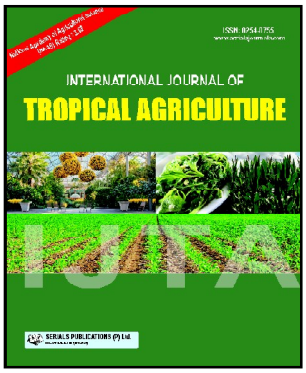

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Influence of Subculturing on *Calotropis procera* (Willd.) R. Br. for Enhanced Shoot Proliferation: An *in vitro* Source of Secondary Metabolites

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Abstract: Plants produce various types of bioactive molecules, phytochemicals and secondary metabolites making them a rich source of different types of medicines. This revival of worldwide interest in medicinal plants reflects recognition of the validity of many traditional claims regarding the value of natural products in healthcare. The plant preparation has very less incidence of adverse reactions and cost effective as compared to modern pharmaceuticals. Hence encouraging both the consuming public and national health care institutions is considering plant medicines and finding ways out to obtain the medicinal constituents from different sources. Several medicinal plants and herbal formulations have been reported for the treatment of various diseases through different mechanisms. The latex of the *Calotropis Procera* is one of the constituents of an Ayurvedic preparation, used for the treatment of asthma, rheumatoid arthritis, nervous disorders as well as for treating diabetes mellitus The free radical scavenging and antioxidant property of the latex of *C. procera* has been shown to be comparable to standard antioxidant, Vitamin C. Medicinal plants indicates that majority of them are flowering plants comprising of trees, herbs, shrubs, climbers etc. It appears that bulk of plant material is obtained from the roots, whole plant, fruits, seeds and bark which are vital for the survival and regeneration of medicinal plants in nature. Destructive harvesting has brought about depletion and scarcity of medicinal plants. Due to rapid depletion of genetic stocks, concerted efforts must be made to evolve new methods for exploiting the phytochemicals for pharmaceutical industries without destroying the nature and environment. The present study is about the methodology adopted to obtain bioactive compounds from *in vitro* cultures which can directly reach to industry maintaining the biodiversity. The influence of subculturing effects on *Calotropis Procera* has been studied to understand maximum availability of the herbal products for the healthcare industries. Subsequent subculturing was done till 6th cycle where maximum shoot proliferation was obtained upto

4th Cycle thereafter resulted in reduced number of shoots. Thus the bioactive components can be taken from the 4th cycle of the subculture and can be used as an *in vitro* source of secondary metabolites.

Key Words: *Calotropis procera*, Ayurvedic preparation, Genetic stocks, Subculture.

Abbreviation: Ms : Murashige and Skoog; SH : Schenk and Hildebrandt; NN : Nitsch and Nitsch); B5: Gamborg's medium; BAP : Benzylaminopurine; AS : Adenine Sulphate

INTRODUCTION

Plants produce diverse types of bioactive molecules, making them a rich source of different types of medicines [1]. This revival of worldwide interest in medicinal plants reflects recognition of the validity of many traditional claims regarding the value of natural products in healthcare. The relatively lower incidence of adverse reactions to plant preparations compared to modern conventional pharmaceuticals, coupled with their reduced cost, is encouraging both the consuming public and national health care institutions to consider plant medicines.Herbal medicines, also referred to as botanical medicine or phyto-medicine, include herbs, herbal materials, herbal preparations and finished herbal products that contain parts of plants or other plant materials as active ingredients. The World Health Organization has estimated that 80% of people in some Asian and African countries rely on herbal medicines for some part of their primary health care and has also advocated traditional medicine as safe remedies for ailments of both microbial and non microbial origin.. Finding healing powers in plants is an ancient idea, especially in India.The *Calotropis procera* under study are wild growing laticiferous plants of family Apocynaceae that have been used in the traditional medicinal system for the treatment of various diseases including those of spleen, liver and abdomen (Kirtikar and Basu, 1935). The latex of *the plant* is one of the constituents of an Ayurvedic preparation, used for the treatment of asthma, rheumatoid arthritis, nervous disorders and diabetes mellitus (Mitra *et al.*, 2002). The latex of *C. procera* has earlier been demonstrated to exhibit anti-hyperglycemic effect in diabetic rats that is associated with its ability to restore hepatic glycogen and normalization of levels of oxidative stress markers (Roy *et al.*, 2005). The free radical scavenging and antioxidant property of the latex of *C. procera* has been shown to be comparable to standard antioxidant, Vitamin C (Mueen Ahmed *et al.*, 2003, 2004). It has anticancerous, ascaricidal, schizonticidal, antimicrobial, anthelmintic, insecticidal, anti-inflammatory, antidiarrheal, larvicidal properties. Calotropis is rich source of cardiac glycosides like calotropin, calotoxin,calactin. It also possess flavonoids, rutin, phenols, saponins [Naz and Bano,2013, Kumar et al., 2013] as its important phytochemicals. In the present study the in vitro studies and influence of subculturing effects on *Calotropis Procera* has been studied to understand maximum availability of the phytomedicines from *in vitro* cultures.

MATERIAL AND METHODS

Collection of seeds

Fresh and healthy fruits were collected from Chhattisgarh region and seeds were collected from the fruits carefully removing the wings thereafter seeds were dried and preceded for surface sterilization.

Surface Sterilization of Seeds

The seeds were surface sterilized using disinfectants. The seeds were first washed in a beaker using 1% Tween 20 for 5 minutes and rinsed using tap water. The seeds were then immediately dipped in 33%

sodium hypochlorite solution and washed with constant stirring for about 15 minutes. The solution was then discarded and the seeds were rinsed using distilled water. Finally, before inoculation, the seeds were sterilized by dipping in 0.1% $HgCl_2$ for 5 minutes. The seeds were then rinsed 2-3 times with autoclaved distilled water. Tween 20 is used as a mild detergent to remove the surface tension and thus allow a better surface contact between the disinfectant and the seeds.

Preparation of medium

Surface sterilized seeds were inoculated on different tissue culture medium supplemented with different concentration of phytohormones to establish axenic shoot cultures. *In vitro* nodes were sub cultured on established medium to study shoot multiplication potential of micro nodes.

Inoculation of the seeds

After washing the seeds in the laminar air hood, they were transferred onto the sterilized petriplate containing autoclaved distilled water. A culture tube containing the media was flame sterilized and forceps were dipped in the test tube containing ethanol and heat sterilized and the seed was immediately inoculated on the media and embedding it in slightly.

Consecutive sub-culturing*:* The micro shoots were sub cultured on the fresh medium every four weeks, for six consecutive sub cultures.

Culture condition*:* All the cultures were kept in culture racks with specific light intensity inside culture room maintained at temperature 25±2° C and a photoperiod of 16 hours light and 8 hours dark.

RESULTS

The seeds were grown in different media. The seeds which do not responded to the media were not considered for further study. Experiments were carried out with the media promoting optimum growth for analysis of multiple shoot proliferation.

Figure 1: 3rd Cycle of Subculture

Figure 3: 5th Cycle of Subculture

Figure 2: 4th Cycle of Subculture

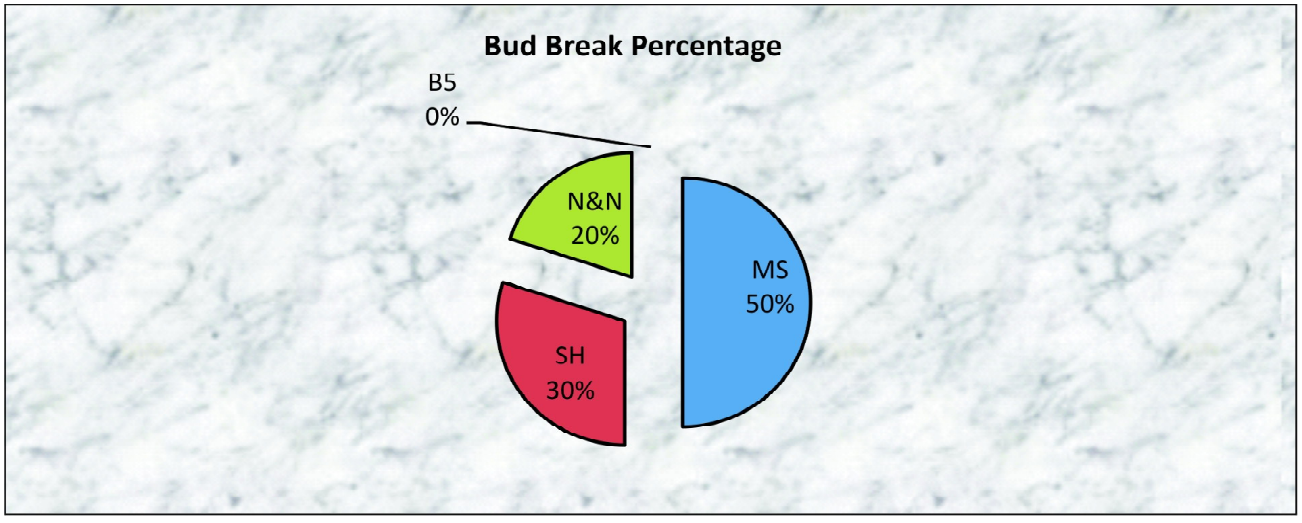
Figure 4: Root Proliferation

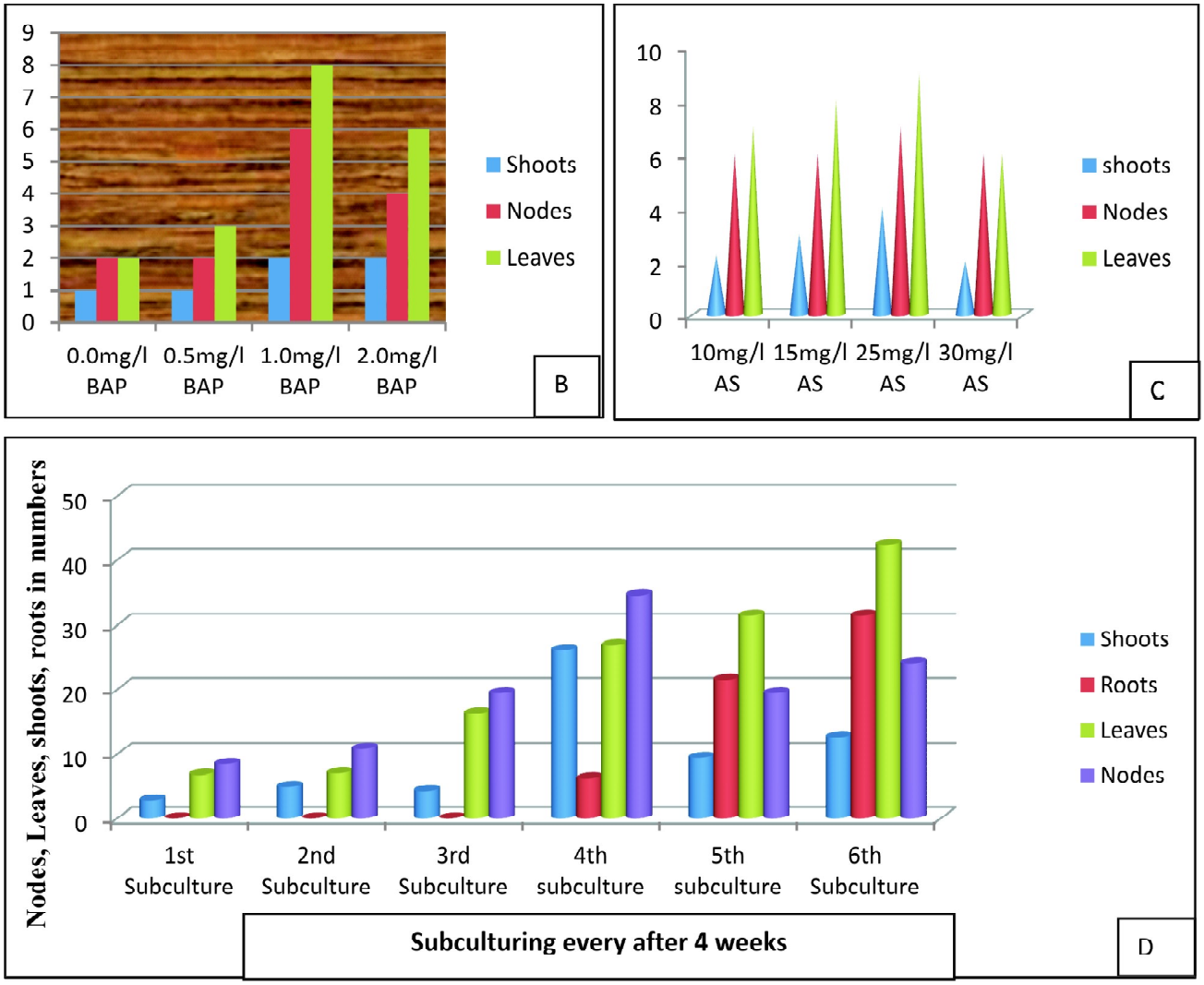
Basal media

Basal B5, MS, N&N, SH medium was prepared and surface sterilized seeds were inoculated and were budbreak response were recorded. 100% budbreak was obtained in MS medium, 60% in SH medium and 40% in N&N medium and 0% in B5 medium. The media selected for further experimentation were MS, N&N and SH medium supplemented with various hormones.

Medium Supplemented with Phytohormone

Various combinations and concentration were tested to check the efficacy in the shoot proliferation. MS medium were supplemented BAP and Kinetin in different concentration and combinations. Best suitable result was obtained in MS medium supplemented 1.0 mg/l of BAP. Later on checked by fortifying with adjuvants and best suitable result was obtained when MS medium supplemented with





Graphs [A]**:** Effect on bud break percentage on MS, SH, N&N and B5 medium [B]: Effect of different concentration of BAP on shoot Proliferation[C]: Effect of adenine sulphate on shoot proliferation [D] Effect of Subculturing on Shoot Proliferation

1.0mg/l BAP and fortified with 25mg/l Adenine sulphate. Than in the same medium combinations the micro nodes were subcultured till 6th cycle to obtain the maximum number of shoots. Maximum shoots were obtained in the 4th cycle thereafter in 5th and 6th cycle there was decrease in number of shoots were observed. 4th subculture also gave rise to root proliferation as well.

DISCUSSION AND CONCLUSION

Calotropis procera is a rich source of phytomedicine which can be further utilize and be exploited to get the important medicinal components out of it. Plant tissue culture is an essential biotechnological tool which has been proven for the development of quality plants and products. Exploitation of medicinal plants for commercial use coupled with the destruction of underground parts, slow reproduction, slow growing and habitat-specific nature, are the crucial factors in meeting the goal of sustainability [Kala,2005,Ghimere, 2005]. The industrial demand for *Calotropis procera* is likely to increase as novel applications are developed. Rapid multiplication techniques and facilities have to be

developed to make improved planting material available in adequate amounts. , Growing the medicinal plants in a controlled environment is an alternative option to minimize variability and for conservation and protection of natural resources. Plant tissue cultures have been used as a source of pharmacologically active secondary metabolites [Vanisree et al., 2004]. The in vitro growth and effect of subculturing effect has been studied in the present research work to identify the maximum supply of secondary metabolites can be obtained. The medium was established in MS medium supplemented with 1mg/l BAP fortified with 25mg/l of adenine sulphate which signifies the importance of the adjuvants to enhance the shoot proliferation as well justified about the factors controlling the in vitro regeneration of *Stereospermum suaveolens* (Shukla et al., 2012) the Maximum shoot proliferation was obtained till 4th cycle which resembles with the results obtained by the authors studied the influence of subculturing on *Dioscorea hispida* (Shukla and Shukla, 2014). The author in the present study has developed a full micropropagation protocol of *Calotropis procera* in her Applied Plant Biotechnology Research Lab at Amity Institute of Biotechnology, Amity University, Noida Uttar Pradesh, India. Tissue culture studies were earlier reported from Calotropis on in vitro differentiation of laticifer from seedling organ and callus cultures (Dhir, et al.,1984). In these studies NAA and 2,4-D have induced laticifers in culture conditions at various concentrations. In vitro regeneration using mature plant parts were known from the reports of Roy and De (1986). Similarly rapid shoot multiplication of Calotropis gigantean has been reported (Amuthapriya and Ravichandran, 2014). Till date full micropropagation protocol with maximum shoot proliferation upto 26 shoots has not been reported yet. This protocol will be applied for conservation and mass multiplication as well as the plants and plant products produced in this study can be used for further biochemical or other studies related to health care industries and institutes.

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