Effect of Culture Media and Hormones Combination on Callus Induction of *Annona Squamosa* L.

Mamta Choudhary, Komal Sharma & Dr. Khushbu Verma*

Faculty of Agriculture & Veterinary Science, Jayoti Vidyapeeth Women's Uniersity, Jaipur, Rajasthan 303122

Abstract- The use of *in vitro* culture techniques is growing with the importance of keeping the plasma germs of plants safe and securing valuable products of medical and commercial importance. *Annona squamosa* L. is an economically important species of the West Indies. In the present study,Callus induction was tested on modified Murashige and Skoog (MS) strains with various growth hormones using a young leaf explant of this species. MS has been found to be better at inducing and maintaining callosities when supplemented with growth hormone equal ratio and high Auxin with low Cytokinin ratio. The hormonal combination of 4BAP+5NAA was recorded as the most appropriate for a high percentage of callus induction (80%). Though, Growth index rate & Tolerance index were highest at 4BAP+4NAA (130.49±2.2561) and 4BAP+4NAA (170.08±2.73) respectively. This study reveals about the best possible combinations to grow callus with best growth. It will help to conserve this tree species & to popularize it for the commercial purpose by opening the way for *In vitro* regeneration & multiplication.

Keywords- *Annona squamosa,* Auxins, Callus induction, Cytokinins, Growth index, Tolerance index

1. INTRODUCTION

Annona squamosa L., the plant of the Annonaceae family, also known as pudding apple, is common in hardwood forests, which are also grown wild in various parts of India. Numerous the compounds have been reported in recent years [1], which have attracted the attention of Chemists and biochemists because of their new structure and magnitude spectrum of bioactivity. Many research studie have show that each part of *Annona squamosa* has medicinal properties [2-3].

- *Mamta Choudhary & Komal Sharma is currently pursuing Ph.D. program in Biotechnology Jayoti Vidyapeeth Women's University, Jaipur, Rajasthan 303122*
- *Dr. Khushbu Verma is currently working as an Assistant Professor at Jayoti Vidyapeeth Women's University, Jaipur, Rajasthan 303122 E-mail: vermakhushbu25@gmail.coom; fbtdepartment@jvwu.ac.in*

The roots are used internally for depression of ghosts and disorders of the spine. The bark is known as a powerful astringent. In Ayurveda, fruits are considered a good tonic enriches the blood used as an expectorant, increases muscle strength; Cooling reduces burns and the tendency to bile; Sedative in the heart and relieves vomiting. The ripe fruits are ripe and the mixture is used with salt against malignant tumor up accelerate the suppuration. Dried unripe fruits are sprayed and mixe with gram flour to poultice to induce suppuration. Due to the singularity of the characteristics of the leaf in healing various conditions, this part was selected for the study. The bark of *A. squmosa* used for diarrhea treatment while the root of this plant used for dysentery treatment.Cooling of the leaf is use to cool and clarify the urine. The leaf be use toward treat hysteria and fainting.The fruits of *Annona squmosa* are haematinic,

refreshing, soothing, stimulating, expectorant and invigorating. They are useful within the cure of anemia and burns. The seeds are abortive with insecticidal also be use for destroy in the hair. Fruits are used to make ice cream and milk drinks. The bark and leaves contain annain, an alkaloid that has many of thes properties [4]. Hypoglycaemic and antidiabetic effects of *Annona squamosa* include be report inside leaf extract [5]. A bioactive acetogenies by cancer movement bioactive has been isolated from *Annona squamosa* bark [6], Flavonoids in leaves [7], aporphinal alkaloids [8] , glycosideand squamolin have been isolated from this plant [9]. The production of leaf fragments in the form of callus is primarily a culture condition required by the explants to survive and grow, to study cell development, to use the products of major with the secondary metabolism and for the preservation of cell cultures. This could also pave the way for the isolation of economically interesting phytochemicals, thus avoiding the collection of plant material from natural sources[10] . In the field of plant biotechnology, calli and cell cultures play a special role in the large-scale production of medical with bioactive compound from plants. Phytochemicals are the main source of pharmaceuticals, flavorings, agrochemicals, dyes, biopesticides and food additives. The phytochemical screening of *Annona squamosa* was found toward have alkaloid, flavonoid, tannins, saponins, glycosides and volatile oils[11] . Plant using various explants and regeneration of plants *In vitro*. From a medical point of view, the importance of this plant and its use will reduce its quantity. That's why it needs conservation with the help of *In vitro* regeneration process. Therefore, the aim of this present study is to discover the best gorwth harmone combination for the the best results for callus induction and growth.

2. MATERIALS AND METHODS

The whole plant of *Annona squamosa* L.(Sitafal) were collected from the Jayoti Vidyapeeth Women's University Jaipur ,Rajasthan. Healthy and young plants be selecte because explant basis. The leaf be to begin with washe with Triton-x100 follow by rinse with organization tap water for 30 minute. Then the explants were treated with sodiam hypochloride for 45 seconds and wash with sterile distill water for 4-5 times. Later, the explants were sterilized with 0.1% murcuri chloride solution for 2 to 3 minutes and finally washed 3-4 times with sterilized distilled water, inoculated into culture tubes inside the Laminar Flow Chamber.

2.1 Culture media and culture maintenance

The basal MS media [12],with 3% sucrose and various concentration combination of BAP+NAA solidified with 0.8% agar was used. Several follow by rinse with organization tap water for 30 minute.The pH of the medium be adjusted to 5.8 after autoclave at 121ºC for 20 minute. The culture be maintain on 25ºC±2ºC with 16 hr photoperiod. before 30 to 45 day, the culture be visually observed for the arrangement of callus texture, color, etc .

Well grown-up callus induced as of cultures were select to sub- culture on top of the same medium after every 4-5 weeks. On the basis of findings and observations, physiological parameters were calculated to find out the best combination result for callus induction.

2.2. Callus culture percentages

All the culture was raised in wide mouth conical flask maintained. Callus culture percentage be calculated used for every hormonal treatment by the following formula.

Callus culture (%) =

$$\frac{(Total\ no\ of\ explant\ with\ callus\ culture)}{Total\ no.\ of\ explants\ inoculated\ for\ each\ treatment} \times 100$$

2.3 Growth indexing of callus

Growth indexing was recorded by analyzing the fresh and dry weight of callus. Below mentioned formulae was used to calculate growth indexing of callus [13].

Growth index (GI) =wf-wo/wo

Where

W_F = final weight of callus

W_0= initial weight of callus

2.4 Tolerance index of callus culture:

Tolerance index of callus was calculated by mentioned formulae [14].

Tolerance index (%) =

$$\frac{Fresh\ weight\ of\ callus\ indication\ medium}{Fresh\ weight\ of\ callus\ hormones\ combination\ n} \times 100$$

3. RESULTS AND DISCUSSION

Experiment was carried out by 5 replicates with be repetition three. Visual explanations of the culture were recorded every week and the percentage of callus culture was calculated. MS medium supplemented with Naphthalene acetic acid (NAA) and 6-Benzyladenine purine (BAP) along or in combinations was use for callus culture. Detail of various hormonal combination with their concentration combination are given in table-1.

TABLE 1

VARIOUS HORMONAL COMBINATION TREATMENTS TESTED FOR CALLUS INDUCTION OF *ANNONA SQUAMOSA* L.

S. No.	Combination of hormones (mgL^{-1})	Callus culture (%)	Rate of callus growth
1.	MS+BAP 0.5	0%	-
2.	MS+BAP+NAA 0.5+0.5	20%	+
3.	MS+BAP+NAA 1+0.5	35%	+
4.	MS+BAP+NAA 2+1	40%	+
5.	MS+BAP+NAA 3+2	50%	++
6.	MS+BAP+NAA 3+3	56%	++
7.	MS+BAP+NAA 3+4	60%	+++
8.	MS+BAP+NAA 4+4	60%	+++
9.	MS+BAP+NAA 4+2	70%	+++
10.	MS+BAP+NAA 4+5	80%	++++

MS = Murashige and Skoog medium, BAP = 6-Benzylaminopurine, NAA = Naphthalene acetic acid

The callus growth rate was recorded per daybasis of callus culture and by comparison in four categorie because very good(++++),good (+++).poor (++) and very poor (+). The callus morphology such as color,texture etc. In the present investigation, fast initiation of callus be recorded on 25 day use MS medium with leaf as explants. The maximum callus induction percentage number (80%) [Fig.1. (a)] was recorded through leaf explants in case of hormones combinations (MS+4BAP+5NAA) followed by hormones combinations (3BAP+4NAA, 4BAP+4NAA, 4BAP+2NAA) with 60% [Fig.1 (b), (c), (d)] slow and good callus initiation other than it showed fast growth by profuse callus be transferred into the fresh media .

It has been also reported in privious studies that BAP + NAA gives the best results in callus induction [15-16].

Similar results were indentified with the same combinations of the effect of variou concentration and combination of plant growth regulator was tested on in vitro callus induction and indirect regeneration of *Mentha piperita* L. plants from young leaves and internodal explants [17]. A green, organogenic and nodular nature of callus was obtained only by leaf explants when grown on MS medium with 1.5 mg / L NAA + 0.2 mg / L BAP, followed by regeneration of the plant on an MS medium. 2.0 mg / L BAP + 0.5 will receive NAA mg / L by leaf callus of *Solanum Tuberosum* L. [18].

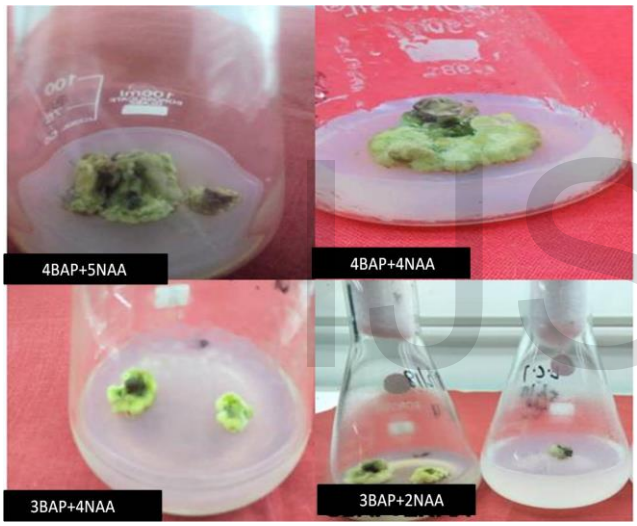

Fig. 1. Effect of culture media,growth regulators and explants on callus culture and growth:(a)Callus from leaf explants in MS+4BAP+5NAA, (b)callus growth in MS+4BAP+4NAA,(c) callus growth in MS+3BAP+4NAA,(d) callus growth in MS+3BAP+2NAA.

Overall, comparable results were indentified in *Brassica Juncea* (L.) with the same combinations of BAP + NAA [19-20], and MS medium containing 1.5 mg/L BAP+0.01mg/L of NAA in the same order related results were indentified in *Indigofera Zollingeriana* with the same combinations of BAP + NAA. [21-22] .

Similar results have been identified in *Annona squmos* the young leaf explants of *Cyclea peltata* cultur on MS medium supplement by different concentration of growth regulators showed of callus. [23] .

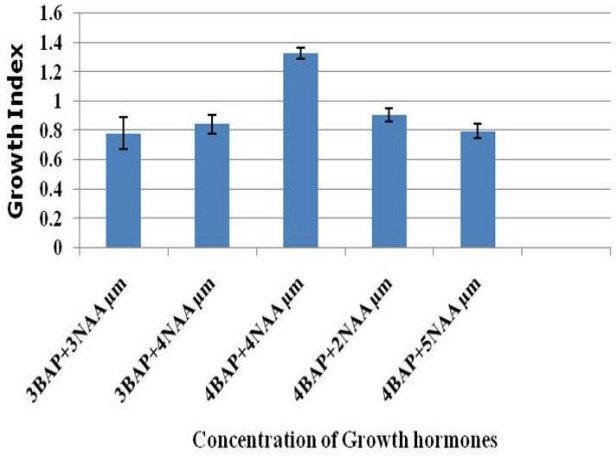

Fig. 2. Callus growth indexing in different concentration of growth hormones

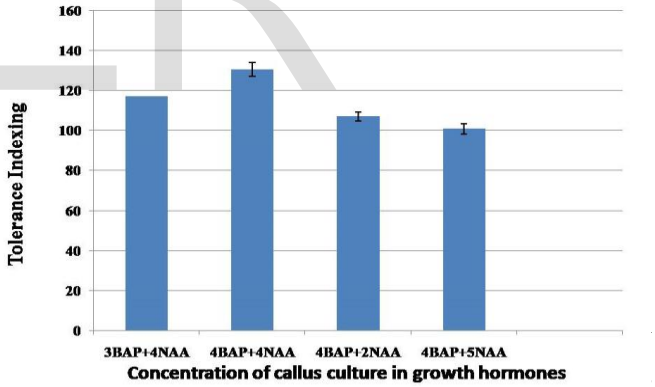

Fig. 3. Callus Tolerance indexing in different concentration of growth hormones

[illegible] reproducible protocol for induction of viable and good quality callus growth indexing percentage (Fig. 3). Related

results be reported in *Brassica carinata* with the same combinations of BAP + NAA [24-25] .

4. CONCLUSION

With this study, a standard with reproducible tissue culture protocol used for rapid induction of callus have been develope use leaf explant of *Annona squamosa*. Use of young fresh leaves on MS media supplemented by concentration of high auxin and low cytokinin be found most suitable for rapid callus culture in *Annona squamosa*. The hormonal combination of BAP+NAA in MS medium is recommended to obtain good quality callus mass with high production percentage. Maintenance of callus be observed in MS four morphologically different type of callus be observed as influenced by different hormonal combination and light condition callus was also derived. It was concluded that use of leaf explant and MS medium supplement by 4BAP and 5NAA and 4BAP+4NAA are most suitable and reproducible protocol for induction of viable and good quality callus of *Annona squamosa L.* The same could be utilized for organogenesis from this critically endangered but commercially important plant species.

ACKNOWLEDGEMENT

Authors are thankful to Hon'ble Chairperson JV'n Vidushi Garg & Hon'ble Founder and Advisor JV'n Dr. Panckaj Garg, Jayoti Vidyapeeth Women's University, Jaipur (Rajasthan) for their kind cooperation, encouragement and providing the facilities of University Innovation Center and other laboratories.

REFERENCES

[1] M. Leboeuf, A. Cavé, P. K. Bhaumik, B. Mukherjee, and R. Mukherjee, ''The Phytochemistry Of Annanaceae," Phytochemistry, vol. 21, pp. 2783-2813, 1980.

[2] A. Atique, M. Iqbal, Ghouse, and A K M., ''Use of *Annona Squamosa* and *Piper Nigrum* against Diabetes. '' *Fitoterapia*., vol. 56 (3), pp. 190-192, 1985.

[3] V. S. N. Rao, P. Dasaradhan, and K. S. Krishnaiah , ''Antifertility effect of some indigenous plants," Indian J. Med. Res., vol. 70, pp. 517-520, 1997.

[4] S. B. Vohora, K. Ishwar, and SA. Naqvi, ''Phytochemical, Pharmacological, Antibacterial and Anti-Ovulatory Studies On *Annona Squamosa,''* Planta Med., vol. 28, pp. 97-100, 1975.

[5] M. Kaleem, M. Asif, Q. Ahmed Qu, and B. Bano, ''Antidiabetic And Antioxidant Activity Of *Annona Squamosa* Extract In Streptozotocin induced Diabetic Rats," Singapore Med. J., vol. 47, no. 8, pp. 670-675, 2006.

[6] R.K. Gupta, AN. Kesari, PS. Murthy, R. Chandra, V. Tandon, and G.Watal , ''Hypoglycemic And Antidiabetic Effect Of Ethanolic Extract Of Leaves Of *AnnonaSquamosa L.* In Experimental Animals,'' J Ethanopharmacol ., vol. 99, no. 1, pp 75-51, 2005.

[7] DC. Hopp, FQ. Alali, ZM. Gu, and JL. Mclaughlin, ''Mono-THF Ring Annonaceous Acetogenins from *Annona Squamosa,* "Phytochemistry., vol. 47, no.5, pp. 803-9, 1998.

[8] D. Craig Hopp, Lu Zeng, Zhe-ming Gu , John F. Kozlowski , and Jerry L. McLaughlin, ''Novel Mono-Tetrahydrofuran Rin Acetogenins, From The Bark Of *Annona Squamosa*, Showing Cytotoxic Selectivities For The Human Pancreatic Carcinoma Cell Line," J. Nat. Prod., Vol. 60, no. 6, pp. 581-586, 1997.

[9] T.R. Seetharaman, ''Flavonoids From The Leaves Of *Annona Squamosa* And *Polyalthia Longifolia,''* Fitoterapia,. vol. 57, pp. 189-198, 1986.

[10] D. Bhakuni, S.Tewari, and M.M. Dhar, ''Aporphine Alkaloids Of *Annona Squamosa,*'' Phytochemistry., Vol. 11, pp. 1819-1822, 1972.

[12] S. Murashige, and F. Skoog, ''A revised medium for rapid growth and bioassays with tobacco tissue cultures,'' Physiol Plant, vol.15, pp. 473-497, 1962.

[13] H. Gregorio, and F.A. Vazquez, ''Plant Cell Culture Protocols 2nd Edition. In V. M. Loyola-Vargas & F. A. Vazquez'', Heidelberg, Springer verlag, Flota Ed. 51–58. 2006.

[14] D. Wilkins, ''the Measurement of Tolerance to Edaphic Factors by Means of Root Growth,'' New Phytologist, Vol.80, no.3, pp. 623-633, 1987.

[15] A. Shafiq, and S. William, ''Effects of NAA and BAP on Callus Culture and Plant Regeneration in Curly Kale (*Brassica Oleraces*L.),'' PJBS, vol. 2, no. 1, pp. 109-112, 1999.

[16] S. Moitreyee, S. Karuna, and S. Sureshkumar Singh, ''Effect Of Culture Media And Growth Hormones On Callus Induction In Aquilaria Malaccensis Lam A Medicinally And Commercially Important Tree Species Of North East India,'' Asian J. Biol. Sci., vol. 6, no.2, pp. 96-105, Issn 1996-3351, 2013.

[17] A. Islam, and M.F. Alam, ''*In Vitro* Callus Induction and Indirect Organogenesis Of *Mentha Piperita* (L.) - An Aromatic Medicinal Plant,'' GSC biological and pharmaceutical sciences., Vol. 04, no.03, pp. 049–060, 2018.

[18] M.K. Ahmet and E. Sezai, '' Callus Induction, Shoot Proliferation and Root Regeneration of Potato (*Solanum Tuberosum* L.) Stem Node and Leaf Explants under Long-Day Conditions,'' Biotechnol Biotec Eq., vol. 29, no. 6, pp. 1075-1084, 2015.

[19] K.K. Sharma, S. Bhojwani and T. Thorpe, ''Factors Affecting High Frequency Differentiation Of Shoots And Roots From Cotyledon Explants Of *Brassica Juncea* (L.) Czern,'' Plant Sci., vol. 66, pp. 247-253, 1990.

[20] H. Mohsen, D. Mohammad, N.Mohsen, Yoosefzadeh, and A. Milad, '' Effect of Plant Growth Regulators on Indirect Shoot Organogenesis of Ficus religiosa Through Seedling Derived Petiole Segments,''Journal of Genetic Engineering and Biotechnology, Vol. 16, pp. 175–180, 2018.

[21] A. Çiğdem, M. Siti, "Multiple Shoot Regeneration of An Important Equatorial Forage Plant Indigofera Zollingeriana Miq.," Ksü Tarim Ve Doğa Derg, vol. 2, no. 5, pp. 672-677, 2018.

[22] H. Mohsen, H.D. Mohammad, ''Indirect Organogenesis through Seedling-Derived Leaf Segments Of Ficus Religiosa - A Multipurpose Woody Medicinal Plant, '' J. Crop Sci. Biotech., vol. 21, no. 2, pp. 129 – 136, 2018.

[23] F. Hook, N. Thoms, K.R. Bhagya and R. Chandrashekar, "Effect of Growth Regulators on Callus Induction from *Cyclea Peltata* (Lam.),''Asian J Pharm Clin Res., Vol 6, Issue 4, pp. 85-88, 2013.

[24] U.S. Rizwan, and M. Iqbal, ''Efficient in Vitro Plant Regeneration through Somatic Embryogenesis from Callus Induction Method For *Brassica Carinata,*" Sarhad journal of agriculture, Vol. 35, Issue 1, Page 314, 2019.

[25] S. Tebogo, W. Remy Kasili and G. Edward, ''Pomegranate Micropropagation, Callogenesis And Genetic Integrity Assessment Using Simple Sequence Repeat Markers,'' Journal of Agricultural Science, vol. 11, no. Issn 1916-9752, E-Issn 1916-9760, 2019.