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In Vitro callus induction studies on *Sida schimperiana* Hochst.Ex A.Rich (Malvaceae) a medicinal plant in Pudukkottai district Tamil Nadu

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Abstract

A viable protocol has been developed for direct callus induction of *Sida schimperiana* to establishment a stable high frequency plant regeneration system. Plant shoot apex and nodal part explant were tested with different combination of Benzyl amino purine (BAP), 2, 4-Dichlorophenoxy acetic acid (2, 4-D), Indole acetic acid (IAA). morpho genic callus induction was observed in highest frequency from hypocotyl explant by culturing in MS medium supplement with in BAP 0.5 mg/L, 2, 4 D 1.0 mg/L, IAA 1.0 mg/L in this protocol morophogenic difference due to explant type is clear for the studied *in vitro* traits. About 80% callus will be observed. This protocol would be useful to create soma clonal variation and to utilize transgenic approach for vital important of *Sida schimperiana.*

Keywords: *Sida schimperiana*, callus induction, medicinal plant, malvaceae, *Sida schimperiana* organogenesis

Introduction

Sida schimperiana Hochst. Ex A.Rich, belongs to the malvaceae family. It is a perennial with a very thick root stock from the summit of whish pocked a large number of procumbent or erect repeatedly forked branches 6-12 in long leaf stalk. Shorter than the leaves which are small ½ in. oblong letuse cunete at the base sparsely covered with stellate hairs, stipules small, ligulate flowers .solitary ,axillary, on very short jointed stalk. Which are sometimes crowded towards the branches ,calyx cyathiform, 5-cleft ,lobes triangular corolla pink fruit globular of 5, glabrous.1seeded, slightly beaked carpels (beak bent inwards) dehiscing along the inner edge. Plant tissue culture is the one of the most powerful tools for induction of fast crop improvements in modern plant breeding age. Recent developments in plant science have clearly shown that biotechnological approaches have contributed the most for the improvements in *Sida schimperiana. Sida schimperiana* callus induction and regeneration through the shoot apex, leaf and nodal cutting is an efficient way for more propagation through the under *in vitro* conditions. The *Sida schimperiana* species highly medicinal plant. It is useful for prenatal abortion, internal worms, amoebic dysentery, cough, influenza, liver disease.

Materials and methods

Sida schimperiana Hochst. ex A.Rich. Plant species was collected in Keeranur village of Pudukottai district, Tamil nadu. The plant specimen was identified with help of Rapinat herbarium Trichy (RHT) St. Joseph's college, (Autonomous).

Plant Material

This is successfully planted in National College herbal garden for further use. The plant Specimens are maintained in the department of Botany, National College. For the initial experiment, healthy nodal and leaf explants were collected from two months old plant.

Source and Choice of Plant materials

Sida schimperiana Hochst.ex A.Rich.,
Three month old plants *Sida schimperiana* Grown and maintained in the green house, Department of Botany National College, Keeranur village were used as the source of explants.

Establishment of Cultures

Glassware and instruments

Glassware Borosil grade consisting of beakers, conical flasks, petridishes, standard flasks, pipettes, measuring cylinder etc. Were used. Explants were cultured in wide neck Erlenmeyer's conical flask (100ml, 150ml, 50ml), culture tubes (150mm long and bottles ($20cm^3$, $60cm^3$).

All the glass wares in use were regularly cleaned to ensure no contamination. Glass wares were thoroughly washed in running water using Labolene' detergent and finally rinsed in distilled water. All the cleaned glassware's were placed in hot air oven for at 100^o C to make them dry. Once used culture vessels autoclaved and washed following the earlier procedure.

Accessories used like scalpels, forceps, spatula, needles, holder were made of stainless steel and were sterilized every time before use. The equipment and instruments used in the laboratory included Laminar Air Flow Cabinet , Hot Air Flow Oven, Refrigerator, Distillation unit, Electronic Monopan balance, Digital electronic pH Meter, Autoclave, Hotplate, Microscope, Rotary Microtome etc.

Basal Media

MS medium (Murashige and Skoog, 1962), B5 medium (Gamborg *et al*., 1968), with different constituents and concentrations were used.

Preparation of Culture Media

Standard procedures were followed for the preparation of media. The media Strength, plant growth regulators and other supplements used is depicted in the Stock solutions of major and minor salts, vitamins and growth regulators were prepared by dissolving required quantity of chemicals in distilled water. The stock solutions of nutrients were stored in amber coloured bottles and of vitamins in tightly liddled volumetric flasks, both under chilled conditions in refrigerator. The growth regulators every fortnight.

The glassware used for the preparation of the media were washed with dilute Labolene and rinsed with double glass-distilled water. The stock solution in the required quantity was pipetted out into a standard flask containing distilled water. 3% sucrose and 100 mg L-1 myo-inositol, as per the specification of the media were added and dissolved in the media. All the plant growth regulators; additives for the different combinations were added before making up the media to the required volume. With an electric digital pH of the medium was appropriated between 5.6 using 5.8 using 0.1 N NaOH or 0.1 N HCL. 0.8% w/v difco bacterial grade agar (in the case of solid medium) was then added to the medium and mixed well.

The solution was then heated on a hot plate and stirred thoroughly, till the dissolved

uniformly. The medium was then poured into pre-sterilized culture vessels. 15 ml was taken in culture tubes (25 mm X 150 mm) and 50 ml was taken in flasks (250 ml). The culture tubes containing the medium were plugged tightly with non-absorbent cotton wool plugs and the flasks with autoclave lids and sealed tight with sealing film.

Preparation of hormones

NAA 10- mg was dissolved in 0.1N NaOH (0.3ml) and diluted with distilled water to make 100 ml and stored in the refrigerator. BAP – Benzyl amino purine (BAP- 10mg was dissolved in 0.HCl (0.4ml) and diluted with distilled water to make 100 ml and stored in the refrigerator. 2, 4-D di chlor phenoxy acetic acid (2, 4-D -10 mg) was dissolved in (0.5 ml) and diluted with distilled water to make 100 ml and stored in the refrigerator. kinetin (kin-10 mg) was dissolved in 0.1N HCL (0.4 ml) and diluted with distilled water to make 100 ml and stored in the refrigerator. GA^3 the same concentration of (GA^3) at 10 mg /100 ml was prepared like 2, 4-D. These hormones were stable to be added before autoclaving the medium.

Sterilization

The sterilization of the culture medium was carried out in an autoclave for 15 minutes at 121^0C and 15 Lbs pressure. After sterilization, the culture tubes and were stored in an air conditioned culture room until further use.

All metal and glass instruments and other accessories used in inoculation cabinet were wrapped in cotton blug and sterilized in an autoclave at 1.06 kg cm^2 pressure for 15-20 minutes at a temperature of 121^0 C. Scalpels, scissors, forceps etc. used were again dipped in alcohol and flamed on a spirit lamp at the time of use.

Explant Preparations and Surface Sterilization

The explants collected from the source plants were coarsely trimmed to a size of 3 cm. and washed in running tap water for 5 minutes followed by washing in distilled water with a few drops of Labolene. After washing these explants in double distilled water, they were immersed in 0.1 % Mercuric Chloride solution and incubated for 5 - 20 minutes. The liquid was stirred by swirling to give proper contact of chemical to the explant. The treated explants were washed three to five times in sterile distilled water. After a final wash in sterile distilled water, the explants were spread on the pre-sterilized petridishes lined with sterile blotting paper inside a laminar airflow chamber. They were then trimmed finely to the appropriate size (1-1.5 cm).

Inoculation and Incubation

Single nodes (1.0-2.0 cm) and inter nodal segments (1.0-2.0 cm) were dissected out and all the inoculation operations were carried out under strict aseptic condition inside a Laminar Air Flow Chamber, which was made sterile by the incessant exposure of germicidal U.V. rays for half hour before use. All operations were carried out using pre-sterilized instruments and glassware. Explants were then aseptically introduced into culture vessels. In order to curtail contamination during drying and inoculation, only a few treated at a time.

The cultures were maintained in the culture room at $25\pm1C^0$ at under 16/8 hr. photoperiod of 2000 lux light intensity provided by white fluorescent tubes with 55%-60% relative humidity or in darkness, as per the treatment. 8 replicates were made in each treatment and all trials were carried out three repeats.

Subculture

Cultures were regularly transferred to fresh medium or regeneration medium as per requirement after every 15-30 days.

Experiments and Observations

All experiments were conducted in 3 replicates twice and data on number of callus obtained through stem, node, and inter node, tendril leaf proliferation as callus induction.

Culture medium

The culture medium used to induce callus consisted of MS basal salts and vitamins supplemented with auxin (2, 4-D, IAA) alone or in combination with cytokinins (BAP).

Subculture

The Calli were subcultured at 15-30 days interval for proliferation.

Data collection and Presentation

Callusing Frequency

Cultures were scored for callus induction at the end of the fourth week (30 days) Frequency of callus induction was computed as the ratio of the number of explants responding to that of total number of that of explants involved and was expressed as percentage as shown below.

$$\text{Frequency of response (\%)} = \frac{\text{Number of explants responding x 100}}{\text{Total no. of explants cultured}}$$

Nature Callus

Visual features of the callus developed were recorded and photographed.

Indirect Regeneration

Explants

Excised nodal segments, internodal segments, leaves (3.0-4.0 cm) tender leaves (2^{nd} or 3^{rd}) from the apex and internodal segments (2.0-3.0 cm) were used as explants for regeneration via callus.500mg of green compact calli was used as explants for indirect regeneration.

Culture medium

MS media was used as basal medium for callus induction Different combinations and concentrations of growth regulators cytokinins (BAP) and auxins (IAA, 2, 4- D).

Culture conditions

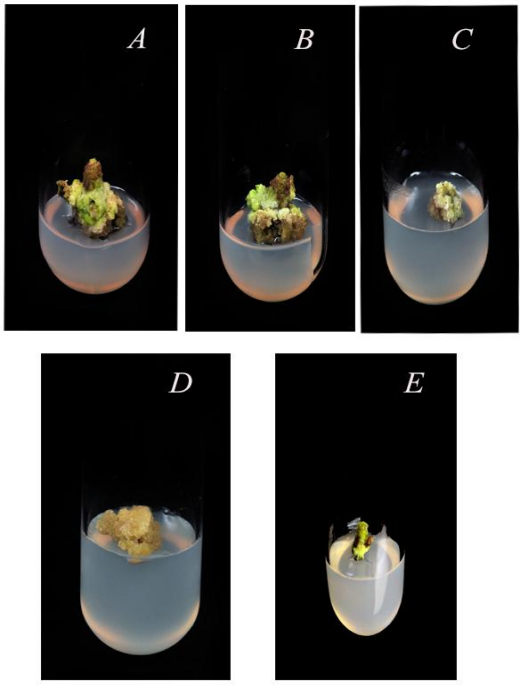
Cultures for callus induction were maintained in a light regine consisting of 16h light at $25^{o}\pm2^{0}$C temperature.

Callus Induction

Hypocotyls, roots, shoot apices with small leaves, cotyledonary nodes, and cotyledons were aseptically excised from placed on the media summarized in for callus initiation.

Results and discussion

Plate-1



A. Compact, Dark green callus.
B. Friable and loose, Green and light green callus.
C. Green and light green, Yellow and colorless.
D. Watery soft, Yellow and colorless.
E. Green and light green callus.

Table 1: Effects of different explant sources and plant growth regulators on callus formation.

Explant sources	Plant growth regulators (mg /L)			Percentage of Callus formation	Texture of Callus	Colour of Callus
	BAP	2,4-D	IAA			
Shoot	0.5	1.0	1.0	82.73±41.01	Compact	Dark green
	1.0	1.0	1.0	70.4±34.21	Friable and loose	Green and light green
	1.5	1.0	1.0	61.8±29.51	Green and light green	Yellow and colourless
	2.0	1.0	1.0	51.43±25.71	Watery soft	Yellow and colourless
	2.5	1.0	1.0	-	-	-
Leaf	0.1	0.2	0.5	55.4±27.49	Friable and loose	Green and light green
	0.2	0.4	0.4	21.9±10.22	Compact and loose callus	Greenand light green
	0.3	0.6	0.3	-	-	-
	0.4	0.8	0.2	-	-	-
	0.5	1.0	0.1	17.83±8.30	Watery soft	Yellow and colourless

The maximum amount of callus induction observed from MS medium supplemented with BAP 0.5 mg/L,2,4-D 1mg/L, and IAA 1 mg/L (Table-1, plate -1). The similar results obtained from (Ramar *et al*., 2013), (Paul *et al*., 2011), (Sudarshana Mysore Shankarsingh *et al* ., 2016), (Ouma *et al* ., 2004), (Ruben Mallon *et al* ., 2011).

Conclusion

An efficient callus induction protocol for *Sida schimperiana* has been developed from these findings. The treatment of BAP 0.5 mg/L, 2, 4-D 1 mg/L and IAA 1mg/L is good for maximum callus induction. Therefore the results reported in the study would be an important step towards the development of good quality and quantity of callus to provide successful platform for regeneration, and genetic transformation.

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Conflict of Interest

No conflict of interest

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