Journal of Advanced Zoology

ISSN: 0253-7214
*Volume **44** Issue **04 Year 2023** Page **752:756***

In Vitro Propagation of Ruta Chalepensis Through and Callus Culture

Saagarika Srinivasan[1], Manjunathan Jagadeesan[2], Thenmozhi. M[*3]

[1,2,3]*Department of Biotechnology, School of Life Sciences, Vels Institute of Science, Technology & Advanced Studies (VISTAS), Chennai, Tamilnadu, India Email: Saagarikas3@gmail.com[1]*

**Corresponding author's E-mail: drmthenmozhi@gmail.com*

Article History

Received: 06 June 2023
Revised: 05 Sept 2023
Accepted: 26 Nov 2023

Abstract

Plant tissue culture is a field that that enables culturing of various plants and parts of plants usually treated under a nutrient medium and in highly sterile conditions. Out of them callus culture is one of the very interesting arenas of plant biotechnology that encompasses many pivotal benefits. The study focuses on such callus enrichment using different hormones that there by enhance its biological activities. The plant namely Ruta chalepensiswas chosen upon wherein the callus growth was noticed. Ruta chalepensis has multiple medicinal activities like anti-cancer, anti-ulcer, anti-diabetic and many more pharmacological properties that yields in treating and curing of illness. Ruta chalepensis the leaf and internode were taken for the study and to analyse which of those at what concentration of plant growth regulators showed a better callus induction. The MS medium as well as Various hormone concentrations was used for the study like auxin and cytokinin from 0.5mg to 2 mg (2,4 – D, NAA, IAA, IBA). Increased concentration of 2,4-D (1.0 mg/L) alone in the MS medium showed profused callus growth. Both the explants used such as leaf and Internode were also tested in the MS medium which was devoid of hormones/plant growth regulators which was treated as control for comparison. From the data obtained, MS medium supplemented with hormones showed better growth rate and callus induction when compared with that of MS medium without hormones/plant growth regulators. Among the plant growth regulators 2,4-D (1.0 mg/L) showed maximum callus initiation from both leaf and internode explants. Further work was carried out in single and combination of the plant growth regulators for callus proliferation and accumulation. Further analysis is being done to study the growth pattern on combination of hormones and fix the hormone concentrations for the mass propagation of callus from the explants.

Keywords: *Ruta chalepensis, callus culture, MS medium, hormone concentrations.*

1. Introduction

Plants are various in numbers and varieties not just in one are but throughout the world, there are certain plants that are cultivated and grown only in certain geographical locations which is due to its climatic conditions, the kind of soil as well as many other parameters. Plants that could aid in many vital properties. It is seen that there are many systems of medicine ranging from allopathy, homeopathy, siddha, ayurveda and yet over the centuries it is noted that there are more advantageous properties seen over the usage of traditional system of medicine that is characterised through the usage of medicinal herbs or plants as a form of drug delivery aiding many hazardous disease conditions of the human body. There is many vital emphases that is been done in the usage of such traditional system of medicine through usage of medicinal plants to the main reason as it justifies the fact of treating across various issues of the human body through nil or mild side effects. These herbs are a good form of pharmacological properties that have the major benefit of dealing with various spectrums of ailments in the human system. Many parts of the plant or the herb that ranges from the shoot, root, leaves, flowers aid in particular aspects of specified functions that thereby helps targeting ranges of diseases. There is definitely a vital arena that is noted with the fruitfulness of such plants with pharmacological activities as it is derived naturally when in relation with the drugs that are used in other system of medicines. There are different plants that have properties such as used for oil extractions that play a predominant role in the field of cosmetology, food flavoring, fragrance, as well as many other herbal features. Out

of these, one of the most vital properties that a plant could carry is the characteristics of it possessing medicinal values. These medicinal plants contribute to many pharmacological values that help in curing infections and diseases, nutritional values. Nevertheless, more understanding is ought to be made with pertinence containing tremendous medicinal values that aid in multiple aids of tool in curing different pharmacological properties and that also concerning in many vital nutritional values.

Various systems of medicines are now enabling the usage and administration of medicinal herbs as a form of drug therapy with the sole reason of them valuing many vital features of pharmacological aids as well as them possessing minor or nil side effects. Out of many such epidemic factors of effects in the human body, one such main prevailing factory condition is that of characterization of low hemoglobin levels in the body that would sound like a very minor effect but can lead to many harmful complications if left untreated. This prevailing problem of lack of hemoglobin could be addressed with the use of various medicinal plants. Such medicinal plants help has magnificent healing properties and aid in curing in various illnesses and one such as increasing hemoglobin levels in the blood, or also can help in increasing the level of active iron form in the body as well can aid in the absorption of various minerals that aid in the increase of the hemoglobin levels in the blood. It is also to be noted that the usage of such medicinal plants is an old treatment method as old as mankind itself. Plants that carry medicinal values, also called as medicinal plants are that those aid in carrying pharmacological properties that could usually help in treating as well as curing illness [1]. One such of these medicinal plants that were being worked uponwas *Ruta chalepensis,* commonly known as *fringed rue* and known as *Aruvada* in Tamil. This plant has been shown to carry many pharmacological properties such as the range from properties ofanti-cancer, anti-diabetic, anti-inflammatory, analgesic and many other tremendous characteristics. It is one of the most magnificent plants that has many important vital properties that could help in many arenas to health and living. It is one of the very old ancient plants that has the commodity in herbal usage and remedies due to the presence of its pharmacological characters.

2. Materials And Methods

Collection of explants and surface sterilization

The plant was procured fresh where in the leaf and the internode plant of *Ruta Chalepensis*was *been* collected from the hills of Nilgiris, Tamil Nadu. Once the collection or the procurement of the plant was done, it was made sure that the plants were kept sterile throughout until timeand for obtaining the same, explants were thoroughly washed initially followed post which tween 20 was added, covered, and left under running tap water for about 30 minutes. Followed by which about 0.1% of sodium hypochlorite was added for about 20 minutes with 5-minute intervals and then washed with distilled water for 5 minutes. Further, sterilization process was taken place under aseptic conditions, in a Laminar airflow chamber. The next step was characterized where the explants were let to be treated under with 0.5% mercuric chloride for 2 minutes and as the final step the explants were profusely washed with sterile distilled water for 5 minutes. The leaf and the internode were cut into sizes ranging from 0.5 cm – 1.0 cm (Ajit and Paratkar 2007; Saeeda et al., 2007, and inoculated into an MS medium m (Murashige and Skoog, 1962) along with different hormonal concentrations.

Culture

For the process of induction of callus, MS (Murashige and Skoog., 1962) were being used. About six individual stock solution of macro, micro, iron, vitamins were prepared and stored. During media preparation, Na2EDTA and FeSO4 were dissolved separately in 100 mL of water. Iron stock was stored in a black bottle to prevent photolysis of chemicals. All the stock solutions were stored in refrigerator and used within one year. Meso-inositol, cytokinin and auxin stock solutions were freshly prepared and used every month. For preparation of medium, all the 6 stock solutions were mixed thoroughly with required amounts of sterile distilled water. Sucrose 3% (30 g/L), 0.1% meso-inositol (100 mg) and required amount of plant growth hormones were added to the medium and the buffered by 1N HCl or 1N NaOH to adjust the medium pH to 5.6 before autoclaving. The medium was solidified by adding 0.8% agar (800 mg/L). Sterile distilled water was used to make the final volume. The medium was poured into culture vials and autoclaved at 15 lb pressure for 15 min at 121ºC. After inoculation with the explants, all culture vials were kept under 16/8 h (light/dark) photoperiod at 25±2ºC.

Callus induction & Initiation

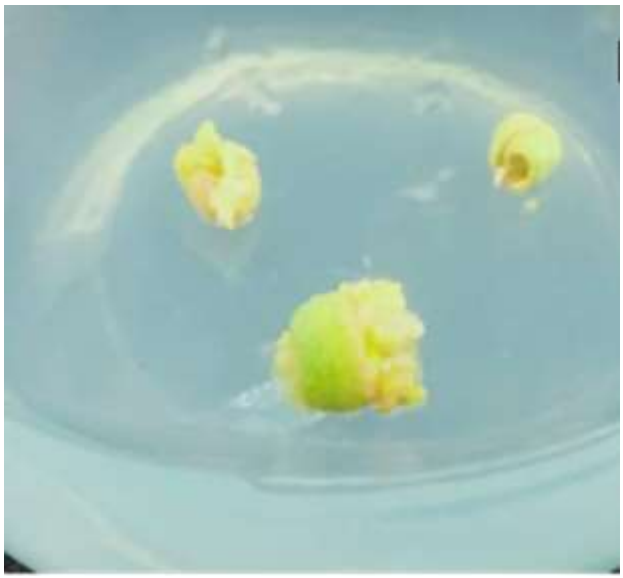
MS medium that was supplemented using various concentration dosages of 2,4-D, NAA, IAA, BAP was tooled for the initiation and induction of the callus. Multiple concentrations of dosages in the mentioned plant growth regulators were performed ranging from 0.5 mg, 1.0mg, 1.5mg, and 2.0 mg to

assess the growth of the callus. The amount of callus induction was calculated using the mentioned formulation Callus induction (%) = (No. of Plants produced/No. of Plants inoculated) X 100.

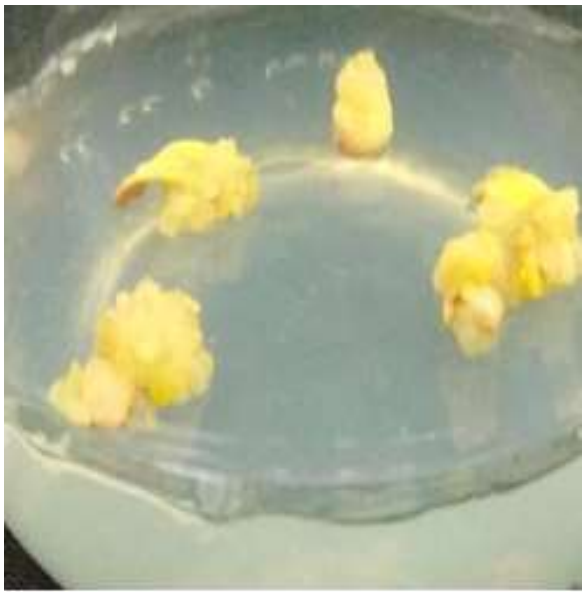
3. Results and Discussion

Effect of 2,4-D on Callus Induction

2,4 – D was being treated using various concentrical dosages ranging from (0.5, 1.0, 1.5, and 2.0 mg/L) wherein the initiation of callus was being examined on the leaf as well as the internode of the explants. Maximum initiation of callus was been noted in the dosage of explants being treated with 2, 4 -D 1.0 mg/L. Evidence quote that the callus induction has seen to be increased at the dosage of 1.0mg/L with regard to 2, 4 -D in a comparatively sufficient duration in wheat, *Triticum aestivum L*. Higher the concentrical dosage of the growth regulators leads to vast detrimental impacts on the explants and was inferred those moderate dosages aid in fairly good induction of callus (M. Y. Zheng & C. F. Konzak 1999).



a. Initiation of callus from leaf explants on MS medium supplemented with 2,4 – D (1.0 mg/L).



b. Proliferation of callus on MS medium supplemented with 2,4 – D (1.0 mg/L) on 20th d

Effect of NAA on Callus Induction

NAA was been treated upon the callus in the dosages of (0.5, 1.9, 1.5, and 2.0 mg/L) and NAA was yet another of a major regulator that was been treated upon the callus to examine the callus induction. It was observed that it was the second regulator that showed induction of callus growth concerning at the dosage of 0.5 mg/L in both the leaf and the internodal part of the plant. Previous studies quote that auxin especially NAA initiate sooner callus induction than that compared other growth regulators, as well as the fact that combinations of auxins together can initiate better callus growth than compared to just

individual concentrations, hence combination studies of hormones can be carried forward (Nic-Can, G.I Loyola-Vargas, V.M. 2016).

Effect of IAA on Callus Induction

Callus induction was also being examined with the treatment of IAA. It was also been treated using various concentrical dosages ranging from (0.5, 1.0, 1.5, and 2.0 mg/L) wherein with regard to IAA there was very minor callus induction observed. The maximum callus induction was seen in the dosage of 1.5 mg/L wherein only about 20% callus induction was noted in both the leaf as well as in the internodes. Past studies when worked upon both 2, 4- D and IAA It was inferred that the callus induction was increased significantly when 2,4-D was treated upon the explants when compared to IAA which showed inhibited growth and callus induction in spring wheat, Triticum aestivum (Shane T. Ball, Hua Ping *et al*1993).

Effect of BAP on Callus Induction

BAP was also being treated using various concentrical dosages ranging from (0.5, 1.0, 1.5, and 2.0 mg/L) wherein the initiation of callus was examined from both the leaf and the internodal areas. Maximum callus induction was observed in the dosage treated with 2.0mg/L of BAP. Shreds of evidence quote that BAP is one of the first-generationcytokinins. Studies have shown that a higher concentrical dosage of growth regulators is linked with increased callus induction, evidence of increased callus induction was noted with an increased dosage of BAP (Shafiq Ahmad, William Spoor 1999).

Hormones used in mg/L in MS medium	**Explants Used**	
	Leaf	**Internode**
2,4-D (0.5)	65.2±0.13	65.1±0.13
2,4-D (1.0)	**94.4±0.89**	**69.2±0.22**
2,4-D (1.5)	84.5±0.22	65.3±0.13
2,4-D (2.0)	70.4±0.05	68.4±0.22
NAA (0.5)	**81.2±0.15**	**75.9±0.22**
NAA (1.0)	73.2±0.05	65.6±0.08
NAA (1.5)	66.4±0.10	57.2±0.33
NAA (2.0)	61.2±0.20	53.8±0.24
IAA (0.5)	40.3±0.89	42.4±0.10
IAA (1.0)	43.6±0.89	49.2±0.15
IAA (1.5)	**45.6±0.10**	**57.2±0.22**
IAA (2.0)	43.9±0.05	53.2±0.42
BAP (0.5)	49.2±0.15	46.9±0.05
BAP (1.0)	65.5±0.13	57.2±0.22
BAP (1.5)	70.2±0.10	61.2±0.05
BAP (2.0)	**73.4±0.31**	**73.2±0.18**

Table 1: Above values represent the Mean ± SD of the triplicate of the experiment where callus induction was noted.

4. Conclusion

With regard to various plant growth regulators that were adopted to examine the impact of the callus initiation using various hormonal concentrations, it was inferred that the Effect of 2, 4-D seen was that the maximum callus growth was seen in at the concentration of 1.0mg/L in both the leaf (90%) and internode (80%). The effect of NAA was the second hormone that yielded callus growth was seen in NAA, at concentrations treated at 0.5mg/L in leaf at (50%) and internode (70%). When IAA was treated upon the explants at the concentration of 1.5mg/L there was callus growth seen in leaf (20%) and internode (20%) and when BAP was treated upon the explants it was noted at the concentration of 2.0mg/L there was callus growth seen up to 50% in leaf and 50% in the internode. It can be concluded and inferred that the many vital advantages are noted upon the process of callus culture.As discussed above, the usage of certain plant growth regulators across various dosage levels was noted to show marked difference in the induction of callus. Certain plant growth regulators showed gradual impact of callus induction. Light could be thrown upon the specificities of this process aiding to getting to secondary metabolites enhancement as well as the process can aid in increased yield in shorter span of time. As the yield increases it can be inferred into the usage of pharmacological arenas due to its

medicinal properties and also due to the higher yield that can be noted in the procedure of plant tissue culture and under the vast spectrum of callus culture and growth.

References:

1. Nic-Can, G.I Loyola-Vargas, V.M. The role of the auxins during somatic embryogenesis. In Somatic Embryogenesis: Fundamental Aspects and Applications; 2016 172-182.
2. Shafiq Ahmad, William Spoor. Effects of NAA and BAP on callus culture and plant regeneration in curly kale (*Brassica oleraces L.*) . Pakistan journal of biological sciences.1999; 2: 109-112.s
3. Mohsen Hesami and Andrew Maxwell Phineas Jones.Application of artificial intelligence models and optimization algorithms in plant cell and tissue culture. Applied microbiology and biotechnology. 2020;104(22):9449-9485.
4. Kim Y, Narayanan S, Chang KO. Inhibition of influenza virus replication by plant-derived isoquercetin. Antiviral Research 2010; 88 (2): 227-235. doi: 10.1016/j.antiviral.2010.08.016
5. Eisinga R, Te Grotenhuis M, Pelzer B. The reliability of a two-item scale: Pearson, Cronbach, or Spearman-Brown. International Journal of Public Health 2013; 58 (4): 637-642.doi: 10.1007/s00038-012-0416-3
6. Sheela KG, Deepa SN. Review on Methods to Fix Number of Hidden Neurons in Neural Networks. Mathematical Problems in Engineering 2013; 2013: 425740. doi: 10.1155/2013/425740
7. Pham QB, Abba SI, Usman AG, Linh NTT, Gupta V et al. Potential of Hybrid Data-Intelligence Algorithms for Multi-Station Modelling of Rainfall. Water Resources Management 2019; 33 (15): 5067-5087. doi: 10.1007/s11269-019-02408-3
8. Elkiran G, Nourani V, Abba SI, Abdullahi J. Artificial intelligence-based approaches for multi-station modelling of dissolve oxygen in river. Global Journal of Environmental Science and Management 2018; 4 (4): 439-450. doi: 10.22034/GJESM.2018.04.005
9. Guddat S, Solymos E, Orlovius A, Thomas A et al. High-throughput screening for various classes of doping agents using a new 'dilute-and- shoot'liquid chromatography-tandem mass spectrometry multi-target approach. Drug Testing and Analysis 2011; 3 (11-12): 836-850. doi: 10.1002/dta.372
10. Ruggieri F, D'Archivio AA, Carlucci G, Mazzeo P. Application of artificial neural networks for prediction of retention factors of triazine herbicides in reversed-phase liquid chromatography. Journal of Chromatography A 2005; 1076 (1-2): 163-169. doi: 10.1016/j. chroma.2005.04.03
11. Ajit, K and G.T Paratkar. Effect of plant growth regulators on indirect organogenesis in Ruta graveolens. L. International Journal of Advanced Research, 3(6) : 1113-1119.