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Effect of Growth regulators on *in vitro* shooting and rooting of *Solanum xanthocarpum* Schradt. & Wendl. and its Micropropagation for future medicinal applications

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Abstract

The medicinal plant *Solanum xanthocarpum* Schradt. & Wendl. belong to family Solanaceae, which is a perennial, prostrate, ramose and prickly plant with green and white fruits but yellowish at maturity. The fruits are known for several medicinal uses like anthelmintic, anti pyretic, laxative, anti-inflammatory and anti-asthmatic. This present investigation aimed at developing rapid micro propagation protocol, which can be used for conservation and mass multiplication of valuable medicinal plant of *Solanum xanthocarpum* to meet our pharmaceutical demand and its conservation. Optimization was made to evolove a standardized *in vitro* technology to conserve, as well as mass propagate this valuable medicinal herb in maximum number. A concentration of 1mg/l benzylaminopurine (BAP) showed highest shoot length, whereas maximum number of shoots per explants were obtained at 3mg/l concentration of BAP. Callus formation initiated at 5mg/l to 8mg/l of BAP. Shoot multiplication followed by subculturing which were performed in every 20 days. Rooting was optimized with different concentrations (0.25mg/l, 05mg/l, 1.0mg/l and 2.0mg/l) of auxin *i.e.* indole-3-butyric acid (IBA). Highest root length and root numbers were obtained at 1.0mg/l(IBA). Primary hardening was done at temperature 25-30^{o}C, humidity 80-90. 70% of survivability was obtained after primary hardening. After secondary hardening 90% survivability were obtained in shade house. After secondary hardening plants were finally transferred into field where 100% survivability was obtained. This standardized micropropagation procedure could be useful for mass multiplication of superior plant material for field cultivation.

Solanum xanthocarpum Schradt. & Wendl. also called as *S. surattense* nightshade, yellow fruit nightshade, yellow berried nightshade, Thai green eggplant, Thai striped eggplant (from the unripe fruit), is a species of *nightshade* native to Asia (Saudi Arabia, Yemen, Afghanistan, Iran, China, Bangladesh, India, Nepal, Pakistan, Sri Lanka, Myanmar, Thailand, Vietnam, Indonesia, Malaysia), and is *adventive* in Egypt. India with its mega-biodiversity and knowledge of rich ancient traditional systems of medicine (Ayurveda, Siddha, Unani, Amchi and local health traditions) provide a strong base for the utilization of a large number of plants in general healthcare and alleviation of common ailments of the people[10]. *Solanum xanthocarpum*, is an annual herbaceous plant. In India, it is mainly grown in Uttar Pradesh, Bihar, Uttaranchal, Punjab, West Bengal, Assam and other North-Eastern States. It grows on all kinds of soil but does well on dry and hot temperate regions. It is a very prickly, diffuse, bright green perennial herb, stems zigzag; prickles compressed, straight, yellow and shining; leaves 5-10 by 2.5-5.7 cm ovate or elliptic, sinuate or sub pinnatified, hairy on both sides, petiole prickly. Flowers are small, in extra- axilliary few flowered cymes. Corolla is purple, lobes deltoid, hairy outside. Fruits are 1.3cm diameter, yellow or white with green veins, surrounded by enlarged calyx[7].

Steroidal alkaloid solasodine is the principal alkaloid present in fruit. Chemical constituents present in fruit are solasonine, solasodine, solamargine, beta-solamargine, solanocarpine and solanocarpidine. Dry fruits contain traces of isochronogenic, neochronogenic, chronogenic and caffeic acids. Petals yielded apigenin, stamens gave quercetin diglycoside and sitosterol. Steroidal constituents namely cycloartanol, cycloartenol, sistosterol, stigmasterol, campesterol, cholesterol, sitosteryl glucoside, stigmasteryl glucoside, solamargine and beta-solamargine from fruit extracts[10]. The whole plant is used traditionally for curing various ailments. Decoction of the plant is used in gonorrhea, paste of leaves is applied to relieve pains, seeds act as expectorant and diuretic, useful in the treatment of catarrhal fever, coughs, asthma and chest pain[11].

Tissue culture is the culture and maintenance of plant cells or organs in sterile, nutritionally and environmentally supportive conditions (*in vitro*). In commercial settings, tissue culture is primarily used for plant propagation and is often referred to as Micropropagation.

Solanum xanthocarpum is a well known medicinal plant in traditional medicinal system and recent scientific studies have emphasized the possible use of *Solanum xanthocarpum* in modern medicine. The present research aims to enhance the production of potential phytoconstituents and propagation of plant by *in vitro* tissue culture method.

Explant collection, sterilization and media preparation:

The plantlets were collected from three different agro climatic regions viz. Raipur, Jagdalpur and Sarguja of Chhattisgarh, India. Plants were identified morphologically according to method of Gostin, (2011). The explants *i.e.* auxilary buds and apical buds were washed thoroughly under running tap water for 10 min followed by treatment with solution

of 0.1% bavistine with 500mg/l of ampicillin solution for 20 min and there after washed thoroughly in sterile water for 3-4 times for 15min to completely remove the fungicide. The explants were then transferred to sterile hood and surface disinfected with 0.1% (w/v) $HgCl_2$ for different time intervals (5-20min) and finally rinsed with sterile distilled water for 3-4 times.

Culture media and culture conditions:

The surface sterilized explants were inoculated on Murashige and Skoog (MS) medium containing all the macro and micro nutrients with 0.7% agar and 3% sucrose. The pH of all media were adjusted to 5.6-5.8. Culture media were autoclaved at 15/psi pressure for 121 degree Celsius for 15-20min. All the cultures were incubated at 25±2^{o}C with 16 hours photoperiod.

Shoot initiation and multiplication:

For multiple shoot induction, the nodal explants with axillary and apical buds were placed on MS medium which was fortified with auxin and cytokinins. MS medium is fortified with various concentrations of cytokinins such as benzylaminopurine (BAP:1.0 mg/l – 8mg/l) were investigated to optimize hormonal requirements for shoot length initiation and multiple shoot induction from auxiliary and apical buds explants. Shoot initiation and multiplication was followed by subculturing of shoot in jars containing MS media with various concentrations of cytokinin (BAP) *i.e.* 0.5mg/ l – 4mg/l. Shoots were sub cultured in every 20 days interval and continued from I to VI stages.

Rooting of multiplied shoots:

After completion of 6th subculture multiplied shoots were inoculated in MS medium containing various concentration of root inducing hormone auxin *i.e.* indole butyric acid (IBA: 0.25 – 2.0 mg/l). All cultures were maintained at 25±2^{o}C in a culture room under cool white fluorescent lamps at intensity of 50µmol m^{-2} s^{-2} with 16 hrs photoperiod. Root length and plant height were measured.

Hardening and acclimatization:

Plantlets with well developed roots were dislodged from the culture medium and washed gently under running tap water to remove the adhering medium. For primary hardening plantlets were transferred to green house in a vermiculite tray containing sand dust, coccopeat and manure where temperature and humidity were maintained as 25-30^{o}C and 80-90% respectively.

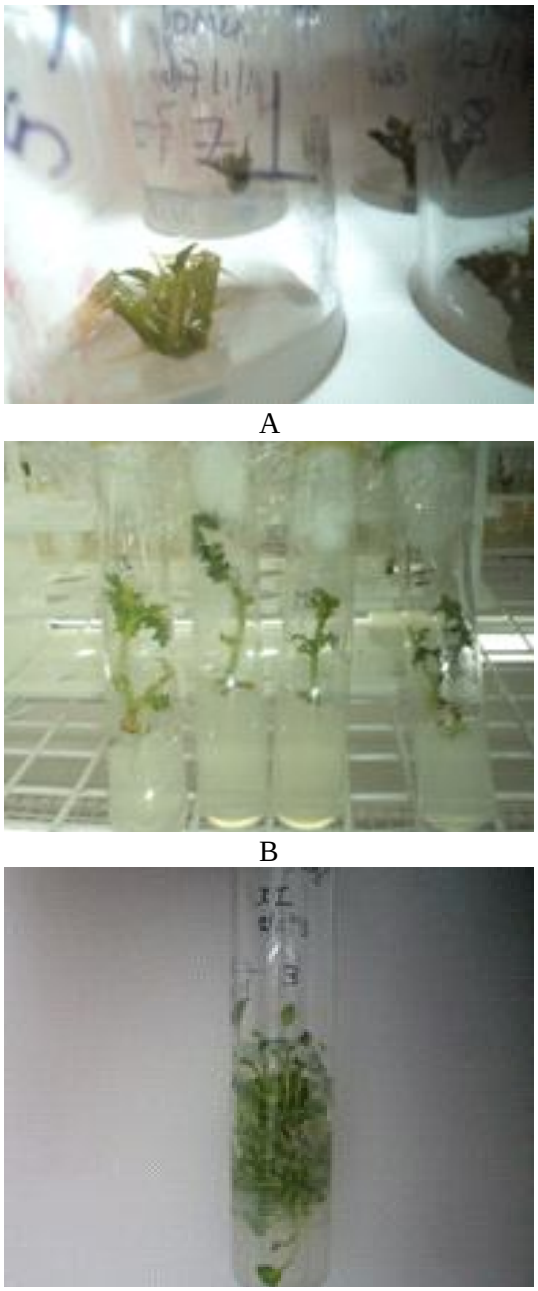
For secondary hardening plantlets were shifted into shade house in plastic bags (10cm diameter) containing garden soil, farmyard manure and healthy leaves and flowers developed after 30 days of transplantation. The plantlets were transferred to field after removing the plastic bags where they grew normally.

The maintenance of living material by traditional method is expensive, laborious and risky. Micropropagation through tissue culture offers an alternative to vegetative practices used in the past and has the potential to providing high multiplication of uniform genotypes, resulting in short term gains[12]. Important secondary metabolites are present in *Solanum xanthocarpum*. Axillary and

apical bud culture could be a valuable techinique for the production of secondary metabolites in large scale.

In the present study the apical and axillary buds were taken as explants and surface sterilization was standardized with two different concentration of $HgCl_2$ *i.e.* 0.1% to 0.2% for various time periods (5,10,15 and 20 min). Highest number of shoots was initiated with 0.1% $HgCl_2$ along with tween eighty for 15min treatment. 16hr light and 8hr dark incubation period and 2000-3000 lux light intensity 25^oC temperature were found as best condtions for shoot initiation. Similar result had been reported that *Solanum nigrum* shoot tip and nodal culture were shriveled within 3 weeks after emergence of 2-3 leaves without forming the multiple shoot (fig 1, A). Shoot multiplication was observed on MS medium supplemented with the various concentration of BAP *i.e.* 0.5mg/l, 1.0mg/l, 2.0mg/l, 3.0mg/l, 4.0mg/l, 5.0mg/l, 6.0mg/l, 7.0mg/l and 8.0mg/l. For initiation of shoot length explants were inoculated in MS medium containing various concentration of cytokinin (BAP) (fig 1, B).

Among these different concentrations, the rate of response of explants to shoot induction and elongation was found to be higher at 1.0mg/l, maximum shoot length were found in 1 mg/l BAP and maximum number of shoot were observed in 3.0mg/l concentration. With increasing the concentration of BAP, the average height of shoots was decreasing and average number of shoots was increasing (fig 1, C). But further increasing the concentration of BAP from 5.0mg/l to 8.0mg/l, callus



A

B

C

Fig. 1: A) Bud breaking from nodal explants. B) Shoot initiation from explants, C) Shoot multiplication from individual explant

formation was started. MS medium without BAP supplementation was maintained as control. It has been reported that multiple shoot induction and elongation of *S. xanthocarpum* was noted higher at 0.5mg/l BAP[1]. Similar results were obtained in *S. villosum* micropropagation study described the same concentrations as useful for direct shoot bud regeneration[4].

Multiplied shoots were subcultured after every 20days in MS containing BAP concentrations (0.5, 1, 2, 3 and 4mg/l). Subculturing was continued up to I to VI stages along with 10 replicates where number of shoots and shoot elongation were observed (Table 1). 1.0mg/l concentration of BAP was found to shows the maximum mean shoot length of 5.0 cm ,whereas the maximum mean number of shoot which is 17.68 was shown by 3mg/l(BAP) (fig 2). Similar findings have been reported that 2.0mg/l BAP with 1.0mg/l IBA were the best concentration for generation of maximum number of shoot buds (9.6)[5].

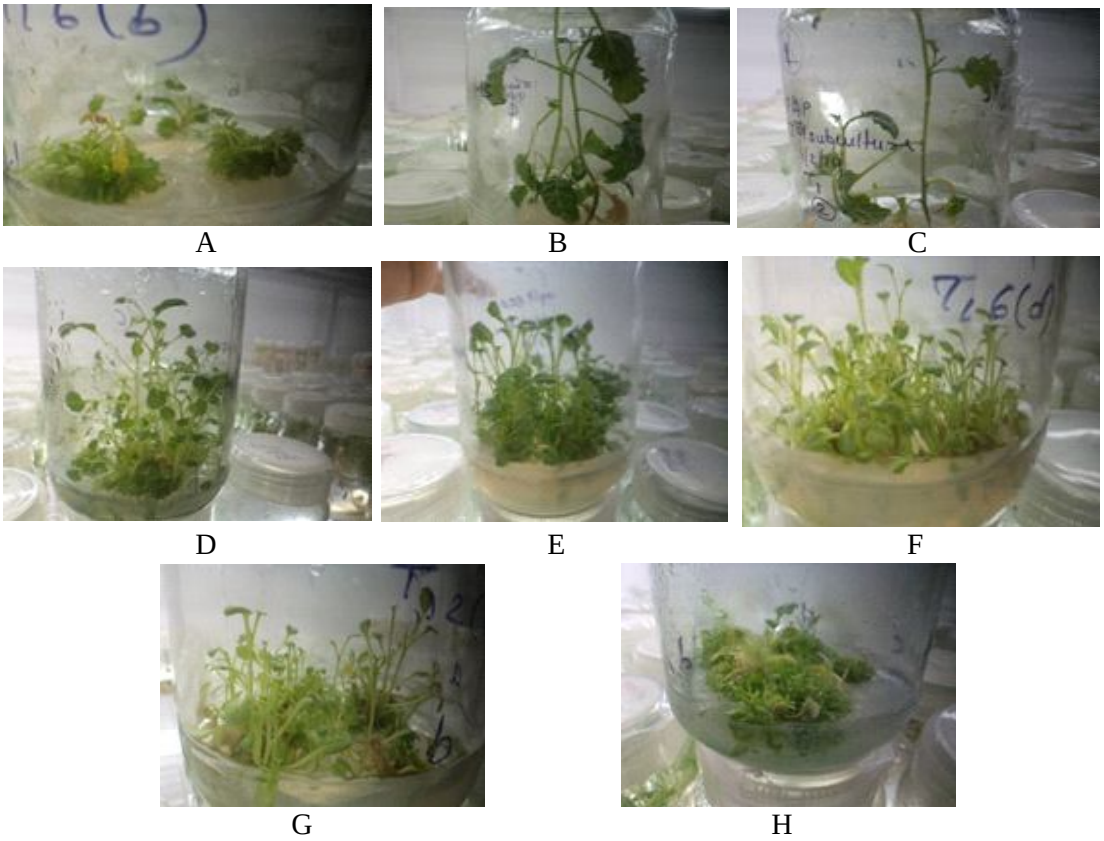


Fig 2: (A) Subculturing of shoots in a jar (B) Subculturing of shoots in MS media (C) Subculturing of shoots in 0.5mg/l BAP (D) initiation of shoot length and multiplication in 1.0 mg/l BAP (E) Subculturing of shoots in 2.0mg/l BAP (F) Subculturing of shoots in 3.0mg/l BAP (G) Subculturing of shoots in 4.0 mg/l BAP (H) Subculturing of shoots in 5.0mg/l BAP.

Table-1: Response of explants of *Solanum xanthocarpum* on MS basal medium supplemented with different concentrations of BAP.

MS + BAP mg/l	Mean number of shoots/ Explants+SD			Mean shoot length (cm)+SD		
	1st week	2nd week	3rd week	1st week	2nd week	3rd week
0.5	2.13±0.56	5.35±1.4	5.82±2.15	1.56±0.4	2.68±0.4	3.1±0.7
1.0	3.62±0.86	4.42±0.82	9.0±1.15	1.39±0.48	1.81±0.4	5.0±0.74
2.0	4.32±1.21	7.82±2.0	11.8±1.48	1.47±0.16	1.63±0.2	3.22±0.65
3.0	2.4±0.75	4.92±1.51	17.68±5.21	0.85±0.42	1.53±0.29	2.44±0.37
4.0	2.9±1.23	3.37±1.38	9.99±5.85	1.25±0.34	1.76±0.28	2.6±0.51

Excised shoots were transferred to rooting medium containing different concentration of auxin *i.e.* IBA (0.25 – 2.0 mg/l) for root induction. Root initiation was observed on MS medium supplemented with all concentration of IBA in 10 days of culture (Fig. 3).

A B C

D E

Fig 3: A) Initiation of root in jar. B) Well rooted plantlets developed in 0.25mg/l IBA (C) Well rooted plantlets developed in 0.5mg/l IBA (D) Well rooted plantlets developed in 1.0mg/l IBA (E) Well rooted plantlets developed in 2.0mg/l IBA .

Table-2: Effect of different concentrations of auxin (IBA) on root initiation

MS + IBA in mg/l	Mean Number of roots+ SD	Mean Root length in cm+ SD	Mean Height of plants in cm + SD
0.25	9.5 ± 5.1	5 ± 2.58	8 ± 4.32
0.5	8 ± 4.32	9.5 ± 2.29	8 ± 4.32
1.0	9.5 ± 5.1	9.5 ± 1.7	8 ± 4.32
2.0	8 ± 4.32	9.5 ± 2.29	8 ± 4.32

The response of root induction with different concentration of IBA has mentioned in Table 2. However the roots were initiated in all the four concentrations but highest response in both root number and root length were more in 1.0 mg/l IBA concentration (fig 3). Maximum mean number of roots which is 9.5 were produced per shoot with the mean length of 9.5 cm were observed in 30 days on MS medium supplemented with 1.0 mg/l IBA (Table-2). In most of studies IBA, NAA and IAA has been used for root induction. High frequency of root was observed at 5µM NAA in *Cichorium intybus*[14]. High frequency of rooting was achieved by IAA in *Syzygium cumini*[3]. IBA in *Eclipta alba*[1] and 10µM IBA was obtained maximum number of roots and 5.5cm mean length in *Solanum nigrum*[8].

The well developed plantlets were taken out after 30 days and washed with sterile distilled water to remove traces of agar and shifted to vermiculite tray containing sand, coccopeat and manure (1:1:1) for primary hardening (fig. 4,A). The survival rate of these plants was 70%. Secondary hardening was performed in plastic bags containing sand, cocopeat and manure in a shade house (fig. 4, B). Percentage of survivability was 90% after secondary hardening. Flowering was appeared after 30 days of hardening (fig 4, C).

A B C

Fig. 4: A) Primary hardened plant in vermiculite tray B) Secondary hardened plant in plastic bag C) Flowering plant after 30 days of secondary hardening

Established plants were transferred to the field for acclimatization, where 100% survivability was obtained (fig. 5). This simple protocol outlined for micro propagation offers a potential alternative system for conserving medicinal plants.

Fig. 5: Well grown *in-vitro* propagated *Solanum xanthocarpum* in field

A way of obtaining genuine crude drug is by large-scale destruction of natural habitat due to population pressure and overexploitation, which have become a major threat to important bio resources[6]. Very much owing to the indiscriminate collection, over exploitation and uprooting of whole plant bearing roots this valuable plant has become vulnerable in various places and endangered in Madhya Pradesh and Chhattisgarh[13]. Research for genetically improving the tree species for higher production of compounds under in culture condition is essential[3]. For the conservation of this species, rapid multiplication and rehabilitation in its natural habitat is necessary. To overcome this threat, a reliable method of quick multiplication like tissue culture and methods of *in situ* as well as *ex situ* conservation could well provide a viable solution to the problem.

Currently, medicinal plants are playing a very important role in making large scale production keeping in view the modern economy which accounts for 70% of India's forest product exports and the demand increase in near future as a new frontier for hardening business. India has been probably the oldest, richest and most diverse cultural traditions in the use of medicinal plants. As medicinal plants have great demand in pharmaceutical industry, it is necessary to establish an efficient protocol for *in vitro* propagation of all important medicinal plants. Above research was focussed on optimization of growth regulators for *in vitro* propagation of *Solanum xanthocarpum*. *S. xanthocarpum* was successfully propagated through axillary and apical buds by using 1.0 BAP mg/l and 1.0 IBA mg/l for shooting and rooting respectively. Studies on its medicinal properties and its comparison with the wild varieties are being continued.

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