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INFLUENCE OF DIFFERENT CARBON SOURCES ON IN VITRO PROPAGATION OF WEDELIA CHINENSIS (OSBECK) MERR.

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Abstract : *Wedelia chinensis* is a procumbent, imperishable herb, with light camphor-like odour. It belongs to the family Asteraceae. The leaves are used for dyeing greyhair, and for promoting their growth. The root is pounded and used as a black color with salts of iron. It's also used in enlarged liver and spleen. A decoction of the herb is deobstruent; used in uterine haemorrhage and menorrhagia. In vitro micropropagation is an effective mean for rapid-fire addition of species. In comparison with the abundant pharmacological data and preclinical studies, information regarding the role of carbon source in Plant Tissue culture of W. chinensis is rare. Hence, this study is tried to quantify the effects of four types of sugars(Sucrose, Glucose, Maltose and Fructose) as carbon sources, and their concentrations, on the in vitro shoot proliferation and rooting of *Wedelia chinensis*. Four types of carbon sources of 0.1 mg/ ml were used to study their effect on shoot regeneration from excised nodal explant. Fructose produced the best shoot elongation. Limited growth of shoot length was observed on medium with glucose and maltose. These results indicate that different carbohydrates significantly affected the success rate of culture.

***IndexTerms - Wedelia chinensis,* Asteraceae, micropropagation, carbon sources.**

INTRODUCTION

Wedelia chinensis is a procumbent, perennial herb, with light camphor-like odour, met with in wet places in Uttar Pradesh, Assam, Arunachal Pradesh and all along the coastal areas. It belongs to the family Asteraceae. The stem is 45cm tall; leaves sub-sessile, linear-oblong, oblanceolate; heads solitary, bright yellow, on long axillary peduncles, upto 4 cm in diameter. The leaves are used for dyeing greyhair, and for promoting their growth; their juice is used for tattooing, the colour produced being a deep indelible blueback. The root is pounded and used as a black dye with salts of iron. The leaves are regarded as tonic and alternative useful in cough, diseases of skin especially alopecia (Kumari and Bhatnagar, 2016). The expressed juice of herb contained an oil-soluble black-dye, tannin, carotene, chlorophyll, phytosterol, wax and resin. The leaves contain wedelolactone and demethylwedelolactone (Meena *et al*., 2011). The leaves are alterative and hair tonic; used for promoting hair growth; useful in cough, cephalalgia and skin diseases. It is also used in enlarged liver and spleen. A decoction of the herb is deobstruent; used in uterine haemorrhage and menorrhagia (Kirthikar and Basu, 2006. Hence, this study is attempted to quantify the effects of four sugars (Sucrose, Glucose, Maltose and Fructose) as carbon sources, and their concentrations, on the *in vitro* shoot proliferation and rooting of *Wedelia chinensis*.

MATERIALS AND METHODS

Nodal segments from mature plants of *Wedelia chinensis* 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 containing different carbon source (Sucrose, Glucose, Maltose and Fructose) supplemented with Benzyl amino purine (BAP) (2.0mg/l) and Indole -3-Butyric acid (IBA) (0.15 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. Rooted plantlets were thoroughly washed to remove the adhering gel and planted specially made plastic cup containing soilrite and kept in the greenhouse for acclimatization. Twenty cultures were used per treatment and each experiment was repeated at least three times. Percentage of success was scored four weeks after culture. 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.	Ammonium nitrate	1650.00	-	-
2.	Potassium nitrate	1900.00	-	-
3. A	Magnesium sulphate	370.00	18.05	10
	Manganese sulphate	16.09	0.845	
	Zinc sulphate	8.06	0.43	
	Copper sulphate	0.025	0.00125	
B	Calcium chloride	440.00	220.00	10
	Potassium iodide	0.83	0.415	
	Cobalt chloride	0.025	0.00125	
C	Potassium dihydrogen Orthophosphate	170.00	8.5	10
	Boric acid	6.02	0.31	
	Disodium molybdate	0.25	0.0125	
4.	Ferrous sulphate	27.84	2.784/100ml	2
5.	Sodium EDTA	37.24	3.724/100ml	
D	Thiamin-Hcl	1.00	0.05	10
	Nicotinic acid	0.50	0.025	
	Pyridoxine-Hcl	0.50	0.025	
	Glycine	2.00	0.1	
6.	Myo-Inositol	100.00		
7.	Sucrose	25g		
8.	Agar- 0.6%			
	PH -5.8			

Table 2 Preparation of Hormonal Stock

Name of the Hormone	Concentration in 100ml	Preparation	Stock concentration
Indole -3- Butyric 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

RESULTS

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	NO. OF LEAVES
1.	Sucrose	2±0.2	1±0.1	1±0.2
2.	Glucose	5±0.1	1±0.2	3±0.12
3.	Maltose	9±0.3	1±0.2	4±0.15
4.	Fructose	9±0.1	1±0.3	4±0.2

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)	NO. OF LEAVES
1.	Sucrose	4±0.3	1±0.12	2±0.03	1±0.3	5±0.4
2.	Glucose	10±0.1	6±0.04	3±0.16	1±0.1	15±0.2
3.	Maltose	12±0.3	3±0.02	4±0.14	1±0.3	18±0.6
4.	Fructose	15±0.5	8±0.23	5±0.16	1±0.5	24±0.1

The type and concentration of the carbon source in the culture medium had significant effects on the number of shoots per explant. Fructose was the most effective of the four sugars tested. The highest number of shoots per proliferated explant (8±0.23) was obtained on medium supplemented with fructose, and the longest shoots (15±0.5) were observed by fructose (Table 4). Cultures grown on the sucrose supplemented media showed very poor response, with low numbers of shoots per proliferated explant. On the fifth day, no roots were observed on the four carbon source. On the tenth day, roots were formed on sucrose, glucose, maltose and fructose. These results indicate that different carbohydrates significantly affected the success rate of culture. Fructose produced the best shoot elongation. Limited growth of shoot length was observed on medium with glucose and maltose.

DISCUSSION

Carbohydrates are necessary in living cells as a source of energy and carbon skeletons for biosynthetic processes. It is well established that carbohydrate requirements depend upon the stage of culture, and may vary from species to species (Thompson and Thorpe 1987). Proliferation of *W. chinensis* cultures in this study was strongly affected by carbon source. The interactions between different types of carbohydrates used were significant for such parameters as shoot number, and shoot length. Medium supplemented with 30 g dm-3 fructose greatly enhanced shoot proliferation and elongation while sucrose. Poor response to sucrose in shoot proliferation might be due to slow break up of sucrose into glucose and fructose. Invertase is required for an efficient conversion of sucrose into glucose and fructose (Pua and Chong 1984), and its levels may vary depending on the species and tissues used. More success with shoot proliferation on the medium containing fructose may be associated with a better utilization of fructose with the sufficient availability of enzymes that help in the hydrolysis of fructose. The enzymatic conversion of fructose into fructose-6-phosphate can make fructose readily available for the tissues and thus used as carbon structure for new growth (Dennis and Greyson 1987). Our results showed that fructose was more effective than other sources of carbon. Similarly, growth of shoots from non-dormant buds of mulberry is not promoted by sucrose, but can be promoted by fructose, maltose or glucose (Oka and Ohyama 1982).

The results of this study provide evidence that as far as root formation is concerned, proliferated shoots of *W. chinensis* can utilize fructose 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 best result at 100% rooting while fructose gave 65% root induction (Sivanesan and Murugesan 2008). This study shows a better rooted plantlets on fructose, and moderate on glucose and maltose and this appears to be the most favorable 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 high concentration of carbohydrates also inhibited the growth of axillary shoots. Responses of in vitro cultures to different kinds of carbohydrates in the medium are tested frequently and results appear species-specific.

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