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ROLE OF DIFFERENT CARBON SOURCES ON IN VITRO MICROPROPAGATION OF OXALIS CORNICULATA (L.)

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ABSTRACT

The response towards nodal explants of O.Corniculata with regard to multiple shoot regeneration was experienced on media containing different carbohydrates Sucrose, Fructose, Maltose and Glucose. The substitution of these sugars shown different responses in different concentrations. Data was collected in terms of regeneration frequency and growth rate. The plant growth regulators BAP and NAA are used on MS Media containing various carbon sources. Among all the concentrations of carbohydrates (1-6%) studied, fructose (2%) showed best performance with maximum shoots (24.4±0.2) and shoot length (13.6±0.1). And also shoot regeneration and shoot multiplication appreciably increased in fructose. This was in line with sucrose, maltose and glucose. The least number of shoots was observed in glucose (10.5±0.4) and the least shoot length (4.4±0.2) with very stunted growth was obtained in maltose. This results noticeably indicates that fructose (2%) is proved to be best for multiple shoot regeneration from nodal explants of O.corniculata. The best response for rooting was obtained in MS Medium supplemented with IBA at 2.0 mg/l.

KEY WORDS

Oxalis corniculata.L, Carbon sources, Invitro regeneration, Fructose, Sucrose, Maltose and Glucose.

INTRODUCTION

In vitro culture of plant cell, tissue and organ is largely determined by the composition of nutrient medium. One of the most important components of nutrient medium is carbohydrate that strongly affects the growth and morphogenesis of *in vitro* cultured plant as it acts as a carbon source and osmoticum both [1]. In plant tissue culture, sugar serves as a carbohydrate supply to provide an optimum culture condition for cell. Among the sugars, sucrose is most popular carbon source used in plant tissue culture. Sugar is a very important constituent and its accumulation in the medium is crucial for *in vitro* growth and development of plants because photosynthesis is deficient, due to the weak development of their leaves, the limited gas exchange and the high relative humidity [2, 3, and 4]. Glucose and fructose are also known to hold up for growth of some tissues [5]. The sugar concentration favoured is very essential for growth and development of plant. In general, with increased sugar concentration, the growth and development increases, until an optimum is reached and then decreases at higher concentrations. The *in vitro* plantlets have need of a carbohydrate supply in order to meet up the energy levels [6]. In broad range sucrose is used as a foremost carbon source for *in vitro* plant culture, since it is the common carbohydrate in the phloem sap of many plants [7]. The current study aims to optimize the finest carbon source for efficient shoot multiplication of *Oxalis corniculata*.

O.corniculata.L a member of *Oxalidaceae* family is an essential medicinal plant disseminated in tropical and sub-tropical parts of India. *O.corniculata*, the creeping wood sorrel, also called procumbent yellow- sorrel or sleeping beauty resembles the common yellow wood

sorrel. The bright yellow flowers and soft green foliage of common yellow oxalis adds a shade of tranquillity to any space. The leaves of this species of oxalis grow in clusters of three and contain a manifestation similar to clover [8]. *Oxalis corniculata* is naturally occurring weed that has been used in traditional medicine for the cure of dysentery and diarrhoea in India [9]. The leaves are used for curing various diseases like cold, fever, cough, stomach-ache, stops bleeding from wounds and as anthelmintic [10]. (The present study was undertaken to optimize the best carbon source for *in vitro* shoot proliferation of field grown *O.corniculata* from nodal explants.

MATERIALS AND METHODS

The Single nodal segments of *Oxalis corniculata* were used for establishment of cultures collected from Herbal garden, Department of Biotechnology, Dravidian University, Kuppam, and Andhra Pradesh, India.

Establishment of *in vitro* cultures:

Nodal explants of *O.corniculata* were collected from the young sprouts of the stock explants were selected as explants. Nodal explants were washed thoroughly in running tap water for 10 minutes, followed by immersing in liquid detergent solution 5% (v/v) tween-20 for 20 minutes and then washed under running tap water. Then the explants were given 0.4% (w/v) bavistin treatment, a systemic fungicide (BASF India Ltd.) for about 15 to 20 minutes. After sterilizing with fungicide, the explants were surface sterilized with 70% (v/v) ethanol for 90 seconds. Then the next treatment is followed by the surface sterilization of explants with mercuric chloride 0.1% (w/v) (Merck india) for 1-3 minutes and thoroughly washed with sterile double distilled water for thrice to eliminate the traces of mercuric chloride before inoculation.

Culture medium:

To observe the effects of carbon sources MS Medium [11] supplemented with different concentrations of carbon sources such as fructose, glucose, sucrose and maltose ranging from 1-6% (w/v) is used throughout the study in combination with BAP and NAA. The P^H of the medium was fine adjusted to 5.8 before autoclaving and solidified with agar 0.8% (w/v). Molten medium was dispensed 15 ml (approximately) into each culture tube (25×150mm) and plugged with non-absorbent cotton. The medium was autoclaved at 15 lbs/sq inch pressure and 121^{0}C for 20 minutes. MS basal medium augmented with various plant growth regulators tested for shoot initiation and proliferation.

Culture conditions:

All the cultures were maintained at 26±2^{0}C temperature and 60-70% relative humidity. The photoperiod of 16 hours day light and 8 hours dark having 3000 lux light intensity by cool white fluorescent tubes (Phillips india Ltd.). The cultures which are obtained were transferred to fresh culture medium after 21 days for development of *in vitro* rooting.

Data and statistical analysis:

In all the cultures, visual observations were recorded such as nodal shoot proliferation, Shoot number per explants, length of the regenerated shoots, root number per explants and average root length. In all the experiments, 20 replicates were taken in each treatment and each experiment repeated thrice. The one-way analysis of variance (ANOVA) appropriate for the design was carried out to detect the significance of differences among the treatment means. The treatment means were compared using Duncan's multiple range test (DMRT) ($p < 0.05$) and were presented as the average ± standard error (SE) using software SPSS 11.5.

RESULTS AND DISCUSSION

Four different types of carbon sources with six different concentrations were used for Shoot multiplication in MS Medium supplemented with 1mg/l BAP + 0.5mg/l NAA. The basal medium supplemented with sucrose, maltose, fructose and glucose with different concentrations (1-6%) were used to evaluate the effects of individual sugars on direct shoot regeneration from nodal explants of *O.corniculata*. The data was recorded after 4 weeks of inoculation. Among all the carbon sources with different concentrations, fructose performed well and this was followed by sucrose, maltose and glucose. The maximum number of shoots was recorded in medium containing BAP and NAA augmented with 2% fructose (w/v) (24.4±0.2) with shoot length (13.6±0.1). And the regeneration frequency is about 92%. The second best carbon source was found to be 3% sucrose (21.4±0.1) and the

maximum shoot length was obtained in 3% sucrose (w/v) (14.3±0.1) cm. The highest regeneration frequency of about 96% was observed in 3% sucrose. In maltose, the maximum number of shoots was obtained at 4% and the shoot length at 3%. The shoots and shoot length obtained in maltose carbon source contain very stunted appearance. The regeneration frequency obtained is 85%. Where as in glucose at 3% the least mean number of shoots (10.5±0.4) and shoot length (10.1±0.2) are obtained and the frequency of regeneration dropped to 82%. From the above results it is clear that, lower concentrations of carbohydrates favour the shoot regeneration and proliferation whereas at high concentration it reduces the shoot organogenesis.

In all the carbon sources, the frequency of shoot regeneration, mean number of shoots and mean number of shoot length was increased profusely up to 1-3% and this was contrary in the concentrations of 4-6%, where the regeneration frequency, shoot number and shoot length was decreased.

Rooting and acclimatization of plants:

The Shoots which were developed *in vitro* were excised from the medium, and it was transferred to fresh MS Medium augmented with different concentrations of auxins such as IAA and IBA. By comparing all the concentrations, the best rooting was obtained in IBA. The highest regeneration frequency of rooting is about 97% and the maximum number of roots (25±0.6) and root length (6.3±0.1) was recorded at 2mg/l IBA.

In vitro developed roots were removed carefully from culture tubes and washed to remove the leftovers of agar (Fig 2F). The plantlets were transferred to polybags containing soil and vermiculite in 1:1 ratio for hardening [12]. Finally, the hardened plantlets were transferred to pots and acclimatized to natural environment.

DISCUSSION

Plant tissue culture techniques are innermost to innovative areas of applied plant sciences, including plant biotechnology and agriculture. The growth and multiplication of shoots *in vitro* are affected by many factors [13]. The exogenously added carbon sources to the medium supplies energy and also to uphold the osmotic potential [1]. The effect of the suitable carbon source and concentration also determines the health and viability of the *in vitro* plants. Sucrose 3% was commonly used as a source of carbohydrate. The carbon source, sucrose is broken down during autoclaving and transformed to glucose and fructose by the action of invertase [2]. Glucose is then utilized first and followed by fructose. It was observed that more and larger roots with an increase in sucrose concentration from 7.64 to 262.93mM [14]. Sucrose was reported to act as morphogenetic trigger in the formation of axillary buds and branching of adventitious roots [15]. Maltose is also used as a carbohydrate source for plant regeneration. But seedling growth was observed in *zeamays* [16] in the presence of maltose. The effect of glucose was emphasized in *Prunus mume. Koehne* [17]. The property could be either due to uptake or insufficient enzyme activity.

In the present investigation, four different carbon sources were used. (Sucrose, Fructose, Maltose and Glucose). In our study, fructose showed best performance compared to sucrose, maltose and glucose. Similar reports were obtained in *Mentha piperita* [18] and *Coffea canephora* [19].

CONCLUSION

In the above study, observations showed that, the rooting and multiplication of shoots of *Oxalis corniculata*. Nodal explants *in vitro* are affected by the type of exogenous carbon source added to the medium. By comparing all the carbon sources, fructose has proved to be superior for multiple shoot proliferation and this was in line with sucrose, maltose and glucose. Further research is required to explore the effects of different variety of carbon source on *in vitro* plant regeneration of *O.corniculata*.

ABBREVIATIONS

NAA- 1-Napthalene acetic acid
IAA- Indole -3-acetic acid
IBA-Indole -3-butyric acid
BAP- 6-Benzyl amino purine
Kn - 6-Furfuryl amino purine

Table 1 Direct shoot regeneration from nodal explants of field grown *O.corniculata* microshoots using different carbon sources.

Carbohydrate (%)	Regeneration Frequency (%)	Mean no. of shoots	Mean shoot length
Sucrose			
1%	75	9.3±0.15^{f}	7.3±0.15^{g}
2%	80	13.3±0.15^{h}	11.2±0.05^{l}
3%	95	21.4±0.1^{n}	14.3±0.15^{o}
4%	76	16.2±0.25^{j}	12.3±0.32^{m}
5%	70	14.0±0.1^{i}	7.16±0.15^{g}
6%	64	8.2±0.25^{e}	6.4±0.2^{f}
Fructose			
1%	85	18.2±0.25^{l}	12.4±0.4^{m}
2%	92	24.4±0.2^{o}	10.2±0.3^{k}
3%	83	20.6±0.1^{m}	13.6±0.1^{n}
4%	76	17.2±0.25^{k}	9.3±0.^{15j}
5%	65	10.4±0.1^{g}	7.2±0.05^{g}
6%	60	7.3±0.15^{d}	6.3±0.15^{f}
Maltose			
1%	55	8.0±0.5^{e}	2.2±0.25^{a}
2%	60	9.7±0.25^{f}	2.8±0.28^{bc}
3%	63	10.4±0.20^{g}	4.4±0.2^{d}
4%	80	13.3±0.28^{h}	3.0±0.1^{c}
5%	75	9.3±0.3^{f}	2.7±0.2^{bc}
6%	70	4.5±0.4^{a}	2.4±0.05^{ab}
Glucose			
1%	65	6.1±0.15^{c}	5.3±0.15^{e}
2%	72	7.1±0.2^{d}	7.7±0.2^{h}
3%	85	10.5±0.4^{g}	10.1±0.28^{k}
4%	82	6.3±0.15^{c}	8.3±0.15^{i}
5%	75	5.5±0.6^{b}	6.1±0.2^{f}
6%	66	4.4±0.4^{a}	4.4±0.2^{d}

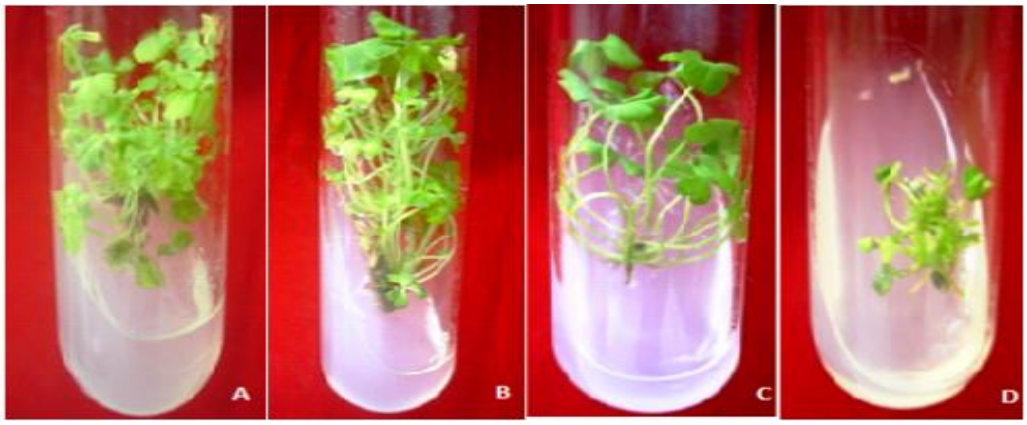
Data represent treatment means ± SE followed by different letter (s) within a column indicate significant differences according to ANOVA and DMRT test (P <0.05).

Table 2: Root organogenesis of microshoots from field grown *O.corniculata* supplemented with various concentrations of IAA and IBA on full strength MS medium.

Plant growth regulators (mg/l)		Regeneration	Mean no. of	Mean Root
IAA	IBA	Frequency (%)	Roots/explants	Length (cm)
0.5	-	78	13.2±0.3^{a}	3.0±0.05^{a}
1.0	-	85	16.5±0.3^{c}	3.8±0.26^{b}
2.0	-	95	17.3±0.28^{d}	4.2±0.25^{cd}
3.0	-	82	14.3±0.28^{b}	3.3±0.15^{a}
-	0.5	78	16.0±0.4^{c}	3.9±0.1^{bc}
-	1.0	86	18.0±0.5^{e}	4.3±0.28^{d}
-	2.0	97	25.0±0.6^{g}	6.3±0.15^{f}
-	3.0	84	21.9±0.3^{f}	5.3±0.1^{e}

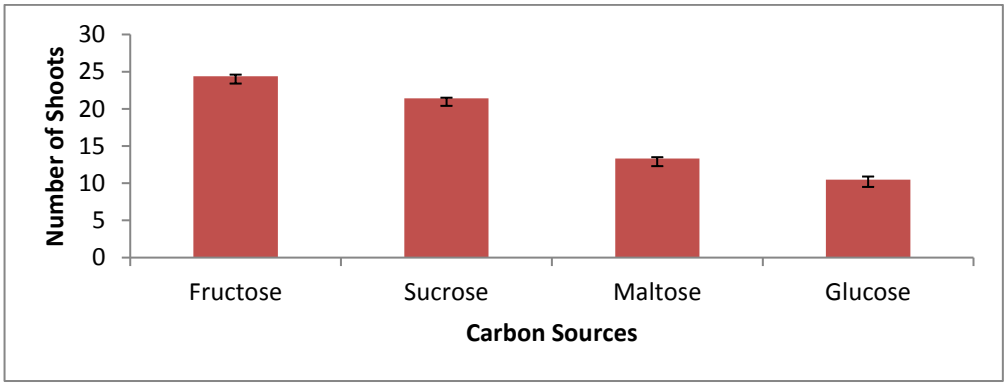
Data represent treatment means ± SE followed by different letter (s) within a column indicate significant differences according to ANOVA and DMRT test (P< 0.05).

Fig. 1: Effect of different carbon sources on *in vitro* shoot proliferation of *O.corniculata* on MS + 1.0 mg/l BAP + 0.5 mg/l NAA Supplemented with different concentrations of carbon source.

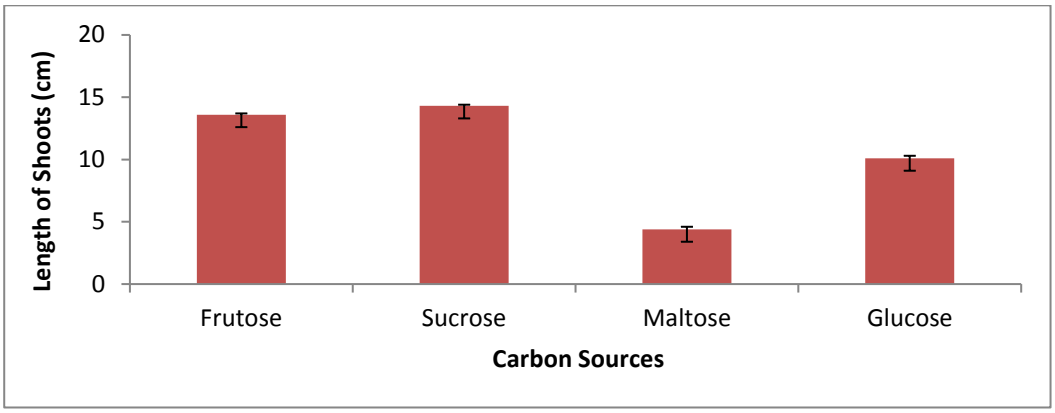


A.MS+3% Sucrose B.MS+2% Fructose C.MS+3% Glucose D.MS+4% Maltose

Fig. 2: E. Rooting of *in vitro* regenerated shoots on MS Medium supplemented with IBA at 2.0mg/l; F. *In vitro* regenerated microshoots with roots ready for hardening; G. Plantlets kept in poly bags for hardening in greenhouse conditions; H. Hardened Plantlets transferred to earthen pots having soil and vermiculite in 1:1 ratio.



A. Effect of different carbon sources on shoot number of *O.corniculata*



B. Effect of different carbon sources on shoot length of *O. corniculata*

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