Volume-4, Issue-12, December-2020: 35-39 P-ISSN: 2659-1561
International Journal of Recent Innovations in Academic Research E-ISSN: 2635-3040

Research Article

Studies on *In vitro* Regeneration of *Sterculia urens* Roxb. Using Cotyledon Explants

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Received: Dec 6, 2020 **Accepted:** Dec 19, 2020 **Published:** Dec 26, 2020

Abstract: In the present study different cotyledon explants (full cotyledon; proximal portion of cotyledon, distal portion of cotyledon) of *Sterculia urens* Roxb. were tested for their regeneration potential on MS basal medium and MS medium supplemented with different growth regulators. Of three different media combinations tested, MS+TDZ (0.1 mg/Lit) showed the maximum conversion efficiency into leaf followed by MS+BAP (1 mg/Lit) and MS+KIN (1 mg/Lit). In all the combinations, the explants were converted into leaf only, no further growth and regenerative potential was observed.
Keywords: *Sterculia urens,* Cotyledons, MS medium, *in vitro* regeneration, leaf.

Abbreviations
MS = Murashige and Skoog, **TDZ**= Thidiazuron, **BAP**= Benzylaminopurine, **KIN** = Kinetin

Introduction

Sterculia urens is a moderate sized tree belongs to the family Sterculiaceae and it grows in the deciduous forests of Andhra Pradesh, Madhya Pradesh, Uttar Pradesh and Rajasthan. The tree yields a gum known as gum karaya, which is economically important to tribal and rural people livelihood (Dhiman *et al*., 2019).

The gum has great demand both within and outside India (Sukhadiya *et al*., 2019) and has numerous applications like as an ingredient in the preparation of emulsions, lotions, adhesives, denture fixative powders, bulk laxatives, food and pharmaceutical industries etc (Shekarforoush *et al*., 2016; Luján Medina *et al*., 2013).

Commercial tapping of karaya is done by wounding, blazing, peeling and by making deep cuts and it is injurious to the trees and ultimately leads to the tree death. Generally the natural propagation of *S. urens* is through seeds but the seed viability decreases as the time progresses (Subhashini Devi *et al*., 2013).

The poor seed germination coupled with over-exploitation has depleted the species from its natural habitat, thereby widening the gap between demand and supply and thus putting pressure on the species. So, there is grave concern about the loss of wild germplasm of *S. urens* (Quazi *et al*., 2017; Shukla and Agnihotri, 2022).

In vitro regeneration of *S. urens* using different explants was reported by several researchers. *In vitro* regeneration of whole plants from cotyledonary node and nodal explants were reported by Subhashini Devi *et al.,* (2011) and Sunnichan *et al.,* (1998), from cotyledonary node by Purohit and Dave (1996), Hussain *et al.,* (2007) and from intact seedlings by Hussain

et al., (2008). The present investigation is intended to know whether the cotyledons of *S. urens* have the capacity to regenerate into plantlets or not.

Materials and methods

Plant material

Seeds were collected from Kovela foundation (Non-Governmental Organization), Visakhapatnam AP, India. Healthy seeds were collected and treated with Con. H_2SO_4 for one min, washed thoroughly with running tap water and with 20% tween for 10 min followed by treatment with bavistin for 5 min.

Then subsequently seeds were sterilized with 0.1% (w/v) $HgCl_2$ for 5min and thoroughly washed with sterile distilled water and were soaked for 24 hours. Surface sterilized 24 hours soaked seeds were taken and surface dried in between Whatman No.1 filter papers. The seed coat was removed using forceps aseptically and the cotyledons were separated and used as explants.

Preparation and sterilization of medium

Murashige and Skoog (1962) MS basal medium and also MS medium supplemented with different concentrations of growth regulators BAP (1 mg/lit), KIN (1 mg/lit), and TDZ (0.1 mg/lit) were used. The pH of all media was adjusted to 5.7 before autoclaving. The medium, glass ware, double distilled water, Whatman No.1 filter papers etc were sterilized at 121^0C at 15 lb/in^2 pressure for 20 minutes. The sterilized medium was poured into culture tubes in the laminar flow unit and plugged tightly.

Inoculation of explants

Surface sterilized cotyledons were dried between Whatman No.1 filter papers and were cut horizontally using sterile surgical blade. The proximal and distal portions of cotyledons and cotyledon as a whole were inoculated on MS basal medium and also MS basal medium supplemented with BAP (1 mg/lit), KIN (1 mg/lit) and TDZ (0.1 mg/lit) with their cut ends touching the medium. For each experiment 100 cotyledons were used. The results were recorded after 21 days.

Culture conditions

All cultures were incubated at 25±2ºC under 16/8 h (light/dark) photoperiod with 60% relative humidity. Light intensity of 40-50 µmol m^{-2} s^{-1} was provided by using cool-white fluorescent tubes.

The cultures were transferred to fresh medium after an interval of four weeks. Each hormonal treatment consisted of 100 samples and all experiments were repeated thrice.

Results and Discussion

Response of Cotyledons

The proximal and distal portions of cotyledons and the cotyledons as a whole when cultured on MS basal medium and MS basal medium supplemented with BAP (1mg/lit), KIN (1mg/lit) and TDZ (0.1mg/lit) did not show any regeneration response.

In all the above media tested the cotyledon differentiated to leaf and there was no further growth and organogenic response (Figure 1, Table 1).

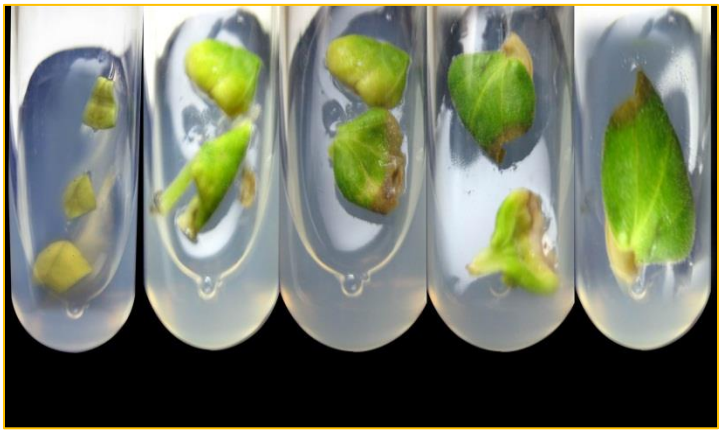

Figure 1. Response of Cotyledons on MS basal medium and MS basal medium supplemented with 1 BAP, 1 KIN and 0.1 TDZ (mg/lit)

Table 1. Culture response of cotyledons on MS medium and MS medium supplemented with BAP, KIN and TDZ

S/N	Type of medium (mg/lit)	No. of cotyledons cultured	No. of explants responded	Type of response
1	MS	FC 100	80	converted to leaf
		PC 100	79	converted to leaf
		DC 100	76	converted to leaf
2	MS+1BAP	FC 100	97	converted to leaf
		PC 100	98	converted to leaf
		DC 100	97	converted to leaf
3	MS+1KIN	FC 100	86	converted to leaf
		PC 100	86	converted to leaf
		DC 100	84	converted to leaf
4	MS+0.1TDZ	FC 100	98	converted to leaf
		PC 100	99	converted to leaf
		DC 100	97	converted to leaf
FC: Full Cotyledons; PC: Proximal Cotyledons; DC: Distal Cotyledons				

In the present study, cotyledon explants were used for regeneration studies. Full cotyledons, either proximal or distal end of cotyledonary explant expanded in to normal leaf upon culture on all media tested. There is no callus formation or further response. The cotyledon is the first leaf with stored food material and has embryonic potential. Generally cotyledonary explants readily produce callus and differentiate into shoots, roots and plantlets under culture conditions. The adventitious shoot formation and plant regeneration from cotyledon explants was reported in several plants species.

Raju and Divya (2020) reported the maximum shoot and root frequency from cotyledon explants of *Syzygium densiflorum***.** Huang *et al.,* (2020) reported the high frequency of *in vitro* regenerated plants through callus mediated organogenesis from cotyledons of *Neolamarkia cadamba.* Mardhiyetti *et al.,* (2021) studied the *in vitro* regeneration of *Sesbania grandiflora* using cotyledons. Curuk *et al.,* (2017) studied the effect of different plant growth regulators (BA, Kinetin, and NAA), different basal media (WPM and MS) and different explants (cotyledon, hypocotyl) on regeneration, caulogenesis, shoot and root formation in carob and WPM containing BAP (0.5 mg/Lit) and KIN (1 mg/Lit) showed the best regeneration capacity for carob cotyledon explants. But in our experiments *S. urens*

cotyledons did not show regeneration into roots or shoots except differentiation into leaf. This may be due to the internal programme of *S. urens* where the juvenile nature of cotyledon is lost by the time the seed germinates.

Conclusion

The present work describes *in vitro* regeneration of *S. urens* from cotyledons. The cotyledons of *S. urens* have no organogenic potential or regeneration potential and further studies using different growth regulators and additives are required in order to produce high frequency shoot or roots from cotyledons of *S. urens*.

Acknowledgments

The authors acknowledge the Head, Department of Biochemistry, Andhra University for providing the facilities to carry out the research work.

Conflict of interest: The authors have no conflict of interest.

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Citation: Subhashini Devi, P. and Satyanarayana, B. 2020. Studies on *In vitro* Regeneration of *Sterculia urens* Roxb. Using Cotyledon Explants. International Journal of Recent Innovations in Academic Research, 4(12): 35-39.