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Effect of Different Carbon Sources on *In Vitro* Shoot Regeneration of *Solanum nigrum* (Linn.) - An Important Antiulcer Medicinal Plant

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Summary

An efficient *in vitro* protocol for mass propagation of *Solanum nigrum* was developed. In the present study, the effect of various carbon sources, sucrose, glucose, fructose, and maltose was investigated on *in vitro* shoot regeneration using nodal explants. The frequency, growth and multiplication rate were highly influenced by the type and concentration of carbon sources used. The highest number of shoots (24.0±0.28) were obtained on MS medium supplemented with 4% fructose, but maximum shoot length (11.0±0.28 cm) was observed on MS medium supplemented with 4% sucrose. The least number of shoots obtained on MS medium supplemented with 1% glucose (3.5±0.50), with a shoot length of (3.4±0.34 cm). Among the different carbon sources used in the present study, fructose at 4% proved to be better choice for multiple shoot regeneration followed by sucrose, maltose and glucose, from nodal explants of *Solanum nigrum*.

Key Words: *Solanum nigrum*, Carbon sources, Fructose, Sucrose, Maltose.

Introduction

Solanum nigrum is an important herbaceous medicinal plant belongs to solanacae family. Solanacae family comprises a number of plants widely known for the presence of natural products of medical significance mainly steroidal lactones, glycosides, alkaloids and flavonoids. The herb is antiseptic, antidysentric and diuretic used in the treatment of cardiac, skin disease, psoriasis, herpivirus and inflammation of kidney. The fruits and leaves have been traditionally used against various nerve disorders [1]. It has very important gastric ulcerogenic activities [2], and is recommended in ayurveda for the management of gastric ulcers. Most prominent medical properties are the presence of alkaloids solamargin and solosonin which yield solasodine as glycone, solasodine has embryogenic, teratonic and antimicrobial activities [3].

Considering the high economical and pharmacological importance of secondary metabolites, industries are deeply interested in utilizing plant tissue culture technology [4]. Mass propagation of plant species through in vitro culture is one of the best and most successful examples of commercial application of plant tissue culture technology.

The growth and multiplication of shoots in vitro are affected by many factors [5], one of which was the concentration and type of exogenous carbon sources added to medium to serve as energy and also to maintain the osmotic potential [6]. In general sucrose is the carbohydrate of choice as a carbon source for in vitro plant culture probably, because it is the most common carbohydrate in the phloem sap of many plants [7-9]. Although sucrose has been the carbohydrate of choice in vast majority of work on in vitro shoot induction and shoot development, it is not always the most effective carbon source for these purpose [10]. However invertases that are released by the explant into the medium split sucrose in to glucose and fructose [11,12]. Thus explants are usually exposed to a mixture of sucrose, glucose and fructose. Sucrose have been proved to be better for shoot regeneration than other carbon sources in micropropagation of Cork Oak [13]; in *Linum usitatissium* [14], medium supplemented with fructose gave consistently higher embryonic culture with higher somatic embryo frequencies.

Therefore, the aim of the present study was to determine the effect of different carbon sources such as sucrose, glucose, fructose and maltose on in vitro shoot regeneration from nodal explants of Solanum nigrum.

Materials and Methods

Plant Material

Healthy nodal explants of *Solanum nigrum* were collected from two month old seed germinated field grown plants growing in the nursery of Biotechnology garden, Sri Venkateswara University, Tirupathi, A.P., India.

Surface sterilization of explants

Young nodal explants of (1.0 – 2.0 cm) were washed thoroughly under running tap water to remove the traces of dust etc., followed by treatment with 10% teepol / tween 20 for 5 minutes. Then the explants were sterilized in 70% ethanol for a minute, and finally with 0.01% Hgcl2 for 1-2 minutes and washed 3-4 times with sterile double distilled water until all the traces of Hgcl2 washed out.

Culture medium

Young nodal explants (1.0 – 2.0 cm) of 4 week old *Solanum nigrum* were inoculated on MS medium [7] supplemented with different carbon sources such as sucrose, glucose, fructose and maltose at (1-4%) and gelled with 0.8%

agar supplemented with cytokinin BAP at (2.0 mg/L). The pH of the medium was adjusted to 5.8 before gelling with agar and autoclaved for 121°C for 15 lbs pressure.

Sub culturing

The cultures were maintained by regular subculture at 4 week intervals on fresh MS medium.

Culture Conditions

The growth room conditions maintained for in vitro cultures were 26± 2°C of temperature and 60 – 70% of relative humidity, light intensity was 3000 lux with a photoperiod of 18 hrs day light and 6 hrs dark. Each experiment was conducted at least thrice with 20 replicates per treatment.

Results

The growth, multiplication rate and other physiological parameters were effected by the type and concentration of carbon sources used.

Effect of different carbon sources on number of shoots regenerated per explant

Among the different carbohydrates used, fructose performed well followed by sucrose, glucose and maltose in terms of inducing multiple shoot number. The results were depicted in (Table 1; Fig. 1) respectively. The maximum shoot number (24.0±0.28) was recorded at 4% fructose, supplemented with MS medium (2mg/L BAP). The next best concentration for obtaining maximum number of shoots was at 4% sucrose, where (18.0± 0.18) shoots were recorded, least number of shoots (3.5±0.50) was obtained in MS medium supplemented with 4% glucose. High frequency of shoot regeneration was observed both at 4% fructose and sucrose respectively, but maximum number of shoots was obtained at 4% fructose only. In maltose, the highest number of shoots (13.0±0.18) was observed at 4% maltose, whereas at 3% maltose gave (9.5±0.50) number of shoots only. But very lesser number of shoots 4.6±0.53 was observed at 1% maltose.

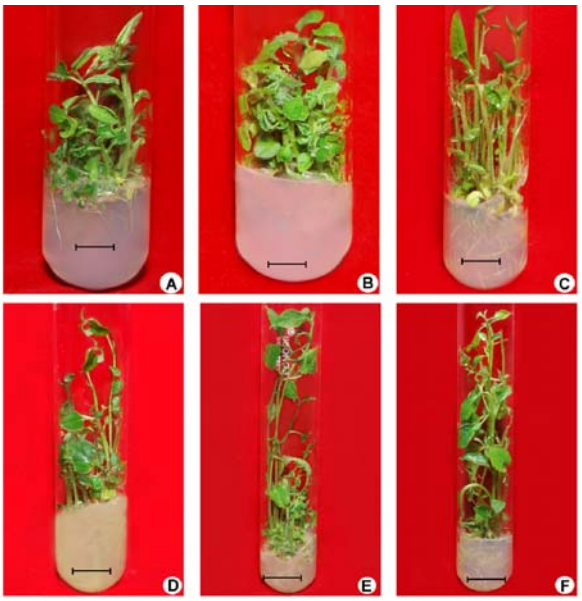

Fig. 1. Multiple shoot initiation from axillary bud explants supplemented with A) MS+2% maltose. A) bar 1 cm= 0.9; B) MS+2% sucrose. B) bar 1 cm = 0.8; C) MS+2% fructose. C) bar 1 cm = 1.1 Elongation of multiple shoots supplemented with; D) MS+4% maltose. D) bar 1 cm = 0.9; E) MS+4% sucrose. E) Bar 1 cm = 1.2; F) MS+4% fructose. F) bar 1 cm = 1.2.

Table 1. Effect of different carbohydrate sources on multiple shoot regeneration from axillary bud explants of field grown *Solanum nigrum* supplemented with 2.0 mg/l BAP.

Carbohydrate (%)	Regeneration frequency (%)	Mean no. of shoots	Mean shoot length	Callus
Sucrose				
1%	65	6.0 ± 0.28	4.2 ± 0.18	C^{+}
2%	74	8.4 ± 0.34	5.5 ± 0.50	-
3%	88	12.5 ± 0.50	7.0 ± 0.18	-
4%	95	18.0 ± 0.18	11.0 ± 0.28	-
Glucose				
1%	60	3.5 ± 0.50	3.4 ± 0.34	C^{++}
2%	65	5.0 ± 0.28	4.0 ± 0.18	C^{+}
3%	72	8.0 ± 0.28	5.2 ± 0.17	-
4%	78	11.2 ± 0.18	6.5 ± 0.50	-
Fructose				
1%	70	8.0 ± 0.28	4.0 ± 0.28	-
2%	72	12.4 ± 0.21	5.4 ± 0.34	-
3%	80	17.0 ± 0.28	6.8 ± 0.31	-
4%	85	24.0 ± 0.28	10.0 ± 0.28	-
Maltose				

1%	54	4.6 ± 0.53	3.5 ±0.50	C^{++}
2%	60	6.0 ± 0.28	4.3 ± 0.31	C^{+}
3%	65	9.5 ± 0.50	5.0 ± 0.28	-
4%	70	13.0 ± 0.18	7.0 ± 0.10	-

Results are mean ± S.E of 20 replicates; C^{+}-Poor callus; C^{++}-Moderate callus

Table 2. Effect of different auxins on *in vitro* rooting using half strength MS medium

Plant growth regulators (mg/l)	Frequency (%)	Mean no. of roots	Mean root length (cm)
IBA			
0.25	95	22 ± 0.56	4.0 ± 0.28
0.5	100	34.2 ± 0.18	5.2 ± 0.18
0.75	94	19 ± 0.23	3.6 ± 0.35
1.0	90	15.4 ± 0.22	2.3 ± 0.31
NAA			
0.25	90	17 ± 0.23	3.5 ± 0.31
0.5	95	26.1 ± 0.43	4.4 ± 0.35
0.75	85	21 ± 0.56	3.8 ± 0.17
1.0	74	14.2 ± 0.18	2.0 ± 0.28
IAA			
0.25	80	14.5 ± 0.34	3 ± 0.28
0.5	90	17.8 ± 0.17	4.3 ± 0.31
0.75	72	10.6 ± 0.35	2.8 ± 0.17
1.0	68	9 ± 0.28	1.5 ± 0.34

Results are mean ± S.E of 20 replicates
Observations: After 4 weeks, values are mean ± SE of 20 independent determinants

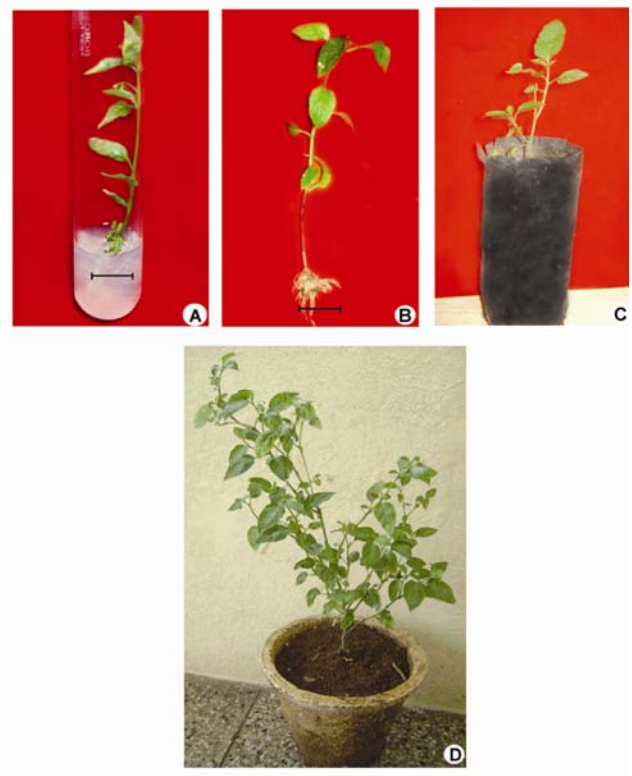

Fig. 2. A) Root induction on *in vitro* derived shoots cultured on half strength MS medium supplemented with 0.5 mg/l IBA. A) bar 1 cm = 1.1 cm; B) Plantlet showing elongated root system. B) 1 cm = 1.0 cm; C) Hardening of plantlet in a polybag containing soil and vermiculite in 1:1 ratio. D) Plantlet transferred to field conditions.

Effect of different carbon sources on shoot length

Shoots induced on MS medium supplemented with 4% sucrose resulted in maximum shoot length (10.0±0.28 cm), when compared to other carbon sources used (Table 1; Fig. 1). The second best carbon source which exhibited positive influence is fructose at 4% which gives (9.0±0.28 cm) of shoot length. The plants grown on glucose and maltose supplemented medium showed reduced shoot length. The length of shoots decreased with further (>1.0%) increase in the level of carbon sources, at low levels (1%) less shoot lengths was recorded.

In vitro rooting

In vitro derived shoots at a length of 5-6 cms were separated from shoot clump and transferred to half strength MS rooting medium supplemented with different auxins such as NAA, IAA or IBA fortified with 3% sucrose. Among the different auxins tried with half strength MS medium, IBA at (0.5 mg/l) resulted in inducing maximum number of *in vitro* roots per shoot (36.2 $\pm$ 0.18) with a maximum shoot length of (5.0 $\pm$ 0.28 cm), (Table 2; Fig. 2).

Acclimatization and hardening

After development of proper root system (after 4 weeks), the plantlets were removed from the culture tubes, washed and planted in root trainers containing soil and vermiculate in 1:1 ratio for acclimatization. Acclimatization of regenerated plants to the external environment in the last stage of micro propagation and its success depends upon different factors as suggested by various researchers [15]. Finally the hardened plantlets were transferred to field conditions with maximum survivability.

Discussion

Different types and concentrations of carbon sources were used to study their effect on shoot multiplication from nodal explants of *Solanum nigrum.* The growth and multiplication of shoots *in vitro* are affected by many factors, one of which is the concentration and type of exogenous carbon source added to the medium [5]. The carbon sources serves as energy and osmotic agents to support the growth of plant tissues [6]. In addition growth and root initiation are highly energy requiring processes that can occur at the expense of available metabolic substrates, which are mainly carbohydrates [16, 12]. In the present study also, growth of *Solanum nigrum* is greatly influenced by different carbon sources supplemented in the media.

Sucrose have been proved to be better for shoot proliferation than other carbon sources in micropropagation of several plant species such as patchouli *Pogostemon cablin* Berth [17] ,*Centella asiatica* [5] , *Peach root* [18]. But in the present study high frequency, maximum number of shoots was induced on fructose supplemented medium. The results obtained are in line with the earlier observations in *Mulbury* [19], where addition of fructose instead of sucrose in the multiplication medium increased the shoot number and also growth of the shoots. The beneficial effect of glucose on direct shoot formation was emphasized in *Prunus mume* [20]. In Indian *Penny wort (Centella asiatica),* in BA and Kn combination, maximum number of shoots were induced in the presence of 3% maltose [5]. Many authors have reported that various sources of carbon such as glucose, fructose, mannitol and sorbitol play an important role in tissue culture of *Asparagus* [21], *Cucumber* [22].

Even though carbohydrates are of prime importance for cell growth, maintenance and differentiation *in vitro*, the fundamental aspects of carbon utilization and metabolism in cell and tissue cultures have to be fully understood [13, 23].

Conclusion

It can be concluded that among the different carbon sources used, fructose performed well followed by sucrose, glucose and maltose in terms of multiple shoot induction. Since fructose and sucrose are the better carbohydrate choices for *in vitro* shoot multiplication of *Solanum nigrum.* However, further research is highly required to explore the effect of different variety of carbon sources on *in vitro* plant regeneration of *Solanum nigrum*.

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References

[1] Perez, G. R. M., L. A. Perez, D. L. M. Garcia and M. H. Sossa. 1998. Neuropharmacological activity of *Solanum nigrum* (L.) fruit. J. Ethanopharmacol. 62:43-48.

[2] Aktar, M. S. and M. Munir. 1989. Evaluation of gastric ulcerogenic effects of *Solanum nigrum*, *Brassica olevacae* and *Ocimum basillicum* in rats. J. Ethanopharmacol. 27:163-176.

[3] Kim, Y. C., C. Q. Ming, A. A. Gunatilaka and D. G. Kingston. 1996. *In vitro* propagation of *Ancistrocladus abbrevattus* airy show (Ancistrocladacae). Plant Cell Tiss. Org. Cult. 57:71–73.

[4] Misawa, M. 1994. Plant tissue culture. An alternative for production of useful metabolites. FAO. Agric. Serv. Bull. Rome pp. 87.

[5] Anwar, H. Md., Md. Taslim Hossain, Md. Raihanali and S. M. Mahbubur Rahman. 2005. Effect of different carbon sources on *in vitro* regeneration of Indian Penny wort (*Centella asiatic*a L.). Pak. J. Biol. Sci. 8 (7):963 – 965.

[6] Lipavska, H. and H. Konradova. 2004. Somatic embryogenesis in conifers: The role of carbohydrate metabolism. In Vitro Cell Dev. Biol. Plant. 40:23-30.

[7] Murashige, T. and F. Skoog. 1962. A revised medium for rapid growth and bioassay with tobacco tissue cultures. Physiol. Plant. 15:473-497.

[8] Lemos, E. E. P. and D. A. Baker. 1998. Shoot regeneration in response to carbon source on inter nodal explants of *Annona muricata* L. Plant Growth Ragul. 25:105-112.

[9] Fuents, S. R. L., M. B. P. Calheiros, J. Manetti-Filho and L. G. E. Vieira. 2000. The effect of silver nitrate and different carbohydrate sources on somatic embryogenesis in *Coffea canephora*. Plant Cell Tiss. Org. Cult. 60:5-13.

[10] Thomson, M. and T. Thorpe 1987. Metabolic and non-metabolic roles of carbohydrates. In: J. M. Bonga and D. J. Durgan (Eds.). pp.89-112. Cell and Tissue Culture in Forestry. Martinus Nijhoff Publishers, Dordrecht.

[11] Goerge, E. F. 1993. Somatic embryogenesis: cell biological aspects. Acta Bot. Neerl. 43:1-14.

[12] De Klerk, G. J. M. and A. Calamar. 2002. Effect of sucrose on adventitious root regeneration in apple. Plant Cell Tiss. Org. Cult. 70:207-212.

[13] Romano, A., C. Norohna and M. A. Martins-Loucao. 1995. Role of carbohydrates in micropropoaton of Cork oak. Plant Cell Tiss. Org. Cult. 40(2):159-167.
[14] Cunha, A. and F. Ferreira. 1999. Influence of medium parameters on somatic embryogenesis from hypocotyls explants and flx (*Linum usitatissium* L.). J. Plant Physiol. 155:591-597.
[15] Bhojwani, S. S and M. K. Razdan 1983. Plant tissue culture: Theory and practice. Elesivier Sci. Pub. Amsterdam. 194.
[16] Thorpe, T. A. 1982. Carbohydrate utilization and metabolism. In: J. M. Bonga and D. J. Durzan. (Eds.). pp. 325-368. Tissue Culture in Forestry. Martinus Nijhoff / W. Junk, The Hague.
[17] Kumara Swamy, M., S. Balasubramanya, M. Anuradha. 2010. *In vitro* multiplication of patchouli through direct organogenesis. Afr. J. Biotechnol. 9(14):2069 – 2075.
[18] Tauquer, A., N. A. Abbasi, I. Hafiz and A. Ali. 2007. Comparison of sucrose and sarbitol as main carbon energy sources in micropropagation of peach root stock GF- 677. Pak. J. Bot. 39(4):1269-1275.
[19] Vijaya Chitra, D. S. and G. Padmaja 2001. Seasonal influence on axillary bud sprouting and micropropagation of elite cultivars of *Mulbery*. Sci. Hort. 92:55-68.
[20] Hisachi, H. and Y. Murai. 1996. Micropropagation of *Prunus mume*. Plant Cell Tiss. Org. Cult. 40(2):159–167.
[21] Mamiya, K. and Y. Sakamoto. 2000. Effects of sugar concentration and strength of basal medium on conversion of somatic embryos in *Asparagus officinalis* L. Sci. Hort. 84:15-26.
[22] Lou, H. and S. Sako. 1995. Role of high sugar concentration in inducing somatic embryogenesis from cucumber cotyledons. Sci. Hort. 64:11-20.
[23] Vu, J. C. V., R. P. Niedz and G. Yelnosky. 1995. Activities of sucrose metabolism enzymes in glycerol-grown suspension cultures of sweet orange (*Citrus sinensis* L.). Env. Exp. Bot. 35(4):455-463.