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RESEARCH ARTICLE

EFFECT OF VARIOUS CULTURE MEDIA ON SHOOT INITIATIONS OF *SARACA INDICA L.* ENDANGERED PLANT

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ABSTRACT

A micropropagation protocol is presented for the conservation of critically threatened woody tree species. The present work was carried out in different types of media and undertaken to develop a basic and simple protocol for shoot induction via micropropagation of *Saraca indica* L. Best result shown in different media in the order as WPM>MS>B5 media. The effect of different mediums on the behavior of in-vitro consecutive micropropagation protocol was developed. Three media were tested (B5, MS and WPM) with different growth hormones for the induction stage. WPM was found to be the best medium for shoot induction. A high frequency of induced shoots was obtained on BAP (6mg/l), KN 1mg/l), and NAA (1.5 mg/l). The low concentrations of growth hormones did no support in vitro shoot induction in *Saraca indica* L. The shoot induction protocol developed in this study provides a basis for germplasm conservation and for further investigation of medicinally active constituents of the elite medicinal plant. Further work for standardization of efficient *in-vitro* protocol for best shoot multiplication and *in-vitro* rooting is underprogress in our laboratory.

Key words: *Saraca indica* L, *In-Vitro* Propagation, Medicinal Plant Conservation, three type's Medium

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INTRODUCTION

Saraca Indica L. belongs to family *Caesalpiniaceae* its locally knows as Sita Ashoka Hempushpa, Anganapriya *Saraca Indica* is a rain forest tree. *Saraca Indica* tree belonging to *Caesalpiniaceace* family. It is found all over India, especially in Himalayas Kerala, and Bengal and whole south region. (Nyeem *et al*.2017). Homeopathic medicinal plants for the several treatment various feminine disorder especially in gynecological disorders. Research on plant materials for their potential medicinal value plant material have been used or the treatment of serious disease throughout the world before the advent of modern clinical drugs (Anitha *et al* .2008). Medicinal plant has been used for the treatment of various aliments throughout the world before the advent of modern synthetic drugs. Herbal medicine has such as amazing influence that numerous alternative medicine therapies extravagance their patient with herbal remedy, Unani and Ayurveda. *Saraca Indica* is one of the most leading plants utilized from ancient times till to date (Bhadauria *et al*. 2012). It is one of the common plants used in Indian system of medicine various parts of the plants are used the treatment of skin diseases, to rheumatic pain). Ashoka active ingredients may have antifungal property, which could be beneficial to medicinal.

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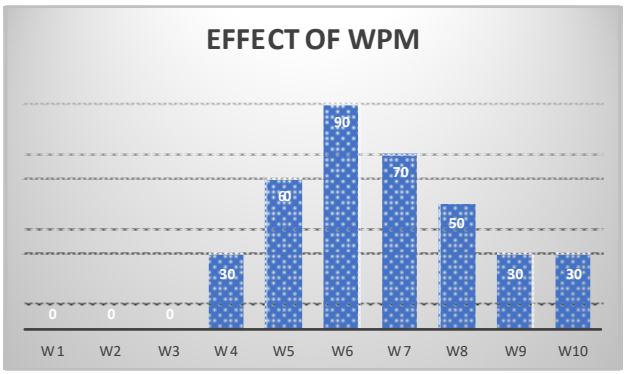
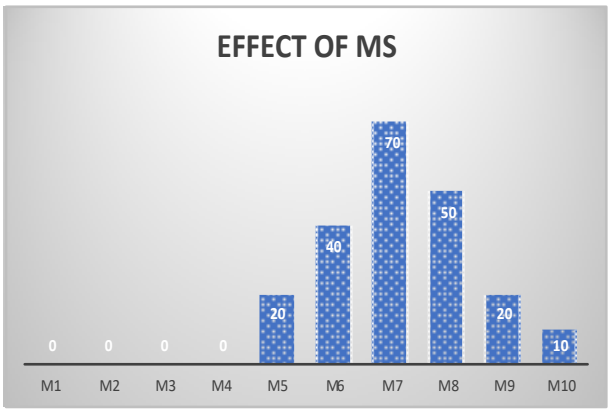
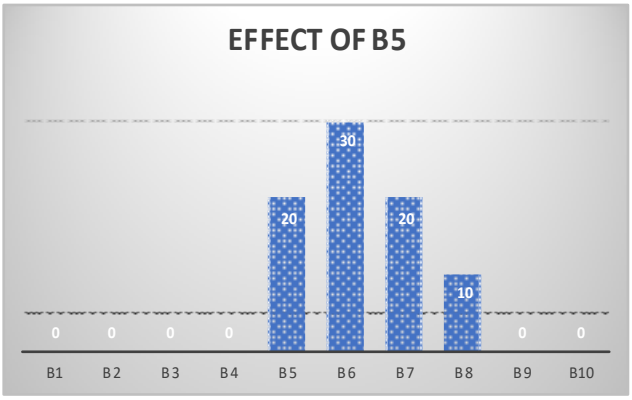
The plant is source of various types of compounds like Antibacterial, Anticancer, Anti-diabetic and Antioxidant etc., which are useful for various medicinal properties and economic importance. It has been, brought to knowledge that Ashoka which is Vada's potentiated completely destroys the brain cancer cells .The need for more intensive research since they play great role in health care. It is also used in uterine tonic Hemorrhagic dysentery & diabetes. As the germination rate is often poor. The seeds have thick and hard seed coatwhich would take very long period for germination. The seed propagation has limitations like low seed production and seed viability for a short period of only two months .(prakash *et al* (1991). The current requirement in our country is too high because this are uses in very popular garden of South America. The annual demand for its bark is 10,724 tonnes and is growing at 15% per annum in India. Commercial exploitation for its bark has led to it becoming vulnerable, resulting in poor regeneration in its natural habitat.(Fatima Shirin *et al*)(2011) *In-vitro* micropropagation is very necessary so the plant requires conservation to meet its demand in medicine and other uses. The biotechnology approach such as plant tissue culture is an alternate and variable methods for and conservation of economically and medicinally important plants (Jaya at all 2012). The media compositions and plant growth regulator's play in vital role in vitro micro propagation there for optimization of these condition is a prerequisite for *in vitro* related work.

Table 1. Effects of different media and concentration of plant growth regulators on *in-vitro* shoot initiation from meristems shoot of *Saraca indica* after approx. -4 weeks of culture.

Plant Growth Regulators mg/l	Shoot induction %		
	WPM	MS	B5
1.0 BAP +005 NAA+0.1 KI	0	0	0
2.0 BAP +0.2 KI	0	0	0
3.0 BAP +0.5 NAA+0.1 KI	0	0	0
4.0 BAP +1.0 NAA+0.5 KI	30	0	0
5.0 BAP +1.0 NAA+0.5 KI	60	20	20
6.0 BAP +1.5 NAA+1.0 KI	90	40	30
7.0 BAP +2.0 NAA+2.0 KI	70	70	20
8.0 BAP +2.0 NAA+20 KI	50	50	10
9.0 BAP +2.5 NAA+2.5 KI	30	20	0
10.0 BAP +3.0 NAA+3.0 KI	30	10	0







There is no reported available in vitro propagation in Bhopal (as pr my deep study). *Saraca Indica* that made us interested to develop micropropagation protocol for this endangered medicinal, economically important plants. The present study was based on under taken to effect of different basal media with different PGR concentration standardize a protocol for *in -vitro* shoot induction of *Saraca Indica plants* by using different explants by tissue culture micropredation to meet its demand in medicine.

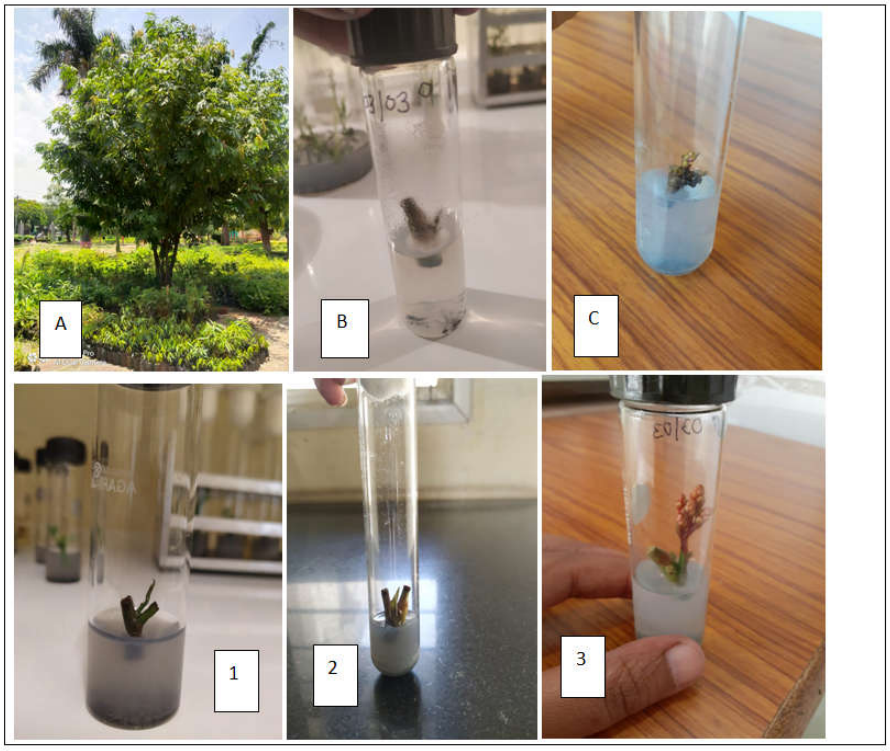
Material ad Methods: -The research was conducted at the Department Biotechnology Institute for Excellence in Higher Education, Bhopal Madhya Pradesh (India).

Collection and Authentication of Plant Material- The shoots were collected in the month of January to April. From mature plants growing inside the Government Hamidia Arts & Commerce College, Bhopal and the plant were identified by collages taxonomists.

Selection of Explants -Meristems were used as explants for this experiment. Explants were cut and reduced to a length of 2 cm using surgical blad. Surface Sterilization Procedure -Shoots were thoroughly washed under running tap water for 30 min to remove all the dirt and soil particles adhering to them, then treated with 5% tween-20 for 5 minutes with constant stirring followed by 3-4 rinses in sterile distilled water and further treated with an antifungal agent (Bavistin) for 2 hours and were further with detergent for 20 minutes and rinsed 4-5 times tap water. Thereafter, again explants were kept immersed in distilled water with few drops of wetting agent, labolene for ten minutes. It was immediately followed by five-time rinses in distilled water to remove traces of labolene. Further sterilization procedures were carried out inside a laminar airflow chamber, where shoots were surface sterilization through 1 minute's treatment in 70% (v/v) for half a minute followed by three times rinses in sterile distilled water. Thereafter mercuric chloride (0.1%) treatment was given to explants for 10 minutes followed by four times rinsed in sterile distilled water. Thereafter shoots were carefully transferred to be placed over sterile Petri plats to remove excess water and were then and were then inoculated into the culture establishment medium (WPM/MS/B5) using sterile forceps under aseptic conditions.

Chemicals and Glass Wear- Meristems induced from shoots were cultured on WPM/MS/B5 basal medium supplemented with 3 % (w/v) sucrose (Sd-fine Chemicals, India) for shoot induction. The pH of the medium (supplemented with respective growth regulators) was adjusted to 5.5-5.8 with 1N NaOH or 1N HCl before gelling with 0.8 % (w/v) agar. In all the experiments, the chemicals used were of analytical grade (Merck and SD-fine Chemicals, India). The medium was dispensed into culture vessels (Borosil, Mumbai, India) and autoclaved at 105 kPa at 125°C for 15 minutes.

The surface-sterilized explants were placed vertically on the culture medium. All the cultures were incubated at 25±2°C under 16h light/8h dark photoperiod with an irradiance of 45 - 50 µ mol/ m^2/s photosynthetically active radiation (PAR) provided by cool white fluorescent tubes (Philip, India) and with 60 - 65 % relative humidity. All subsequent subcultures were done at four weeks intervals. Culture media consisted of WPM/MS/B5 (Morishige and Skoog 1962) supplemented with 3% (w/v) sucrose and 0.8% (w/v) agar (Himedia, India) was evaluated for their effects on *in-vitro* growth and development of *Saraca Indica* for induction of shoots, explants were cultured on WPM/MS/B5 medium supplemented with different concentration of cytokines, including BAP (6mg/l), NAA (1.5. mg/l) and Kinetin (1.0mg/l) either individually or in combination.



Photos A. Mature Plant B. Inoculated shoot C. Different stages of initiated shoots (1/2/3)

Application of tissue culture to plant conservation in India has been largely restricted to economically important species. However, the approach could usefully be extended to conserve all threatened plants so that vital biodiversity and the ecological network is sustained can be preserved (Jiten Chandra *et al*., 2011) *Saraca indica* is categorized as a rare and endangered species. According to literature survey, this is found out that, in the southeast coast of India, medicinal plant *Saraca Indica* is being endangered. Ths species is currently listed as a globally vulnerable species by the IUCN (2013)(Ch.Mohan *et al*)(2016)

Shoot Induction- Meristems node was excised and inoculated by vertical orientation on the culture medium containing different concentration of BAP (0.5-10 mg/l), NAA (0.1-2.0 mg/l) and Kinetin (0.1-2.0mg/l). Ten single explants were assigned randomly to each treatment and the culture was kept under 16 h light/day photoperiod at 25±2°C shoot induction the effects of different treatments were quantified and the data were subjected to presented. Medium lacking growth regulators served as control.

Results And Discussion -MS medium supplemented with different levels of BAP, KN, or NAA were tried to induce shoots from meristems explants of *Saraca Indica* nodal explants showed initiated on higher concentrations of BAP (6 mg/l), KN (0.1mg/l), or lower levels of NAA (1.0 mg/l) however these levels failed to induce shoot formation. The combined effect of cytokinin's, KN in combination with BAP or NAA, was tested on in vitro nodal in. *Saraca Indica* interestingly, the above cytokinin's when combined resulted in axillaries and meristem as well as bud break. A combination of KN (1.0mg/l) plus BAP (6.0 mg/l), NAA (1.5mg/l) showed maximum (90WPM/70MS/40B5, %)shoot induction. The purpose of this study was to develop an *in - vitro* propagation method from mature nodes of *Saraca Indica* a medicinally important plant.

In the present work we have, for the first time in Bhopal District established a rapid and reproducible method for high-frequency from mature node segments *of Saraca Indica*, Similar observations were previously reported with previously paper the fresh calli produced in all the treatments were white and hard which turned brown after two week(M.L. mini *et al* 2013) . Earlier studies have compared the effectiveness of different type's PGR concentrations. In the present investigation, the interaction of KN with NAA or BAP with MS basal media was established that resulted in efficient shoot regeneration. Further, the *in - vitro* regenerated shoots are using further experiments. However, nodal segments incubated on medium supplemented with KN + NAA+BAP produced healthy shoots and overall shoot. Quality did not differ much when these media formulations were used.

The synergistic action of a combination of two or more cytokinin's resulting in to shoot induction from various explants has also been reported for *Gymnocladus dioicus* L. (Geneve, 2005); Eclipta Alba (Baskaran and Jayabalan, 2005); and Momordica tuberose Roxb (Aileni *et al*., 2008). In vitro clonal propagation of Ashoka the micro shoots rooted on MS medium supplemented with 4.0mg/l of IBA. (R. Rama Subbu *et al*) (2008). The role of cytokinin's in shoot differentiation from nodal segments was reported in several plants species but only a few reports were successful in inducing organogenesis from mature node explants (Ramesh *et al*., 2002). The present work was aimed to develop in vitro organogensis protocol for rapid and large scale production of planting material (shah *et al* 2016). The greatest number of multiple shoots (8.68 shoots/explants) was obtained from explants cultured on medium containing 0.5 mg/l−1 BA. Generally, increasing BA concentrations led to a significant decrease in the number of formed shoots. Meanwhile, it was the observer that BA at 0.5 mg/l−1 gave the highest significant average of shoot length as 4.61 cm.

In this concern, (Baskaran and Jayabalan (2005). On Saraca Indica Here we report on shoot induction from mature node explants of Saraca Indica. In conclusion, in vitro growth and development from nodal explants of Saraca Indica was highly influenced by the type of BAP+NAA+KN+MS basal medium combination used for propagation. The results presented also demonstrate that mature nodal explants of Saraca Indica offer great potential as a source tissue for shoot induction. The procedure reported in this study may facilitate improvement, conservation, and mass propagation of this medicinally important plant species.

Conclusion

It can be concluded that the WPM showed a high% of shoot induction as compared to MS, B5 media. The protocol defined in this study as outlined below and is demonstrated in fingers. The findings have several implications for managing the diversity of this species as well as the restoration of its degradation. In conclusion, our results suggested that the tissue culture technique could be successfully used as a rapid method to propagate *Saraca indica* plants using MS medium supplemented with 0.1 KNmg/l,6 BAP with 1.5 NAA. The present experiment has shown that it is possible to *in vitro* shoot induction and use for further experiments' and done by plantlets.

Abbreviation: BAP: 6-Benzyllaminopurine, NAA: Naphthalene Acetic Acid, KN: Kinetin, MS: Murashige and Skoog Medium), Mg: Mile Gram and PGR: Plant Growth Regulators Composition

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