CORPUS PUBLISHERS

Archives of Agriculture Research and Technology (AART)

Volume 3 Issue 1, 2022

Article Information

Received date : February 01, 2022

Published date: February 22, 2022

*Corresponding author

Rosa M Longoria E, Department of Biological Sciences, Autonomous University of the West, Mexico

Keywords

Contamination; *In vitro, Lycopersicum esculentum*; Plant regeneration; Tissue culture; Culture medium

Research Article

Establishment of Tomato Plants (Solanum Lycopersicum) under in vitro Conditions

Rosa M Longoria E*, Félix G Rubén, Lizarraga S, Glenda J and Beltrán P Hugo

Department of Biological Sciences, Autonomous University of the West, Mexico

Abstract

Tomato cultivation in the state of Sinaloa represents a third of the production in Mexico. Technological innovation techniques have now been incorporated into its production, moving from soil cultivation to stick cultivation and under controlled conditions in greenhouses; offering products with low or no agrochemical residues, seeking to reverse contamination problems in addition to these practices increasing the value of the product. The objective of this research was to develop an efficient and reproducible system for the in vitro propagation of tomato plants (*Solanum lycopersicum*) without the use of exogenous growth regulators, using dry mature seeds of six tomato varieties (hybrid brigade, hybrid maya; sun 6200; sun 6366; calista; DRD 8561). Under sterile conditions, the seeds were cultivated in a medium with salts (MS) Murashige and Skoog [1], without growth regulators, under controlled conditions (photoperiod of 16 $h.day^{-1}$) at 25 ± 20oC. After five weeks of incubation, vitro plants were obtained, which presented a positive response to the aforementioned conditions, obtaining stems of good size, presence of leaf tissue, and abundant root growth, showing efficient and rapid tissue reproduction and easy handling. This work presents an efficient plant regeneration system with the advantages of not using exogenous growth regulators and easy handling from adventitious shoots, demonstrating that the micropropagation technique helps to produce more plants in less time.

Introduction

Agriculture is one of the main sources of development in the economy of our country. Mexico is one of the most important producers and exporters of vegetables, its main market is North America. Tomato *(Solanum lycopersicum)* exports grew at an interannual rate of 6.7% from January to July 2021, to one million 180,586 tons, the production of red tomato in the state of Sinaloa was 12.7% according to data from the Ministry of Agriculture 2021. In Sinaloa, the production of vegetable seedlings is a common practice; most farms produce their seedlings in greenhouses, although there are private greenhouses dedicated to the production and sale of seedlings. Due to the economic importance of vegetables and the fact that in recent years a significant reduction in production has been observed in the state of Sinaloa due to the presence of phytopathogenic agents, it is necessary to develop highly efficient strategies for better management of diseases to maintain the required demand [2]. On the other hand, it is important to mention that various ecological imbalances such as global warming, the increase in physical and chemical degradation of the soil, irregular rainfall, and the use of chemical fertilizers are factors that play an important role in crop productivity. Due to modernization, the use of chemical fertilizers has been widely adopted to increase yield, and this leads to various health hazards after consumption; therefore, there is a need to balance these effects by using natural resources for agriculture in a positive way [3]. Tissue Culture is an invaluable tool for solving basic problems applied to plant biology; since employing this technique a strict control is obtained as the material is confined in an aseptic microenvironment, occupying very little space and, by clonally propagating, it allows the maintenance of the genotype [3,4]. As an application of plant tissue culture, *in vitro* micropropagation can be mentioned, which in recent decades has gained great importance as an alternative for the mass production of plants with agronomic characteristics of interest, resulting in benefits in horticulture; being used to increase or replace the vegetative propagation techniques used until today [5,6]. Several studies have reported the regeneration of explants, adventitious shoots, apical meristem, cotyledons, petiole stems, leaves, anthers, and inflorescences *in vitro* tomato plants; revealing that the frequency of the regeneration of adventitious shoots differed depending on the type of explants and the concentration of growth regulators added to the regeneration medium, which plays an important role in the regulation of plant growth, therefore it is not there is an established protocol for *in vitro* propagation in tomato plants [5,7-11]. The objective of this work was the establishment of a micropropagation protocol that would allow rapid in vitro regeneration without the use of exogenous growth regulators, allowing the evaluation of six varieties of tomato *(Solanum lycopersicum)*; hybrid brigade, hybrid maya; sun 6200; sun 6366; calista and DRD 8561, provided by a commercial company, ensuring the maintenance of said material for long periods. In the present study, an efficient and rapid regeneration protocol was established, obtaining seedlings without the use of exogenous growth regulators.

Materials and Methods

Desinfección and establishment of plant material

Plant material preparation was performed under sterile conditions using a laminar flow chamber (horizontal laminar flow hood, Figure model CFH-120) as shown in Figure 1a. To obtain adventitious shoots, mature seeds of six varieties of tomato *(Solanum lycopersicum)* were used; hybrid brigade, hybrid maya; sun 6200; sun 6366; calista and DRD 8561, provided by a commercial company. The surface sterilization of the seeds was carried out under aseptic conditions as follows: washing with 80% Tween for 3 min with constant agitation; subsequently in 70% ethanol solution for 1 min under agitation, after the time was elapsed and a 1.2% sodium hypochlorite solution was added, leaving under agitation for 15 min. Once this time was over,

the seeds were washed in sterile water, the surface of the seeds was dried with absorbent paper, and seeds of similar size to the six varieties were selected and placed on filter paper previously moistened with sterile water and pre-cultured. For three days, in a growth chamber under controlled conditions with a photoperiod of 16 light hours. After a time, 12 seeds were placed in each culture box (Magenta) containing 50 mL of MS Murashige and Skoog 1962 medium (Medium Plant; Sigma) supplemented with 7 gL^{-1} of Agar, and 3% commercial sugar without regulators. Of growth, adjusting a pH of 5.7 before sterilization (121°C/15min). Subsequently, the culture boxes were transferred to a growth chamber under controlled conditions (photoperiod of 16 $h.day^{-1}$) at 25 ± 20oC (Figure 1). Where the time in which 50% germination was reached was determined.

Plant multiplication

From the plants obtained from seeds after 8-10 days, explants of 2-3 cm in length were cut, which were established in MS culture medium (Murashige and Skoog), carrying out evaluations at 15 and 30 days. Subsequently, the elongated shoots were extirpated individually from the generated plants, explants of 1-3cm in length were cut, to be subcultured in MS medium for rooting; after two weeks, new shoots emerged repeating the process for the generation of new plants (Figure 2).

In the multiplication phase, 5 explants per flask were placed, in a randomized complete block design with three replications, planting eight explants per culture box (magenta) which were sealed with parafilm paper to reduce the risk of contamination; subsequently, they were pre-incubated at 25 ± 1°C in the dark for 48 hours, and transferred to a growth chamber under controlled conditions (photoperiod of 16 $h.day^{-1}$) at 25 ± 20oC (Figure 1). Subsequently, germination percentage, shoot formation/explant, number of shoots/explant, length (cm) of shoots/explant were quantified (Table 1). The plants that were obtained were established individually in pots using vermiculite as a substrate. To maintain high relative humidity and counteract the sudden change in conditions, a transparent polyethylene cover was placed on each one, removing them in periods of 3 to 5 days. The plants were kept in a growth chamber under controlled conditions (1,100 lux and 28°C), adding Steiner's (1980) diluted nutrient solution to the irrigation water for approximately 25 days. The plants established in the substrate had to meet the following requirements: thick and strong stems, healthy and abundant roots, the height of 13-15cm, light green leaves free of pests and diseases, to be able to adapt to future transplants under different conditions. (field, shade mesh, or greenhouse). It is important to mention that all the experiments were carried out with three repetitions for each of the varieties under the same conditions.

Analysis of Data

The data generated during the study were statistically analyzed by performing an analysis of variance (ANOVA), the differences in the means between treatments were performed Tukey's Multiple Range Test ($p<0.05$) with the support of the Statistical Program Sigma Stat version 3.5.

Results

Tomato seeds of the hybrid brigade, hybrid maya; sun 6200; sun 6366; calista and DRD 8561, began to germinate after four days in culture medium (Figure 1b). After two weeks, the young seedlings grew with a root but without the apical part. Two weeks later, elongated explants and differentiated adventitious buds emerged in all treatments. On the other hand, the number of adventitious shoots obtained from immature plant parts was higher than in explant reports [12-14]. The explants of the six tomato varieties used in the experiment presented organogenic capacity after preculture; the rate of differentiation in adventitious shoots, as well as elongated shoots in all the subcultured explants, was higher as the weeks passed. After seven weeks of incubation, *vitro* seedlings were obtained for each of the varieties that were established under the aforementioned conditions (Figure 1c & Figure 1d). After a time of 15 and 30 days of establishment, the length of shoots (cm) was measured in the plants of each variety, presenting the minimum values at 30 days for the hybrids brigade and maya, in the formation of shoots the varieties calista and DRD 8561 presented the lowest values; on the other hand, the lowest number of shoots per explant was observed in the brigade hybrid (Table 1). The percentage of formed explants exceeded 50%, increasing this percentage in the following weeks of culture; each vitro plant was transplanted into pots with sterile medium (vermiculite), however at this stage 40% of the seedlings were lost, those that survived were placed in the shade and with high relative humidity. However, during the first fifteen days of acclimatization, 15% of the seedlings were lost, after six weeks the seedlings showed good development with good-sized stems, the presence of leaf tissue, and a profuse growth of the root system (Figure 3). The results obtained confirm that the tissue culture technique generates a good regeneration frequency, which suggests that morphogenic activity takes place in these explants in the absence of growth regulators (Figure 3).

As well as the management of the tissue in the hood was improved, minimizing the presence of contamination in the culture medium.

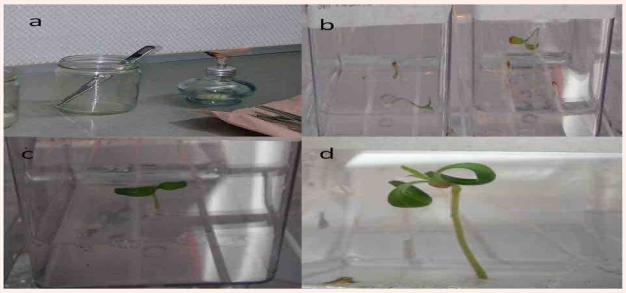

Figure 1: Establishment of six varieties of tomato seeds MS culture medium (Murashige and Skoog). (a) Laminar flow chamber under sterile conditions. (b) Seeds of similar size were established in the MS culture medium without growth regulators. (c and d) *vitro* plants after five weeks of establishment.

Figure 2: Vitro plants regenerated of different explants grown on MS culture medium (Murashige and Skoog) under controlled conditions without growth regulators varieties: brigade hybrid, maya hybrid; sun 6200; sun 6366; calista and DRD 856.

Figure 3: Vitroplants are established in vermiculite substrate.

Table 1: Variables evaluated in the *in vitro* establishment in MS culture medium (Murashige and Skoog), of six varieties of tomato seeds *(Solanum lycopersicum)*.

Variety	Germination Percentage (%)	Shoot Length 15 days (cm)	Shoot Length 30 days (cm)	Explants Budding (%)	Number of Shoots/ Explant
Hibrido brigade	100	1.23±0.01	1.28 ± 0.05	62^{ab}	1.28 ± 0.05
Hibrido Maya	100	1.21±0.02	1.26 ± 0.06	62^{ab}	1.82 ± 0.06
Sun 6200	100	1.23±0.01	2.17 ± 0.06	83^{a}	2.17 ± 0.06
Sun 6366	100	1.21±0.01	1.88 ± 0.06	75^{b}	1.88 ± 0.06
Calista	100	1.21±0.01	1.58 ± 0.05	52^{ab}	1.58 ± 0.05
DRD 8561	100	1.21±0.03	1.49 ± 0.06	54^{ab}	1.49 ± 0.06

*Means with the same letters in the columns do not present a significant difference $p<0.001$

Discussion

There is a close relationship between plant tissue culture and modern biotechnology for the obtaining and regeneration of plants, tissue culture is regularly used, followed by the regeneration of the complete plant, opening an immense range of possibilities both for the improvement of cultivated plants, as well as for obtaining new products from it [6]. For the propagation of plants in vitro, it is necessary to consider the components of the culture medium and the conditions in which the seedlings develop as fundamental factors [15]. The culture media contain all the minerals and vitamins necessary for the growth and differentiation of plants, the concentrations are in the appropriate proportions and can be used in a considerable number of plant species, without interaction between them, hindering their assimilation [16,17]. Different sources of explants have been used for shoot formation in different cultivated and wild tomato species, such as leaves, stems, roots, cotyledons [18-20]. The results obtained in this study showed the importance of the preculture time and its influence on the induction of shoots and roots; obtaining a reasonable regeneration rate. From the germination of six varieties of tomato (Solanum lycopersicum); brigade hybrid, maya hybrid; sun 6200; sun 6366; calista and DRD 8561, used to carry out this work, complete plants were obtained in a time of 11 weeks, presenting an efficient regeneration system, obtaining on average seedlings of 15 cm in height, considering an average yield in the different micropropagations of 67% for the derivatives of internodes which generated 17± 2 complete plants in six weeks of cultivation with a 50% rooting response; and an average propagation of 25 ± 2 plants generated under in vitro conditions in MS culture medium without growth regulators in seven months for each germinated seed; where each plant generated in vitro could continue its development under greenhouse conditions before being taken to the field, which, when transplanted, develop naturally in such a critical stage as an adaptation to new conditions.

The protocol developed in six varieties of tomato *(Solanum lycopersicum)*; brigade hybrid, maya hybrid; sun 6200; sun 6366; calista and DRD 8561, differs from those reported about the absence of growth regulators, which allows it to be used as a basis for the conservation and propagation of other species.

Conclusion

The results obtained in this work indicate a probability of success in the micropropagation process of this species, as well as further research related to the establishment of future micropropagation protocols. The advantages of this regeneration method it was the non-use of exogenous growth regulators, it is easy handling, and the short time to obtain seedlings. However, feasibility studies must still be carried out in the field, since the adaptation to the external environment of the seedlings generated in vitro must be carried out after a gradual adaptation process.

Thanks

The authors thank the Universidad Autónoma de Occidente, Research and Postgraduate Department, through the Research Support Program for the support provided to carry out this project.

References

1. Murashige T, Skoog F (1962) A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiology Plant 15(3): 473-497.
2. Márquez IM, Redman RS, Rodríguez S, Roosinck MJ (2007) A Virus in a Fungus in a Plant-Three Way Simbiosis Requiered for Thermal Tolerance. Science. American Association for Advanced of Science, Washington 315(5811): 513- 515.
3. Roca WM, Nolt B, Mafla G, Roa J, Reyes R (1991) Elimination of viruses and propagation of clones in cassava (Manihot esculenta Crantz). In: Rock WM, Mroginski LA (Eds.). Tissue Culture in Agriculture: Fundamentals and Applications. 697p IATTC, Cali, Colombia.
4. Slack S (1980) Meristem tip culture. Plant Disease 64(1): 15-17.
5. Plana D, Álvarez M, Lara RM, Florido M, Álvarez F, et al. (2005) A new *In Vitro* regeneration protocol in tomato (*Lycopersicon esculentum* Mill.) Tropical Crops 26(2): 17-20.
6. Vinoth S, Gurusaravanan P, Jayabalan N (2012) Effect of seaweed extracts and plant growth regulators on high-frequency *in vitro* mass propagation of *Lycopersicon esculentum L* (tomato) through double cotyledonary nodal explant. J Appl Phycol 24: 1329-1337.
7. Young R, Kaul V, Williams EG (1987) Clonal propagation *in vitro* from immature embryos and flower buds of *Lycopersicon peruvianum* and *L esculentum*. Plant Sci 52(3): 237-242.
8. Branca C, Bucci G, Domiano P, Ricci A, Bassi M (1990) Auxin: structure and activity on tomato morphogenesis *in vitro* and pea stem elongation. Plant Cell Tiss Org 24: 105-114.
9. Compton ME, Veillux RE (1991) Shoot root and flower morphogenesis on tomato inflorescence explants. Plant Cell Tiss Org 24: 223-231.
10. Gubis J, Lajchova Z, Farago J, Jurekova Z (2004) Effect of growth regulators on shoot induction and plant regeneration in tomato (*Lycopersicon esculentum* Mill.). Biol Brat 59(3): 405-408.
11. Jiménez P, Barrera P, Huachi L, Vera A, Caicedo C (2020) Propagation *in vitro* of Quishuar (Buddleja incana RUÍZ & PAV). The Farm: Journal of Life Sciences 31(1): 71-81.
12. Álvarez M, Rodríguez J, Plana D, Santana N (1999) Radiosensitivity to 60Co gamma rays in tomato callus (*Lycopersicon esculentum*, Mill) variety Amalia. Tropical Crops 20(4): 35-39.
13. Fuentes AD, Soto N, Alfonso D, Oramas P (1998) Study of the culture conditions for the regeneration of tomato plants (*Lycopersicon esculentum* Mill), from cotyledons and leaves of the variety Campbell 28. Biot applied 15: 242-245.
14. Pratta G, Zorzol R, Picardi L (1997) Intra and interspecific variability of in vitro culture response in *Lycopersicon* (tomatoes). Brazilian Journal of Genetics 20(1): 75-78.
15. Cano C, Dickson S, González GS, Bago A (2008) *In vitro* cultures open new prospects for basic research in arbuscular mycorrhizas. En: Mycorrhiza. State of the art, Genetics and molecular biology, Eco-Function, Biotechnology, Eco-Physiology, Structure and Systematics. Ed.: Varma, A. Springer-Verlag, Berlin Heidelberg pp. 627-654.
16. Cassells AC, Gahan PB (2006) Plant Tissue Culture Dictionary pp. 265.
17. Mohammadi GE, Rezaee DY, Prasad R, Varma A (2008) Mycorrhizal fungi: What we know and what should we know? In: Mycorrhiza. State of the art, Genetics and molecular biology, Eco-Function, Biotechnology, Eco-Physiology, Structure and Systematics. Ed.: Varma, A. Springer-Verlag Berlin Heidelberg p. 3-28.
18. Pozueta RJ, Houlné G, Cañas L, Schantz R, Chamarro J (2001) Enhanced regeneration of tomato and pepper seedling explants for Agrobacterium-mediated transformation. Plant Cell Tiss Org Cult 67: 173-180.
19. Cano A, Moreno V, Romero V, Bolarin MC, Caro M (1998) Evaluation of salt tolerance in cultivated and wild tomato species through in vitro shoot apex culture. Plant Cell, Tissue and Organ Culture 53: 19-26.
20. Chyi YS, Phillips GC (1987) High efficiency Agrobacterium mediated transformation of Lycopersicon based on conditions favorable for regeneration. Plant Cell Rep 6(2): 105-108.