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Impact of Different Carbohydrates and Their Concentrations on *in Vitro* Regeneration of *Solanum viarum* (Dunal)—An Important Anticancer Medicinal Plant

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ABSTRACT

In this present study, the effect of various carbon sources such as sucrose, glucose, fructose and maltose was investigated on *in vitro* shoot regeneration of *Solanum viarum* using axillary bud explants. The frequency, growth and multiplication rate were highly influenced by the type and concentration of carbon source used. Among the different concentrations (1% - 6%) of carbohydrates studied, the maximum number of shoots (22.6 ± 0.50) and shoot length (5.92 ± 0.13 cm) was obtained on MS medium supplemented with 4% (w/v) fructose. The least number of shoots (1.5 ± 0.32) was obtained on 6% maltose and the least shoot length (1.2 ± 0.23) was observed on 6% glucose. Among the four types of carbon sources that were employed in the present study, fructose at 4% proved to be better choice for multiple shoot regeneration followed by sucrose, glucose and maltose from axillary bud explants of *S. viarum*.

KEYWORDS

***Solanum viarum*; Carbohydrates; Plant Regeneration; Multiple Shoot Induction**

1. Introduction

Solanum viarum (Dunal) is a much branched, prickly shrub one to two meters tall at maturity. It is a member of economically important family *Solanaceae*. This plant grown in India as a richest source of steroids [1], which yields an economically important source of glycoalkaloide solasodine which is present in the fruit is a precursor for the steroid disogenin [2]. This is used in the synthesis of steroid hormone for treating cancer, addison's disease, rheumatic arthritis and for providing contraceptives [3]. This glycoalkaloid solasodine is a nitrogen analogue of disogenine. Solasodine though 16-Dihydropregnenolone (16-DPA) is converted to group of compounds like testosterone, methyl testosterone and corticosteroids like predinisolene and hydrocortisone. This steroid compounds have anti-inflammatory, anabolic and antifertility properties [4]. Considering its high economical and pharmacological importance of secondary metabolites industries are deeply interested in utilizing the plant tissue culture technology for large scale production of these substances [5]. In general sucrose is the carbohydrate of choice as a carbon source for *in vitro* plant cultures because it is the most common carbohydrate in phloem sap of many plants [6]. Sucrose was proved to be the best choice as carbon source in previous reports on *Mentha piperita*, *Stevia rebaudiana* and *Solanum nigrum* [7-9]. However, invertases that are released by the explants into the medium split sucrose into glucose and fructose [10]. Thus the explants are usually exposed to a mixture of sucrose, glucose and fructose. Therefore, the

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aim of present study was to determine the effect of different carbon sources such as sucrose, glucose, fructose and maltose on *in vitro* shoot regeneration from seed germinated axillary bud explants of *S. viarum*.

2. Materials and Methods

2.1. Plant Material and Surface Sterilization

Seeds were collected from ripen and dried fruits of healthy plants of *S. viarum* (D.) that are cultured in the herbal garden, Dravidian University, Kuppam, A. P., India. Matured seeds were first washed under running tap water for 30 minutes to remove any adherent fruit tissue and dried juice which might serve as an agent for fungal contamination. Then the seeds were thoroughly washed with bavistin 0.4% (w/v) a fungicide under sterile conditions for an hour and rinsed thrice with sterile distilled water. This was followed by surface sterilization with 5% Tween-20 (v/v) for 15 minutes and the seeds were sterilized in 70% alcohol (v/v) for 2 minutes. Then the seeds were washed with 0.1% $HgCl_2$ (w/v) for 2 minutes. Finally the seeds were washed thrice with sterile distilled water to remove the traces of $HgCl_2$. The seeds were inoculated on MS medium [11] containing 0.8% (w/v) agar supplemented with various concentrations of cytokinins (BAP and Kinetin) and auxins (NAA, IAA and IBA) prior to autoclave, the medium was adjusted to the P^H of 5.8 and sterilized for 20 minutes at 121˚C for 15 lbs pressure. The primary shoots formed *in vitro* were separated aseptically and cultured on MS medium supplemented with BAP (2.0 mg/l). Axillary buds were excised from the regenerated shoots were used as explants as a prerequisite for further experiments.

2.2. Culture Medium and Culture Conditions

Young nodal explants (1.0 - 2.0 cm) from *in vitro* seed germinated of four week old *S. viarum* were inoculated on MS medium supplemented with different carbon sources such as sucrose, glucose, fructose and maltose separately at (1% - 6%) and gelled with 0.8% (w/v) agar supplemented with cytokinin BAP at (2.0 mg/l). The culture room conditions maintained for *in vitro* cultures were 26˚C ± 2˚C and 60 to 70% relative humidity. Light intensity was 3000 lux with a photoperiod of 18 hours day light and 6 hours in dark. After sufficient elongation and multiplication of regenerated shoots, the micro shoots were carefully excised and rooted on half strength MS medium with NAA, IAA and IBA (0.5 - 2.0 mg/l) separately. The rooted plantlets were then transferred to polycups containing sterile soil and vermiculate (1:1) subsequently the plantlets were transferred to the green house after a month and planted in the soil.

2.3. Data Analysis

The variables such as shoot regeneration frequency, number of shoots per explant, shoot length, number of roots per shoot and root length were recorded.

2.4. Statistical Analysis

All experiments were conducted with a minimum of 20 explants. All assays were repeated at least three times. The experimental data were statistically analyzed by one-way ANOVA using the DMRT (Duncan's Multiple Range Test) ($P < 0.05$) and were presented as the average ± standard error (SE).

3. Results and Discussion

3.1. Effect of Different Carbon Sources on Regeneration of Shoots

Among the different carbohydrates used, fructose performed well followed by sucrose, glucose and maltose in terms of inducing multiple shoot number. The results are depicted in (**Table 1**) respectively. Fructose has been reported to be effective in preventing hyperhydricity and helps in production of adventitious shoots [12]. The maximum mean shoot number (22.6 ± 0.50) was recorded at 4% fructose, supplemented with MS medium (2.0 mg/l BAP) with maximum frequency of shoot regeneration (95%) **Figure 1(a)**. Different types and concentration of carbon sources were used to study the effect on shoot multiplication from axillary bud explants of *S. viarum*. The growth and multiplication of shoots *in vitro* are affected by several factors, one of which is the concentration and kind of exogenous carbon source added to the medium [13]. For the growth of plant tissues the carbon source serves as the energy and osmotic agent [14] for various energy requiring processes that can occur at the expense of available metabolic substrates for the growth and root initiation [15].

The next best concentration for obtaining maximum mean shoot number was at 4% sucrose, with maximum number of shoots (12.6 ± 1.25) were recorded (**Figure 1(b)**, **Table 1**). Sucrose has been proved to be better for shoot regeneration than other carbon sources in micropropagation of *Linum usitatissimum* [16]. Sucrose is the most common carbohydrate in the phloem sap of many plants. In contrast glucose was also better for inducing shoot proliferation in case of *indica* and *japonica* [17]. In our investigation next to sucrose maximum mean number of shoots (8.72 ± 0.75) was recorded at 4% glucose, and (4.15 ± 0.55) was observed at 4% maltose respectively (**Figures 1(c)** and **(d)**). Whereas at 3% maltose gave (3.55 ± 0.53) number of shoots only. But very least mean number of shoots (2.5 ± 0.25) was observed at 1% mal-

Table 1. Effect of different carbohydrate sources on multiple shoot regeneration from axillary bud explants of *in vitro* grown *S. viarum* supplemented with 2.0 mg/L BAP. Data represent treatment means ± SE followed by different letter(s) within column indicate significant differences according to ANOVA and DMRT test (P < 0.05).

Carbohydrate source	Concentration (%)	Regeneration frequency (%)	Mean no. of shoots	Mean shoot length (cm)
Maltose	1	65.00	2.52 ± 0.25^{abc}	1.25 ± 0.66^{a}
	2	68.00	2.15 ± 0.42^{ab}	2.17 ± 1.25^{b}
	3	70.00	3.55 ± 0.53^{cde}	2.23 ± 1.53^{b}
	4	75.00	4.15 ± 0.55^{df}	2.65 ± 0.48^{cd}
	5	83.00	3.7 ± 0.42^{cde}	2.12 ± 0.27^{b}
	6	62.00	1.5 ± 0.32^{a}	1.32 ± 0.54^{a}
Glucose	1	65.00	2.15 ± 0.41^{abe}	2.15 ± 0.10^{bc}
	2	69.00	4.55 ± 0.42^{efg}	2.25 ± 0.85^{b}
	3	73.00	7.75 ± 0.34^{jk}	2.85 ± 0.35^{d}
	4	88.00	8.72 ± 0.75^{k}	3.57 ± 0.10^{ef}
	5	70.00	4.9 ± 0.62^{fg}	3.89 ± 0.04^{f}
	6	65.00	2.8 ± 0.34^{bc}	1.21 ± 0.23^{a}
Sucrose	1	55.00	2.3 ± 0.35^{ab}	2.32 ± 0.53^{bc}
	2	60.00	6.85 ± 0.40^{ij}	2.40 ± 1.25^{bc}
	3	65.00	11.5 ± 0.83	3.52 ± 0.6^{ef}
	4	90.00	12.6 ± 1.25	4.81 ± 0.37^{g}
	5	80.00	6.15 ± 0.74^{hi}	2.23 ± 0.11^{b}
	6	60.00	4.23 ± 0.65^{ef}	2.01 ± 0.5^{b}
Fructose	1	60.00	5.15 ± 0.35^{fgh}	1.24 ± 0.32^{a}
	2	75.00	7.25 ± 0.44^{gh}	2.35 ± 0.30^{bc}
	3	90.00	15.5 ± 0.45	3.35 ± 0.20^{e}
	4	95.00	22.6 ± 0.50	5.92 ± 0.13^{h}
	5	85.00	6.15 ± 0.33^{hi}	3.51 ± 0.17^{e}
	6	65.00	$3.0 \pm 0.42b^{cd}$	1.50 ± 0.15^{a}

BAP = benzylaminopurine.

tose. Maltose aids as both a carbon source and as an osmaticum, compared to sucrose there is a gentler rate of extracellular hydrolysis, it is taken up more gradually, and hydrolysed intracellularly more slowly.

In our study also the growth of *S. viarum* is greatly influenced by different carbon sources supplemented with in the growth medium. High frequency, maximum number of shoots was induced on fructose supplemented medium. These findings are consistent with the previous reports in Mulberry [18], where addition of fructose instead of sucrose in the medium increased the shoot number and also growth of the shoots. Several reports indicate that the carbohydrate source can influence the degree and type of differentiation and thus the efficiency of plant regeneration [19]. It is well established that carbohydrate requirements depend upon the stage of culture and may show difference according to the species [20]. Shoots induced on MS medium supplemented with 4% fructose resulted in maximum mean shoot length (5.92 ± 0.13 cm) when compared to the other carbon sources used. The least mean shoot length (1.21 ± 0.23 cm) was observed at MS medium supplemented with 6% glucose **Table 1**. Shoot length declines at higher concentration of glucose as carbon sources might be due to the embarrassment of organogenesis.

3.2. *In Vitro* Rooting

In this present study the shoot lets formed were excised and transferred on half strength MS media with IAA, IBA and NAA seperately of varied concentrations (0.5 - 2.0 mg/l) **Table 2**. IBA (1.0 mg/l) produced the maximum number of roots (18.3 ± 0.15) with root length (5.9 ± 0.16 cm). As IBA concentration increases the number of roots were decreased. These results are in line with *Aegle marmelos* [21]. The maximum regeneration frequency (100%) was observed with IBA (0.5 mg/l) (**Figure 1(e)**). Whereas the least number of roots (8.4 ± 0.23) with least root length (1.6 ± 0.32 cm) were noticed in IAA (2.0 mg/l). NAA (1.0 mg/l) was also gave the second highest number of roots (16.2 ± 0.31) with root length (4.8 ± 0.18 cm). The maximum root regeneration frequency (100%) was observed with IBA (0.5 mg/l).

3.3. Acclimatization and Hardening

After the development of plant lets with well developed roots (after 4 - 5 weeks) were successfully acclimatized and eventually established in green house. A mixture of sterile soil and vermiculate (1:1) ratio supported maximum percentage of survival. Gradual acclimatization was done with decreasing humid conditions and transition to the field condition (**Figures 1(g)** and **(h)**).

4. Conclusion

In the present study, growth of *S. viarum* is greatly influenced by different carbon sources supplemented in the

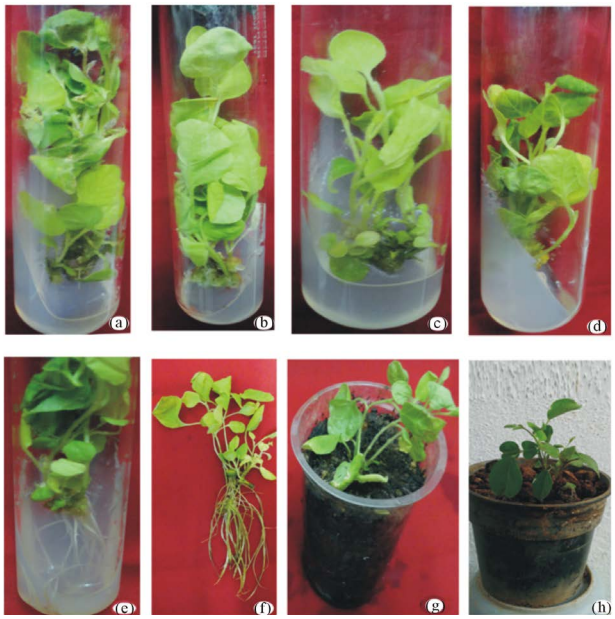

Figure 1. Effect of different carbon sources on multiple shoot regeneration on MS medium + BAP (2.0 mg/l) of *S. viarum*. (a) Multiple shoot formation from nodal explanton MS medium supplemented with fructose (4%); (b) Multiple shoot formation from nodal explanton MS medium supplemented with sucrose (4%); (c) Multiple shoot formation from nodal explanton MS medium supplemented with glucose (4%); (d) Multiple shoot formation from nodal explanton MS medium supplemented with maltose (4%); (e) Direct rooting of regenerated shoot lets on MS medium after 40 days of culture (IBA 0.5 mg/l); (f) Well rooted plants ready for hardening; (g) and (h) Tissue cultured plants in polycups and pots containing sterile soil andvermiculate. (1:1 ratio).

Table 2. Root organogenesis of *in vitro* derived shoot lets of *S. viarum* supplemented with various concentrations of IAA, NAA and IBA using half strength MS medium. Data represent treatment means ± SE followed by different letter(s) within column indicate significant differences according to ANOVA and DMRT test ($P < 0.05$).

Plant growth regulators	Concentration (mg/L)	Frequency (%)	Number of roots/shoot	Mean root length (cm)
IAA	0.5	85.00	10.4 ± 0.36^{ab}	3.1 ± 0.25^{de}
	1.0	90.00	12.3 ± 0.14^{d}	4.3 ± 0.32^{g}
	1.5	78.00	14.5 ± 0.42^{f}	2.7 ± 0.15^{c}
	2.0	86.00	8.4 ± 0.23^{a}	1.6 ± 0.32^{a}
NAA	0.5	91.00	13.5 ± 0.52^{e}	3.4 ± 0.12^{e}
	1.0	87.00	16.2 ± 0.31^{g}	4.8 ± 0.18^{h}
	1.5	85.00	14.4 ± 0.66^{f}	3.8 ± 0.33^{f}
	2.0	75.00	11.6 ± 0.45^{c}	2.1 ± 0.25^{b}
IBA	0.5	100.00	14.5 ± 0.41^{f}	4.2 ± 0.24^{g}
	1.0	98.00	18.3 ± 0.15^{h}	5.9 ± 0.16^{i}
	1.5	89.00	13.7 ± 0.32^{e}	3.8 ± 0.36^{f}
	2.0	93.00	10.3 ± 0.48^{b}	2.8 ± 0.30^{cd}

IAA = indole acetic acid, NAA = naphthalene acetic acid, IBA = indole-3-butyric acid.

media. It can be concluded that *S. viarum* cultures have quite selective carbohydrate requirements. Among the different carbon sources used, fructose performs well followed by sucrose, glucose and maltose in terms of multiple shoot induction. Our results could contribute to the improvement in the micropropagation of this economically important medicinal plant on commercial scale to meet the present day demand. However, further research is required to explore the possible growth promoting factors in these carbon sources.

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