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Pharmaceutical Sciences **Research Article……!!!**

"ROLE OF DIFFERENT CARBON SOURCES ON IN VITRO NODAL PROPAGATION OF *ALTERNANTHERA SESSILIS* (L.) R.Br. *ex* DC."

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KEYWORDS:

Alternanthera sessilis, Amaranthaceae, micropropagation, carbon sources.

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ABSTRACT

Alternanthera sessilis (L.) R.Br. *ex* DC. is a Perennial herb. It belongs to the family Amaranthaceae. It is used as a topical treatment for the common skin problem cane vulgaris. The antioxidant carotene is found in large amounts in the plant. In India and Sri Lanka, it is used for treatment of gastrointestinal problems. *In vitro* micropropagation is an effective mean for rapid multiplication of species. In comparison with the abundant pharmacological data and preclinical studies, information regarding the role of carbon source in Plant Tissue culture of *A.sessilis* is rare. This study attempted to quantify the effects of four types of sugars(Sucrose, Glucose, Fructose and Maltose) as carbon sources, and their concentrations, on the *in vitro* shoot proliferation and rooting of *A. sessilis*. Four types of carbon sources of 0.1mg/ml were used to study their effect on shoot regeneration from excised nodal explant. Among the different sugars, sucrose was found to be superior for plant regeneration. These results indicate that different carbohydrates significantly affected the success rate of culture. Sucrose produced the best shoot elongation. Limited growth of shoot length was observed on medium with fructose and maltose.

INTRODUCTION:

Alternanthera sessilis (L.) R.Br. *ex* DC. is a Perennial herb. It belongs to the family Amaranthaceae. It has many-branched. Its habit and dimensions vary greatly depending on the humidity level: in dry conditions, it is erect and can reach 30 cm in length; in wet conditions, it is prostrate, then erect with stems 10cm-1 m long. In flooded areas, it is a floating herb reaching several meters in length. It is used as a topical treatment for the common skin problem cane vulgaris. The antioxidant carotene is found in large amounts in the plant (Jerajoni *et al*., 2004). In Southeast Asia, young shoots and leaves are ingested as vegetables. In folklore, *A. sessilis* was used for treating sick individuals. Gayathri *et al* (2006) stated that *A. sessilis* is used for the treatment of biliousness, dyspepsia associated with sluggish liver, chronic congestion of liver, acute and chronic pyelitis, cystitis, gonorrhea and snake bite in Sri Lanka. In India and Sri Lanka, it is used for treatment of gastrointestinal problems. *In vitro* micropropagation is an effective mean for rapid multiplication of species in which it is necessary to obtain a high progeny uniformity. Therefore, the interest in using these techniques for rapid and large-scale propagation of medicinal and aromatic plants has been significantly increased. In comparison with the abundant pharmacological data and preclinical studies, information regarding the role of carbon source in Plant Tissue culture of *A.sessilis* is rare. In vitro propagation of *A.sessilis* through nodal segments or axillary buds has previously been reported by Bennici and Schiff, 1997, Boro *et al*., 1998 and Flores *et al*., 1982. This study attempted to quantify the effects of four sugars as carbon sources, and their concentrations, on the *in vitro* shoot proliferation and rooting of *Alternanthera sessilis*.

MATERIALS AND METHODS

Nodal segments from mature plants of *Alternanthera sessilis* were used in the present study. The explants were collected, washed thoroughly under running tap water for 15 min, treated in 1% Savlon for five min and were surface sterilized with 0.1% $HgCl_2$ for different lengths of time to ensure contamination-free culture. Thereafter the explants were washed three - four times with autoclaved distilled water to remove traces of $HgCl_2$ inside a laminar air flow cabinet. The surface sterilized shoots were cut into 1.0 - 1.5 cm long segments, each containing a single node. The cut segments were then cultured individually on MS medium supplemented with Indole-3-Acetic acid (0.50 – 2.0mg/l) and Benzyl Amino Purine (0.50-2.0 mg/l). The pH was adjusted to 5.7 ± 0.1. All media were steam sterilized under 1.1 kg/cm^2 pressure at 121°C. Cultures were grown at 26 ± 1°C under 16 h photoperiod with a light intensity of 2000 - 3000 lux. Well rooted plantlets were carefully removed from culture vessels, washed under running tap water to remove the remnants of agar. After proper removal of agar dip the plantlet in the IAA solution (0.1%) for

1 minute and transferred to tray containing sand . For 1 week, the potted plantlets were kept under transparent polythene membrane to ensure high humidity, and then they were kept in open in diffuse light for hardening. After 7 days, the surviving plants were transferred to pots containing garden soil and maintained in green house for acclimatization. Data collected were analyzed and results were presented in the tables.

TABLE – 1. COMPOSITION OF MURASHIGE AND SKOOG(MS) MEDIUM(1962)

Group	Components	Final concentration (mg/l)	Stock solution (mg/500ml)	Volume of Stocksolution to be taken(ml/l)
1. 2.	Ammonium nitrate Potassium nitrate	1650.00 1900.00	- -	- -
3. A	Magnesium sulphate Manganese sulphate Zinc sulphate Copper sulphate	370.00 16.09 8.06 0.025	18.05 0.845 0.43 0.00125	10
B	Calcium chloride Potassium iodide Cobalt chloride	440.00 0.83 0.025	220.00 0.415 0.00125	10
C	Potassium dihydrogen Orthophosphate Boric acid Disodium molybdate	170.00 6.02 0.25	8.5 0.31 0.0125	10
4. 5.	Ferrous sulphate Sodium EDTA	27.84 37.24	2.784/100ml 3.724/100ml	2
D	Thiamin-Hcl Nicotinic acid Pyridoxine-Hcl Glycine	1.00 0.50 0.50 2.00	0.05 0.025 0.025 0.1	10
6. 7. 8.	Myo-Inositol Sugars Agar- 0.6% PH -5.8	100.00 30g		

TABLE – 2 PREPARATION OF HORMONAL STOCK

Name of the Hormone	Concentration in 100ml	Preparation	Stock concentration
Indole -3- Acetic acid	**100mg**	**Dissolve in 1 ml of 0.1NNaoH and made up the volume to 100 ml by SMF**	**1mg/ml**
Benzyl Amino Purine	**100mg**	**Dissolve in 1 ml of 0.1N Hcl and made up the volume to 100 ml by SMF**	**1mg/ml**

Table 3. Effect of carbon source on the shoot and root proliferation on the fifth day

S.NO	CARBON SOURCE	SHOOT LENGTH (mm)	NO. OF SHOOT	ROOT LENGTH (mm)	NO. OF ROOT
1.	FRUCTOSE	5±0.3	1±0.4	-	-
2.	GLUCOSE	14±0.4	1±0.2	1±0.1	2±0.12
3.	SUCROSE	21±0.6	1±0.1	2±0.2	3±0.01
4.	MALTOSE	9±0.25	1±0.2	1±0.2	1±0.02

Table 4. Effect of carbon source on the shoot and root proliferation on the tenth day

S.NO	CARBON SOURCE	SHOOT LENGTH (mm)	NO. OF SHOOT	ROOT LENGTH	NO. OF ROOT (mm)
1.	FRUCTOSE	10±0.05	1±0.2	-	-
2.	GLUCOSE	30±0.32	1±0.01	2±0.03	5±0.10
3.	SUCROSE	40±0.10	1±0.12	3±0.02	6±0.02
4.	MALTOSE	20±0.60	1±0.01	1±0.02	2±0.07

RESULTS

A perusal of the data in Table 3 & 4 reveals that effect of different concentration of sugars on shoot regeneration in *Alternanthera sessilis*. Four types of carbon sources were used to study their effect on shoot regeneration from excised nodal explant. Among the different sugars, sucrose was found to be superior for plant regeneration. The highest length of shoot from proliferated explant was observed by on medium supplemented with sucrose(Fig.1(a)), (table 4). The medium supplemented with Glucose showed moderate level of shoot growth. Cultures grown on the maltose supplemented media showed very poor response, with less numbers of shoots per proliferated explant. Rooting percentage depended on the type and concentration of the carbohydrate present in the medium (Fig. 2a). Maximum rooting was observed in the medium containing sucrose. On the fifth day, no roots were observed on fructose (Table 3). On the tenth day, roots were formed on glucose, maltose and sucrose except fructose (Table 4).

These results indicate that different carbohydrates significantly affected the success rate of culture. Sucrose produced the best shoot elongation. Limited growth of shoot length was observed on medium with fructose and maltose. For acclimatization, plantlets were removed from rooting medium after three weeks of incubation and transferred to plastic pots containing autoclaved soil trite covered with perforated polythene bags to maintain humidity and were kept under culture room conditions for one week. After three weeks, polythene bags were removed

and transferred to green house and placed under shade until growth was observed (Figure 2b). Then they were planted under normal garden conditions.

Fig. 1. ALTERNANTHERA SESSILIS

Fig. 1(a) SHOOT INITIATION ON DAY 5

Fig 2. SHOOT INITIATION ON DAY 10

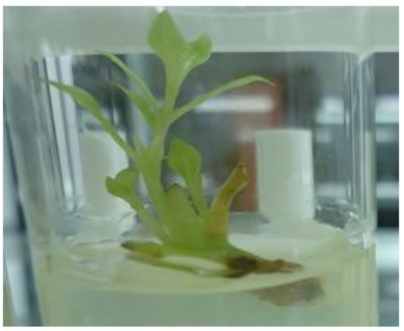
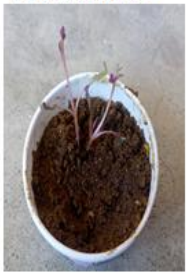


Fig 2(A) ROOT INITIATION

Fig 2(B) HARDENING



DISCUSSION

Several reports have documented that the carbon source affects the *in vitro* morphogenesis of different plant species(Fuentes *et al*, 2000). Carbohydrates are required in living cells as a source of energy and carbon frameworks for biosynthetic processes. The carbon sources serves as energy and absorbent agents to advocate the growth of plant tissues(Lipavska and Konradova, 2004). In addition growth and root induction are massively energy claiming processes that can occur at the expenditure of available metabolic substrates, which are substantially carbohydrates (Calamar and Klerk, 2002). Sucrose have been validated to be better shoot proliferation than other carbon sources in micropropagation(Kumara Swamy *et al*., 2010). In the present study high percentage of seedling growth was attained on sucrose supplemented medium. In *Solanum nigrum* L., among the different carbon sources fructose at 4 % proved to be better choice for multiple shoot rejuvenation followed by sucrose, maltose and glucose(Tauquer *et al*., 2007) Shoot length declines at higher concentration of carbon sources may be due to the inhibition of organogenesis. Still, further exploration is required to know the impact of carbon sources on development of shoots and the physiological changes during growth of *A. sessilis*. Proliferation of shoots from *A. sessilis* of this study was firmly affected by carbon source. The relations between different types of carbohydrates used were significant for such parameters as shoot

number, shoot height, and length. Medium supplemented with sucrose greatly enhanced shoot proliferation and elongation while fructose didn't stimulate rooting of microcuttings. The results of this study give evidence that as far as root formation is concerned, proliferated shoots of *A. sessilis* can use sucrose better than glucose and Maltose Carbon source, each at 3 % concentration (fructose, glucose, lactose, maltose and sucrose) was tested for root induction in *Withania somnifera* and sucrose gave the high percentage result at 100% rooting while fructose gave 65% root induction (Sivanesan and Murugesan 2008). This study shows a better rooted plantlets on sucrose, and moderate on glucose and maltose and this appears to be the most favourable source of carbon for this species. The role of carbohydrates during cell division and cell differentiation is closely related to their effect on plant metabolism and development (Rolland *et al*., 2006). Gabryszewska(2011) has shown inhibitory effect of high concentration of sucrose in growth of axillary shoots of *Syringa vulgaris*. In this study we can found that carbohydrates also inhibited the growth of axillary shoots. Responses of *in vitro* cultures to different kinds of carbohydrates in the medium are tested constantly and results appear species-specific. Although carbon sources are of high significance for *in vitro* organogenesis, carbon metabolism in vitro is still not easily understood (Kozai, 1991).

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