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Effective Method for *In-vitro* Micropropagation of *Solanum xanthocarpum* Schard and Wendl from Seeds and Inter-nodal Explants

(Subtitle: Invitro Micropropagation of *Solanum xanthocarpum* Schard and Wendl)

Asha Poorna.C[4*]
drpoorna2015@gmail.com
[4*]Biotechnologist, Department of Dravyaguna
Government Ayurveda College
Trivandrum-695001
Kerala, India

Dr. Jollykutty Eapen[1], Dr. A. Shahul Hameed[2],
Dr. M.S. Deepa[3] and Dr. V.C. Indulekha[3]
[1]Principal and Professor, [2]HOD and Professor,
[3]Associate Professors, Department of Dravyaguna,
Government Ayurveda College
Trivandrum-695001, Kerala, India

Abstract:- This study was aimed for the development of callus and multiple shoots from germinating seeds and inter-nodal buds of *Solanum xanthocarpum*. The experimental result shows a good response of this plant to ½ Murashige and Skoog composition than to full strength medium. The medium was supplemented with different concentrations of 6- Benzyl amino purine, Indol Acetic Acid and naphthalene acetic acid separately and in combination. By increasing the concentration of growth regulator auxin Indol Acetic Acid and naphthalene acetic acid with a high concentration of cytokine 6- Benzyl amino purine resulted in the formation of callus alone which was whitish or brownish friable callus. The addition of 6-Benzyl amino purine alone in low concentration leads to the initiation of green compact callus. Shoot formation can be commenced by reducing the concentration of BAP and its optimum was observed as 0.5 mg/L, without any other growth regulators. Germinated seeds also observed to be very efficient in the production of green compact callus at this concentration. These calluses were cut and placed in a medium with the same concentration to induce shoot formation. The maximum number of multiple shoots (9-11 plantlets) was callus formed from seeds, than nodal explant resulting callus. Regenerated shoots were subjected to initiation of rooting in MS medium without growth regulators and successfully hardened and transferred to the greenhouse and later to the garden.

Keywords:- *Solanum xanthocarpum, Micropropagation, Regeneration, Callus, Shoot Formation.*

I. INTRODUCTION

Solanum xanthocarpum Schard and Wendl belong to family Solanaceae and it is also known as Yellow berried nightshade. This herbaceous plant is commonly known as Nidigandhika and Kantakari in Sanskrit, Kantakari in Bengali, Kandankattiri in Tamil, and Kandankathirichunda in Malayalam. It is an important medicinal plant in Ayurvedic point of view. In Materia Medica *Solanum xanthocarpum* is having an important place as expectorant and antipyretic. It is one of the members of Dasamula of Ayurveda and all part of this herb is used in different Ayurvedic preparations (Mohan et. al., 2007). It is mainly used for lung diseases, fever, asthma, TB and kidney disorders (Rita and Animesh 2011). In Ayurveda this plant is defined as pungent, bitter, gastrointestinal, substitute astringent and root decoration is generally used as febrifuge, effective diuretic and expectorant. The entire plant and fruits are used by Acharyas like Charaka and Sushrutha for the internal treatment of tympanitis, bronchial asthma, piles, misperistalsis, and dysuria and rejuvenation (Kumar 2017). In Charaka Samhita Kantkari Ghrita is indicated for cough and asthma (Sharma P.V, 1983). The plant is known to have pest repellent property and used as molluscicide (Changbunjong et. al., 2010). Commonly people use the fruits for treating throat infection and other inflammatory problems because it is used as folklore medicine and the *khodh* tribes of Orissa use the decoction from fruit for the treatment of Diabetics Mellitus (Kumar and Pandey 2014, Vadnere et. al., 2008). There are also reports related to the use of this plant for wound healing (Kumar et. al., 2010).

Genus *Solanum* consists of about 1500 species and is found all around the world. *S. xanthocarpum* is very much fond of the hot dry climate and grows in the temperate zone. In India it is mostly found in Bihar, Punjab, UP, Uttaranchal, West Bengal, Assam, and the other North Eastern States. It is observed that the recent changes in climatic condition of Kerala have very much affected the growth and survival of this plant so it is considered as threated species. *S. xanthocarpum* is a diffuse annual herb of height 1.2 meter that spread close to the ground with zig-zag stems full of prickles. They have numerous branches and the young ones covered with dense stellate tomentum, compressed prickles, straight. The matured branches are zigzag and covered with yellow shining prickles. Leaves are 5 to 10 and are ovate or elliptic (2.5 to 5.6cm) sub pinnate or sinuate, obtuse or sub-acute, stellately hairy on both sides and petiole is full of prickles. Leaves become nearly glabrous on aging and armed with long sharp yellow prickles on the nerves and midrib. Flowers are extra axillary

with few-flowered cymes and are small with purple or violet corolla, deltoid lobes, and hairy outside. The berries are 1.3-2.0cm in dia, premature berries have white with green veins and turn yellow when ripe, surrounded by enlarged calyx.

Triterpenoids and phytosterols are separated from the methanol extract from different parts of the plant using HPTLC (Preet and Gupta 2018). The species mainly contain steroidal alkaloids, solasonine and solamargine and solasodine serve as an important intermediate in the synthesis of steroidal hormones. Solasodine is present in large quantities in fruits. The other main constituents present in fruit are solasonine, solasmargrine, β-solamargine, solanocarpidine. Dry fruits contain traces of caffeic acids, isochronogenic, neochronogenic and chromogenic (Arora and Ansari 2019, Reddy and Reddy 2014). In the traditional system of medicine *S. xanthocarpum* have high potential, but less exploited in the modern system of medicine due to lack of experimental research. Researcher, when correlated with traditional knowledge with a modern experimental methodology for testing efficiency of drug and safe use of the herbal drug, will bring out the potentiality of this plant to the field of medicine. Tissue culturing technique is used for the mass production and propagation of plants which help in the conservation and protection of endangered species. Techniques in Biotechnology can be used for the improvement of the yield of secondary metabolites and their medicinal application. Overexploitation due to high medicinal value and damage of the habitat this plant is being considered as endangered and so it needs ex-situ conservation through tissue culture.

II. MATERIAL AND METHODS

A. Sterilization of Media

The chromic acid solution was used to wash all glassware before the experiment and it prepared from potassium dichromate and sulphuric acid in the ration 2:1 w/v. All other equipments like forceps, glasswares, and scalpels used in this experiment were autoclaved for sterilization. MS (Musarhige and Skoog, 1962) medium supplement by HiMedia India (PT101) was used throughout the study along with growth hormones of different concentrations provided by HiMedia. BAP was used for shoot multiplication, NAA and IAA used as a rooting hormone. The pH of the medium was adjusted to 5.8 with 1N NaOH or 1N HCl before autoclaving.

B. Sterilization of Explant

The explants of *S. xanthocarpum* were collected from the Medicinal garden of Govt. Ayurveda College Trivandrum and seeds from a vendor nearby. The nodal explant with axillary bud and seeds were materials surface sterilized with 70% ethanol for 1min and washed thoroughly in running tap water for 30 min. This was followed by washing with tween 20 for 3min and rinsed in distilled water and it was again treated with hypochlorite (10%) for 2min. The plant material was washed in running water and then treated with 0.1% $HgCl_2$ for 2min (treatment depends on the morphological property of the explant) and then washed 5times in sterile distilled water under aseptic condition.

C. Multiple Shoot Propagation

Multiple shoots can be induced by inoculating the nodal explant with the axillary bud in ½ MS medium supplemented with different concentrations (0.5-3µM) of growth hormones 6-benzyl amino purine (6-BAP). Shoot propagated from callus of the length of about 1.5 to 2cm were subcultured into ½ MS supplemented with auxin for root propagation – indole acetic acid (IAA- 1 to 3µM). All cultures were maintained at 25ºC+/-2 with a photoperiod of 16hours. Cultures that develop a root of length more than 1cm were dislodged from the culture medium and washed gently under running water to remove the attached medium to roots. The plants then planted in cups with autoclaved garden soil and supplemented with farmyard manure and sand and kept in the greenhouse for 2weeks.

III. RESULTS AND DISCUSSION

Seeds of *S. xanthocarpum* were kept for germination in cotton, soaked with sterile distilled water under aseptic conditions. They were also cultured in MS medium. It was observed that seeds kept on cotton soaked in water have germinated faster than that kept in MS medium. In cotton soaked in water, the seed has taken only about 5 days to germinate, while in the medium it has taken 15days to germinate. The plant shows slow response in full strength MS medium after 3week of inoculation however the response was fast in the half-strength medium. So for further studies, half strength medium was preferred. The seeds and inter-nodal stem cutting were planted on MS and ½ MS medium supplemented with different concentrations BAP (0.5-3mg/L) and combination of BAP with NAA (0.5, 1 and 2 mg/L) was done. It was observed that stem cutting with inter-nodal buds can be used for the effective propagation of these plants. *S. xanthocarpum* prefers to grow in ½ MS than full strength MS medium. Low concentrations of growth regulators are required for the production of the shoot. The calluses formed are mostly creamy, yellow and white which are friable in the high concentration of BAP supplemented ½ MS medium (Table 1.). Initiation of callus from cut end of explant due to mitosis of cells of that region, and it might be due to wound reaction or effect of exogenous growth regulators. The nature and texture of callus varies related to the cytokine and auxin : cytokinin ratio used in the medium.

When BAP in combination with NAA supplemented in ½ MS medium, resulted in yellow, white and creamy and brown compact or friable callus. Friable callus falls apart easily and can be used to generate cell suspension only. Seeds are cultured in MS medium without growth supplement (Fig.1A and B) and seed germinated after 15days of incubation at 23ºC. The plantlets produced were sub-cultured into ½ MS medium supplemented with a low concentration of BAP (0.5mg/L) and shown in Fig.1C. A low concentration of BAP (0.5mg/L) has promoted the production of compact yellow callus which turns green after 3weeks (Fig.1C). This compact green callus when sub-cultured in fresh ½ MS resulted in the development of shoot (Fig.1D), then the combination with BAP and NAA.

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S. No	Conc. of BAP mg/L	% of callus responding	Callus Morphology	Conc. of BAP+NAA mg/L	% of Callus Responding	Callus Morphology
1	0.5	100	Light green compact	0.5+0.5	90	Yellow friable
2	1	90	White friable	1+1	90	White friable
3	2	80	Creamy friable	2+2	90	Creamy compact
4	3	90	Brown compact	3+3	90	Brown compact

Table 1:- Effect of Growth Hormone on Callus Formation in Inter-nodal Explant of *Solanum xanthocarpum*

About 9-10 shoots were produced from one callus. Creamy white callus formation observed in medium supplemented with a high concentration of growth hormone BAP (0.5mg/L) and NAA (1mg/L) (Fig. 2.). The plantlets stated producing callus after 22- 25 days of incubation in ½ MS medium. The white and creamy friable callus has increased in biomass and remained the same for a long period while white and creamy compact callus has changed to light brown and latter dark brown after 90-95 days of incubation. Single strength MS medium supplemented with a low concentration of BAP at the beginning of short multiplication was observed to be very much effective and ½ MS medium free of growth regulators are observed to be more effective in root induction. Recently isolated callus results in great morphogenesis response for induction of shoots than aged ones.

The direct revival of the plant was also identified without the intervening callus phase (Fig. 3). A dark pretreatment of shoot culture for a few days observed to be effective for the production of viable protoplast. Production of protoplast from seeds grown plants also observed to produce plantlets from green compact callus which latter developed to new plants. Direct regeneration of plant found to be more effective and economic in propagation of this plant as it take only 45-50 days for the production of new plantlets.

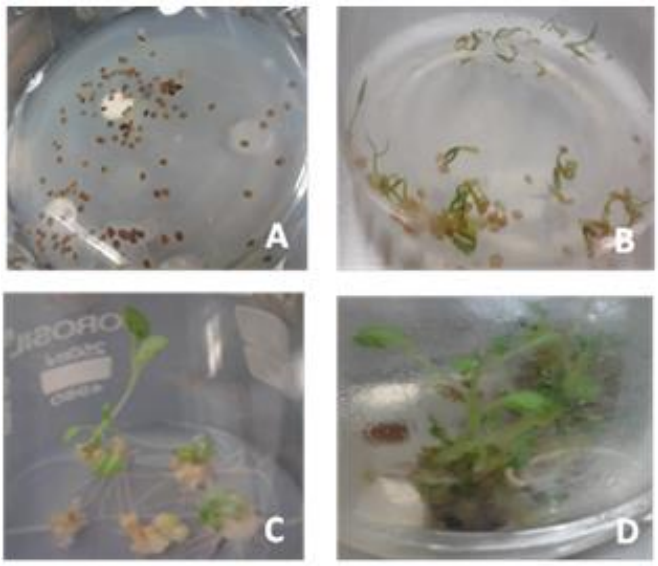

Fig 1:- A. Seeds cultured in MS medium without any growth hormone and germination after 15 days of incubation. B. Plantlets germinated form seeds after 15days of incubation at 25ºC with 16/8h photoperiod. C. Plantlets from germinated seeds cultured in ½ MS medium supplemented with 0.5 mg/L BAP which promotes callus production. D. Development of shoots from subculture compact green callus in ½ MS supplemented with 0.5 mg/L BAP.

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Fig 2:- Callus produced from inter-nodal stem cutting in in MS medium supplemented with 0.5 mg/L BAP and 2 mg/L NAA

Direct regeneration of plantlet from explant was possible in *S. xanthocarpum* and it was reported after explants taken from ie. hypocotyl, cotyledon and leaf of *Solanum surattense* explained (Ramaswamy et. al., 2000). The results indicate that the presence of auxins in medium plays a vital role in the initiation of callus on stem and root while cytokinins requirement was very low. Callus initiation using IAA and NAA in low concentration in MS medium was explained (Sundar and Jawahar 2011) in their observation high concentration of growth regulators reduces callus proliferation. Cytokinins in low concentration were observed to be suitable for induction of organogenic callus formation when compared to auxin used in both explants were reported in the literature. Callus formation was one of the most important steps during meristem culturing (Uddin et.al. 2004). These cells are made of unorganized parenchymatous tissue formed by vigorous mitotic division of explant and have no polarity.

Callus induction after 20-25 days was reported in *S. xanthocarpum* in MS medium supplemented with 11.4 µM/L of IAA and 10.7µM/L of NAA and 8.8µM/L of BAP (Sundar and Jawahar 2011). *Solanum hainanace* has reported producing maximum shoot and shoot elongation in MS medium containing BAP and shoot and regeneration in MS medium with IBA, kinetin and BAP (Loc and Kiet 2011). *Solanum nigrum* (L) leaf as explant, when propagated in MS, has produced the maximum number of shoots (Bhat et. al., 2010). *S. xanthocarpum* nodal explant with meristematic axillary bud was cultured ½ MS medium supplemented with IBA (0.25mg/L) and FAP (0.5mg/L) together with activated charcoal (1%) for hairy root induction by inducing *Agrobacterium rhizogenes* in explant by stabbing method (Khatodia et. al., 2013).

We depend on medicinal plants for their secondary metabolites or phytochemicals and they possess a wide range of these bioactive compounds in varying proportions. Of these alkaloids from plants are the largest groups of phytochemicals that are reported to have remarkable use to human beings. Solasodine a specific alkaloid isolated from various plants in the *Solanaceae* family reported various pharmacological activities (Kaunda and Zhang 2019). Related to in vitro micropropagation studies there are a few reports related to the production of *S. xanthocarpum.* Mesophyll protoplast of *S. xanthocarpum* used as explant in MS medium supplemented with different concentrations of 2,4-D (2,4- dichloro phenoxy acetic acid) naphthalene acetic acid, kinetin and organic acids for callus induction (Praveen et. al., 1982). Dark pretreatment for 7days observed to be effective for production of viable protoplast and it was reported that yield of protoplast from seed grown plants were poor and they divide occasionally to produce 2-4 callus and they did not grow further (Praveen et. al., 1982).

MS medium supplemented with 2, 4-D alone produced brown callus and brown colour of callus show sensitivity of plant tissue to 2, 4 –D as in *S. nigrum* (Sridhar and Naidu 2011). According to their report the colour of callus was mainly due to the location of phenolic secondary metabolic in cells. If the accumulation of the phenolics was in the cytoplasm it undergoes oxidation and polymerization and oxidized product appears brown. 2,4-D in combination with BA was reported as potent hormonel combination for stimulating green callus induction. Plant propagation mainly occurs through callus generated from organogenic callus and it was produced from explants. So the sources of explant and its physiological state are very important and critical factor for organogenic callus induction. (Hams, et. al., 1983).

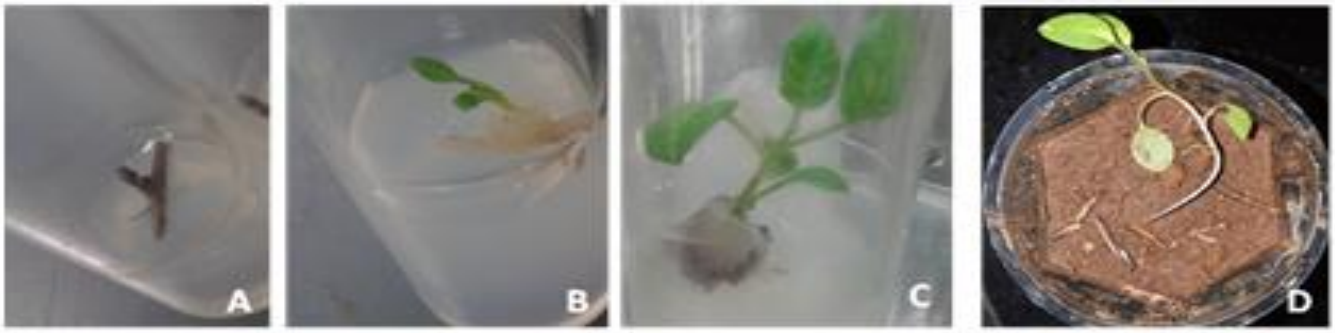

Fig 3:- A. Direct regeneration of *S. xanthocarpum* from stem inter-nodal explant in ½ MS medium supplemented with 0.5µM BAP. B. Plantlet produced from inter-nodal bud in ½ MS supplemented with 0.5µM BAP after 18days. C. Plantlet produced from inter-nodal bud transferred to ½ MS supplemented with 1 mg/L NAA for rooting. D. Mature plant developed in ½ MS supplemented with 0.5 mg/L BAP and 1mg/L NAA hormone for root propagation, after 45 days planted in soil mixed with manure.

Callus induction was required for the adventious improvement and induction of somaclonal variations and embryoids. BAP alone supplement medium shown high frequency of shoot production in *S. nigrum* (Sridhar and Naidu 2011).

There are reports related to the use of seeds for the propagation of plants (Ashakiran et. al., 2013). In this study the hypocotyls, inter-nodal and nodal segments were also used as explants and they were cultured in MS medium supplemented with BAP (0.75mg/L) and kinetin (0.25mg/L) reported to be effective in induction of shoot from callus and elongation of shoot observed to be high at 0.5 mg/L BAP. A similar result was observed in our study where it was higher in ½ MS-related to full strength MS medium. Immense exploitation of this plant for medicinal uses would have resulted in the disappearance of this plant from different parts of Kerala so there is a need to conserve the germplasm through micropropagation which is very much in need during these days.

Rooting is the most important step in vitro plant regeneration before the acclimation stage and the presence of auxins influences root production. In this study, the auxins used are NAA and IAA (0.5, 1 and 2mg/L) concentration. The presence of NAA in 1mM/L concentration observed to be very effective in root initiation. *S. surattenes* root incitation was observed in MS medium supplemented with 0.4mg/L of IBA combined with 20% TBE with maximum 6.2 numbers of roots of 5.8cm in 4 weeks (Gurusaravanan et. al., 2017)

Micropropagation and in vitro adventitious root development are main two concepts of tissue culture. Of which *in vitro* adventitious root development is very much acceptable by Ayurveda industries related to the increasing demand for high-quality raw material and decreasing sources of medicinal plants. In the Ayurvedic concept "*Anukta dravya grahana*" that defines the importance to recognize the properties of an undocumented drug or medicinal plant before using it as an Ayurvedic drug (Kusuma and Joshi 2010, Ashakiran 2013, Kumawat and Dasari 2018). The efficiency of tissue culture plant or plant part also has to be determined even if they are genetically and botanically identical and the Rasanirdharana method can be combined efficiently with phytochemical screening (Dhyani 2008).

IV. CONCLUSION

In conclusion, it was observed that direct regeneration of plant was more effective than any other method and it was observed to be more economical. The protocol developed for the propagation of *S. xanthocarpum* can be used for the propagation of this medicinal plant on large scale as well as for the conservation of this plant. Further research related to the estimation of solasodine content in callus needed to find out whether the callus produced can be used as a substitute for the plant for this compound.

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