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In vitro propagation of medicinal plant *Sida cordifolia* under different growth regulators and salt stress

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ABSTRACT

The demand of medicinal plants is increasing in pharmaceutical industries due to the presence of different secondary metabolites. Due to this reason, different plant species are likely to become endangered or extinct due to their medicinal importance. *Sida cordifolia* (Malvaceae) is one of them. It is a very valuable medicinal herb mentioned in Indian Ayurveda and other medicinal systems throughout the world. It is commonly known as Bala. It is used to treat asthma, chronic dysentery, asthma, gonorrhea and as a fat burning supplement. The entire plant is rich in secondary metabolites. It constitutes important bioactive compounds such as ephedrine, vasicinone, vasicinol, palmitic, stearic acid. Tissue culture technique will provide an efficient method for propagation and conservation of this medicinal plant. For direct shoot regeneration from nodal explants under *in vitro* conditions, BAP performed better than Kn. Shoot regeneration was found maximum at 2.0 mg/l BAP. The plantlets were also tested for their salinity tolerance. Root, shoot length and biomass was not affected by low levels of salinity stress. However, high levels of salinity stress (200mM) reduced the shoot, root length and biomass drastically. For root induction, half-strength MS medium with 2.0 mg/l IBA and 3% (w/v) sucrose was found to be the best. Regenerated plants were found to be morphologically similar to the mother plants. This protocol could be helpful for mass cultivation and germplasm conservation of *S. cordifolia.*

Key words*: Sida cordifolia, BAP, Salinity stress.*

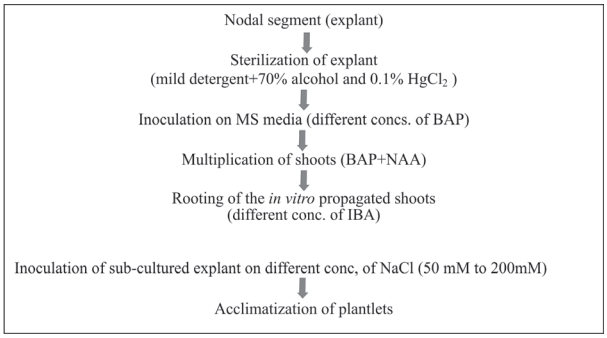
Introduction

The demand for medicinal plants is increasing in pharmaceutical industries due to the presence of different secondary metabolites. Due to this reason, different plant species are likely to become endangered or extinct due to their medicinal importance. WHO has emphasized on the use of medicinal plants as the best source of herbal drugs. For the potential use of herbal medicine, it is necessary to study the medicinal plants found in folklore (Ali *et al*., 2001; Nair *et al*., 2005). Different parts of medicinal plant such as flowers, barks, leaves, fruits, stems and roots possess a variety of pharmacological activities. The genus *Sida* L. (Malvaceae) is distributed mainly in the tropics and subtropics. The species *S. cordifolia* is native to Africa, tropical and temperate Asia and South America. About 17 species of the genus *Sida* have been reported in India (Sivarajan and Pradeen, 1996). *Sida cordifolia*, commonly known as Balais a small, erect, downy shrub with chordate-oblong or ovate- oblong leaves. Roots of the plant are 5-15 cm long (Rangari, 2000). It is a valuable medicinal herb mentioned in Indian Ayurveda and other medicinal systems throughout the world. Its root is extensively used as diuretic, astringent and tonic (Rastogi and Malhotra, 1985). It is also used for the treatment of infections in blood, urinary disorders, neurological problems, bile, piles, leucorrhoea, gonorrhea, chronic dysentery,asthma (Agharkar *et*

al., 1991). The entire plant is rich in secondary metabolites. It constitutes important bioactive compounds such as ephedrine, vasicinone, vasicinol, hypaphorine, betaine, chloine, flavonoids, saponins (Ghosal *et al*., 1975). It also contains appreciable amount of total phenolic contents (5.6 ± 1.3 mg/ g), carotenoids (0.3 ± 0.1 mg/g), Total soluble protein (6.5 ± 0.1mg/g) (Arshad *et al*., 2020). Due to its medicinal importance and extensive collection, population of *S. cordifolia* is declining in the wild. Germplasm conservation of this traditional medicinal plant through *in vitro* protection is important to support pharmacological and genetic improvement programs. To overcome different environmental restraints *in-vitro* cultures acts the best option for the surplus and continuous supply of important active ingredients in plants (Davies and Deroles, 2014). However, only a limited data are available on the micropropagation of plants especially from Malvaceae family (Hasson and Poljakoff-Mayber, 1995; Zapata *et al*., 1999) and to date, there are limited studies on regeneration of *S. cordifolia* from different explants. Also, there are no data available on the micropropagation of *Sida cordifolia* under salt stress. Hence, the present study provides a protocol for direct regeneration of *S. cordifolia* through nodal segment for the conservation and cultivation of *S. cordifolia*. Also, the regeneration capacity of *S. cordifolia* under varying salt stress regimes has been evaluated.

Materials and Methods

For *in vitro* propagation of *Sida cordifolia*, explants were procured from nursery, Kurukshetra University, Kurukshetra. Standardization of *S. cordifolia* was performed in Environmental Biotechnology Lab, Institute of Environmental Studies of the University. Procedure for micropropagation is described below:



MS media was fortified with different growth regulators, 3% (w/v) sucrose with 0.8% (w/v) agar. BAP (0.5 mg/l to 4.0 mg/l) and in combination (BAP 0.5 to 3.0 mg/l with 0.05 mg/l NAA) was used as growth regulator. For rooting medium IBA was used. The pH of the MS medium was set to 5.8 and then autoclaved at 121°C for 15 min. The cultures were incubated at 25±2°C with relative humidity between 70–80% under a 16-h photoperiod. A photosynthetic photon flux density of 60 µmol m^{-2} s^{-1} was provided by cool white fluorescent light.

Shoot length and root length was measured by using centimeter scale. Biomass was calculated by measuring the dry weight of shoot and root in oven at 60^0C in an oven for 48 hours.

Results and Discussion

Surface sterilization is the first and the most important step for tissue culture technique. For surface sterilization of nodal segments of *Sida cordifolia* $HgCl_2$ at a concentration of 0.1% showed better shoot regeneration. Below this concentration, explants showed contamination on MS media while above this concentration, explants turned black (Fig. 1).

In vitro propagation of *Sida cordifolia* under different growth regulators

For *in vitro* propagation of nodal segments of *Sida cordifolia* BAP performed better than Kn. Shoot regeneration was found maximum at 2.0 mg/l BAP (Fig. 2). At this concentration, number of shoots regenerated was maximum and also the days required

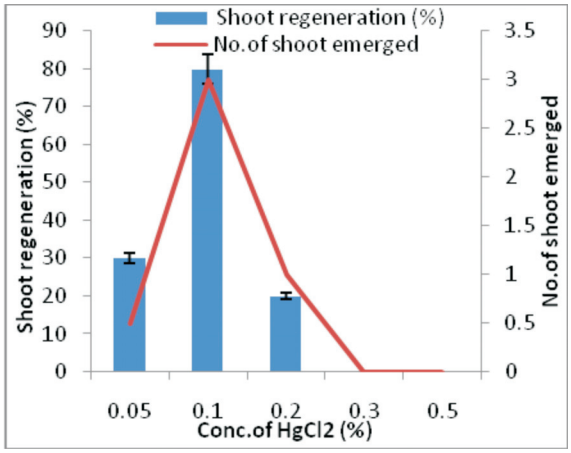


Fig. 1. Shoot regeneration of *Sida cordifolia* under different concentration of $HgCl_2$

for shoot regeneration was less. Shoot regeneration was negatively affected by BAP above 2.0 mg/l. Similar outcomes of bud breaking has been reported with BAP in *Mentha piperita* (Mehta *et al*., 2012). The present study also confirms similar response in *S. cordifolia*.

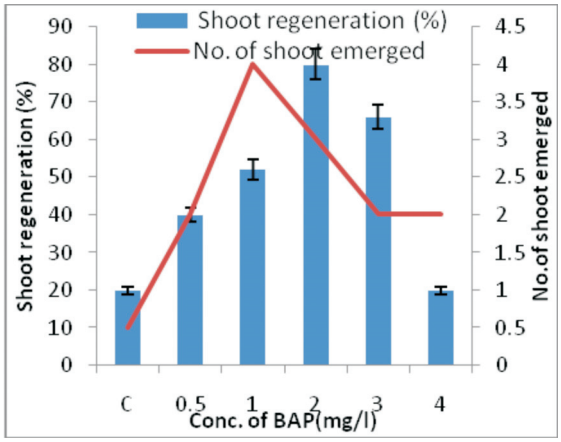


Fig. 2. Shoot regeneration of *Sida cordifolia* under different concentration of BAP

To increase shoot multiplication, different concentrations of BAP was combined with NAA. Auxin in combination with cytokininresulted in multiple shoot buds after 2 weeks of culture. The frequency of shoot formation and also the average number of shoots produced per explant was increased as compared to the use of BAP only.Among the various combination of growth regulators,BAP (2.0mg/l) and NAA (0.05mg/l) performed better than the other combinations (Table 1). Also, the number of shoot regenerated was maximum at this concentration. Similar results of effectiveness of BAP with NAA have been reported in *Liliumlongiflorum* (Deswiniyanti and Lestari, 2020); *Saussureacostus* (Khan *et al*., 2021).

Table 1. *In vitro* propagation of *Sida cordifolia* on MS media with different combinations of BAP and NAA

S. No.	Conc. of BAP + NAA (mg/l)	No. of shoot emerged/ explant	Shoot regeneration (%)
1.	C	0.5	20
2.	0.5+0.05	4	45
3.	1.0+0.05	5	65
4.	2.0+0.05	8	95
5.	3.0+0.05	66	3

Root induction: For root induction, 2-3cm regenerated plantlets of *S. cordifoila* inoculated on half-strength MS medium with 2.0 mg/l indole-3-butyric acid and 3% (w/v) sucrose showed optimum results. Reduced strength of basal medium is generally used for rooting of adventitious shoots (Hu and Wang, 1983). Roots started to appear after 16 daysof inoculation from cut ends of the shoots on rooting medium. The highest response with 95% root induction and an average of 5.8 roots per shoot was obtained after 32 days culture on half strength basal MS medium supplemented with 2.0 mg/l IBA.68% survival rate of plants was obtained by transferring rooted shoots to a soil, sand, and vermiculite (1:1:1, v/v/v) mix. For hardening 7 weeks period was necessary for the successful establishment of the plants. Regenerated plants were found to be morphologically similar to the mother plants.

In vitro propagation of *S. cordifolia* under salt stress

Subculturing of explants on different conc. of NaCl showed that low levels of salinity had no effect on shoot- root length and biomass of plantlets. At higher conc. morphological parameters decreased (Table 2). However, high levels of salinity stress (200mM) reduced the shoot, root length and biomass drastically. The highest shoot regeneration with highest biomass was obtained on MS media with 50mM NaCl .Above 100mM shoot regeneration was reduced.

Table 2. Effect of salinity stress on morphological parameters of *Sida cordifolia*

S. No.	Conc. of NaCl	Shoot regeneration (%)	No. of shoot emerged	Shoot length(cm)	Root length (cm)	Biomass (g)
1.	C	20	0.5	0.2	0.1	0.01
2.	50mM	75	5	2.5	1.4	0.4
3.	100mM	61	3	1.2	0.8	0.2
4.	150mM	45	1	0.7	0.3	0.1
5.	200mM	10	1	0.4	0.2	0.05

Conclusion

Sida cordifolia is recommended in traditional medicine in different parts of world such as Brazil, China and India for different medical conditions including asthma, bronchitis, nasal congestion, inflammation of oral mucosa and neurodegenerative diseases. Also, pharamaceutical companies mainly depend upon on medicinal plants, which are being depleted rapidly. The excess use of medicinal plants present on natural sites lack efforts on systematic cultivation methods. So, for mass multiplication, *in vitro* propagation method especially for this plant is necessary. Under *in -vitro* conditions, BAP alone and in combination with NAA proved better for regeneration of *S.cordifolia.* Under different levels of salinity stress under in vitro conditions, *S. cordifolia* was able to tolerate the moderate levels of salinity concentration. The present work on this important medicinal plant species provides a baseline for future mass propagation of the declining population of *Sida* under salt stress conditions. This may ensure sustainability and management of *S. cordifolia* in natural ecosystems.

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Conflict of Interest Statement

The authors have no conflict of interest.

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