytokinins must supply by the culture media, abscisic acid and gibberellins among other components in high concentrations.

Bobkov (2014)[37] studied the effect of the use of severe temperature stress treatments and growth regulators in low concentration during induction and resulted callus was embryo-like structures and then regenerated plants.

Nafie *et al*, (2013)[38] initiate that the presence on MS medium supplemented with 1.5mg L-1 2, 4-D in combination with 0.1mg L-1 of 24-Epibrassinolide give positive results. The successful establishment of a regeneration system is affected by many factors, such as culture medium, specific plant growth regulators, appropriate genotype, optimal explants, etc.

Organogenesis

This refers to the production of plant organs i.e. roots, shoots and leaves that may arise directly from the meristem or indirectly from the un differentiated cell masses (callus). Plant regeneration via organogenesis involves the callus production and differentiation of adventitious meristems toward organs by altering the application of plant growth hormones in nutrient medium. Skoog and Muller (Skoog F, Miller CO 1957)[39] were the first who demonstrated that high ratio of cytokinin to auxin stimulated the formation of shoots in tobacco callus while high auxinto cytokinin ratio induced root regeneration.

Plant Growth Regulators

Some chemicals occurring naturally within plant tissues (i.e., endogenously) have a regulatory rather than nutritional role in growth and development. These combination, which are generally active at very low concentrations, are known as plant hormones. Synthetic chemicals with physiological activities similar to plant growth substances or compounds having an ability to modify plant growth by some other means are usually termed PGRs. There are specific recognized classes of plant growth substance. Until recently, only five groups were realized, namely:

Auxins
Cytokinins
Gibberellins
Ethylene
Abscisic acid (ABA)

Auxins and cytokinins are by far the most important plant growth substances for regulating growth and morphogenesis in plant tissue and organ cultures; in these classes, synthetic regulators have been discovered with a biological activity, which equals or exceeds that of the equivalent natural growth substances. No chemical replacement to the natural gibberellins or ABA are present, but some natural gibberellins are extracted from cultured fungi and are available for use as exogenous regulants.

Auxins

Auxins are mostly used in plant tissue culture and usually form an integral part of the nutrient media. Auxins promote, mainly in combination with cytokinins, the growth of calli, cell suspensions, and organs and also regulate the direction of morphogenesis. At the cellular stages, auxins control basic processes such as cell division and cell prolongation. After all they are capable of initiating cell division, they are involved in the formation of meristems giving rise to either unorganized tissue or defined organs. The choice of auxins and the concentration administered depend on:

The type of growth and/or development required.
The rate of utilization and transport of the applied auxin to the target tissue.
The subdue (oxidation and or conjugation) of auxin in the medium and within the explants.
The sensitivity of the plant tissue to auxin (and other hormones as well).
The cooperation between applied auxins and the natural endogenous substances.

The most commonly detected natural auxin is IAA which may be used in plant tissue culture media, but it tends to be oxidized in culture media and is rapidly metabolized within plant tissues. However, for many purposes, it is necessary or desirable to use one of the many synthetic analogues of IAA. These analogues have different structures but similar biological properties and are also called auxins. The syntheticauxins 2, 4-dichlorophenoxyacetic acid (2, 4-D), α-naphthalene acetic acid (NAA), and indole-3-butyric acid (IBA) are commonly used in the tissue cultures. Indeed, all active auxins are weak organic acids. The comparable degree of activity of individual auxins in various growth processes is very variable. It differs not only from plant to plant but also from organ to organ, tissue to tissue, cell to cell, and also with the age and physiological state of the plant (tissue; Davies 2004)[40]. In tissue culture, depending on other hormones present in the medium, changes in auxins concentrations may change the type of growth, e.g., stimulation of root formation may switch to call us induction. In this respect, each tissue culture system is unique, and the effects of different concentrations of auxins and other hormones must be tested for each case individually, and only to some extent, the results can be transferred to other cultures. Plants-like other higher organisms-have to possess intra-organismal communication system(s) working over relatively long distances. As no nervous system is present, the main signaling systems are hormone dependent (Libbenga and Mennes 1995)[41]. Auxins are a component of such systems. Auxins and cytokinins impact at several levels in many different processes of plant development.

Cytokinins

Among PGRs, cytokinins have proven to be the most important factor affecting shoot regeneration, and their significant effects may be related to the histological changes in induced tissues (Magyar-Tabori *et al*, 2010)[42]. Cytokinins are N6-substituted adenines with growth regulatory activity in plants that promote cell division and may play a role in cell differentiation (McGaw and Burch 1995)[43]. Cytokinins added to the medium are vital important during tissue culture of plants because they induce division and organogenesis (Howell *et al*, 2003)[44] and affect other physiological and developmental processes (Heyl and Schmulling 2003[45], Ferreira and Kieber 2005[46], Van Staden *et al*, 2008)[47]. The success of a culture is affected by the type and concentration of applied cytokinins, because their

uptake, transport, and metabolism differ betweenvarieties and they can interact with endogenous cytokinins of an explant (Werbrouck *et al*, 1996[48], Strnad *et al*, 1997[49], Van Staden *et al*, 2008)[47].

Gibberellins

Plant tissue cultures can generally be induced to grow and differentiate without gibberellins, although gibberellic acid (GA3) may become an essential ingredient of media for culturing cells at low densities (Stuart and Street 1971)[50]. GA3 is investigated to break the dormancy of different types of seeds at a critical concentration. It stimulates seed germination via the synthesis of α-amylase and other hydrolases (Shepley *et al*, 1972)[51]. Thus, in recent papers, GA3 has been used to break dormancy and morphogenesis (Chaturvedi *et al*, 2004[52], Shahzad *et al*, 2007[53], Parveen *et al*, 2010[54], Balaraju *et al*, 2011[55]).

Basal media

The primary components of plant tissue culture media are the mineral nutrients. How quickly a tissue grows and the range and quality of morphogenetic responses are strongly influenced by the type and concentration of nutrients supplied.

Toma and Rasheed (2012)[56] conduct a research work on propagation protocol from seed culture and regeneration ability through cultures of callus derived from hypocotyls of *Asparagus densiflorus* were successful. Good seed germination was achieved by cold and wet seed separation prior to culture and using sloped surface medium equipped with 2mgl-1 BA. The foremost parameters for explants multiplication of hypocotyls were recorded while using BA alone at 1.0mgl-1 by producing 8.13 shoots/explant of 7.81cm mean length and 33.00 leaves per explant. Using IBA at 1.25mgl-1 as compared to IAA or NAA was the best auxin for rooting of Asparagus shoots. By the addition of 0.5+ 0.3mgl-1 of both BA and NAA, a compact green callus was produced. The highest roots number (12.4 roots/ explant) and the longest roots (4.98cm) were gained from the addition of 1.25mgl-1 IBA. A successful high survival rate (95%) was found with Asparagus plantlets which gradually acclimatized into the out-air conditions.

Esserti *et al*, (2016)[57] studied the effect of seaweed extract (SE) from *Fucus spiralis* (Fs), *Cystoseira myriophylloides* (Cm) and *Laminaria digitata* (Ld) on *in vitro* plant tissue culture was tested. About 25% of Murashige and Skoog (MS) medium with additional combination of 25 % of SE from Cm medium increased adventitious shoot regeneration from *Nicotiana benthamiana* leaf discs explants by 620 %, when compared to the formal regeneration medium. The effect of SE was also examimed on *in vitro* micro-propagation of *Nicotiana benthamiana*, plum, grape and apricot by estimating shoot length, internodes and number of leaves. When seaweed extract used alone but at lower concentrations (2.5 and 12.5 %), from Fs and Cm resulted in at least the same efficacy as MS alone for micro-propagation of *Nicotiana benthamiana* shoots. The enhancement of root was in *Nicotiana benthamiana* and grapevine, and was correspond with their excessive concentrations of IAA when compared to SE from Ld. This finding, in addition to mineral analysis data, suggests that SE of Fs and Cm contained necessary nutrients and growth regulators to allow their use as medium for *in vitro* plant culture. The composition, type, and strength of basal medium also played an important role in shoot multiplication. Full solidity of MS medium was found beneficial for multiple shoot production in *H. antidysentrica* (Mallikarjuna and Rajendrudu 2007)[58], *Actinidia deliciosa* (Abbas *et al*, 2010)[59], *Albizia lebbeck.* (Rajeswari and Paliwal 2008)[60], *Acacia nilotica* (Akbas *et al*, 2007)[61], *P. santalinus*. (Perveen *et al*, 2011)[62] and *V. negundo* (Ahmad and Anis 2011)[63]. Modification in the MS medium such as MS salts reduced to one half, one third, one fifth, or three fourth has been found effective in *Acacia Senegal* (Badji *et al*, 1993)[64], *A. mearnsii* (Huang *et al*, 1994)[65], *Anacardium occidentale* (Das *et al*, 1996)[66].

Sawardekar *et al*, (2010)[67] studied somatic embryogenesis and direct regeneration in Tur. The experimental material comprised of three genotypes of pigeonpea viz., AKT 8811, ICPL 87 and Konkan

Tur-1 with three types of explants *viz.,* leaf disc, hypocotyl and embryo axes. All the genotypes showed callus induction on basal media MS and B5 supplemented with different combinations of 2, 4-D and BAP.

Mao *et al*, (2006)[68] carried out research on shoot initiation response was observed from its apices of 3-5-day-old seedlings. MS salts, B5 vitamins, 8.88lM N6 benzylaminopurine, 1gl-1 casein hydrolysate, 342lM L-glutamine, 3% sucrose, 0.3% phytagel, adjusted to pH 5.8 are used in the optimal medium for maximum shoot initiation. A change in pH from 5.8 to 7.0 had no effect on shoot initiation and on number of shoots per explants. Using this preferred medium, 77% frequency of the highest shoot initiation was obtained, reaching a maximum of eight shoots per explants. For shoot extension, 14µM gibberellic acid was supplemented in the shoot initiation medium. Presence of indole butyric acid in the rooting medium had no effect on root induction. The regenerated plants were developed and fertile normally.

CONCLUSION AND FUTURE PROSPECTS

Tissue culture techniques in tomato are advancing rapidly. However, there is still an extended thanks to go before hybrid cultivars are often raised via tissue culture in an economically feasible way. In order to supply plants from transformed cells, techniques like as regeneration and somatic embryogenesis are necessary. These techniques also are necessary for producing an outsized number of elite transgenic plants. At present day, the knowledge on large-scale propagation of tomato through somatic embryogenesis is lacking. Somatic embryogenesis in tomato has not reached a stage where good quality embryos showing high vigor can be produced at a reasonable cost. Facts on plant establishment and field performance of the tissue culture raised tomato plants is scarce. Numerous reports on genetic instability of tomato under *in vitro* conditions exist, thus demanding the necessity to guagesomaclonal variations arising from tissue culture. Despite the potential, and vast amount of the research undertaken on this subject, plant part culture has not become an integral a part of tomato-breeding programs. Techniques have been developed for interspecific and intergeneric somatic hybridization through protoplast fusion. These techniques need to be exploited to overcome the serious difficulties due to high incompatibility barriers to hybridization. In view of this anti-GMO consumer attitude, a wise mixing of plant breeding, biology and tissue culture techniques should be implemented to take advantage of these fields for both the development of latest cultivars and for his or her multiplication. Somaclonal variations arising from tissue culture also can be exploited to help with tomato breeding. Photoautotrophic micro-propagation is another potential area in tomato which will be exploited. In conclusion this report, an easy germination seeds and tissue culture protocols established for tomato in Nigeria.

ACKNOWLEDGEMENT

The authors wish to express their sincere gratitude to Department of Biotechnology, School of Life Sciences, Noida International University, Uttar Pradesh, India for providing necessary facilities to carry out this review work.

CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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