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Effect of carbon sources on Shoot Regeneration of Radish (*Raphanus sativus* L.) Var. Beeralu Rabu

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ABSTRACT

Efforts were made to develop a culture medium for successful *in vitro* propagation of radish. The effect of various carbon sources such as sucrose, glucose, maltose and dextrose were investigated on *in vitro* shoot regeneration of Rabu (*Raphanus sativus* L.) Var. Beeralu using aseptic hypocotyl explants. Numbers of regenerated shoots were evaluated one month after establishment. Test shows that there were significant effects at $p<0.05$ level on radish shoots, regenerated in different carbon sources. The frequency, growth and multiplication rate were highly influenced by the type and concentration of carbon source used. The maximum number of shoots (6 shoots/explant) on MS medium supplemented with 3% dextrose and maximum shoot length (4cm) was obtained by 4% dextrose. Regeneration frequency was 100% in 4% dextrose. The least number of shoots were observed by each and every concentration of glucose except 4%. Among the four types of carbon sources that were employed in the present study, dextrose at 4% proved to be better choice for multiple shoot regeneration followed by sucrose, maltose and glucose from hypocotyl explants of *Raphanus sativus* L Var. Beeralu Rabu.

Keywords: Carbon sources, Dextrose, *Raphanus sativus*, Regeneration, Glucose

INTRODUCTION

Radish (*Raphanus sativus* L; Brassicaceae) is an important vegetable crop that is cultivated throughout Asia (Cho *et al*., 2008). Roots and leaves with different shapes and sizes are edible parts of this crop and it can be used to medicinal purposes, ornamental as well as culinary purposes. Radish is used as vegetable or salad in Sri Lanka (http://www.agridept.gov.lk) as well as other countries. Radish is one of the vegetables that can be grown in all agro ecological regions in Sri Lanka throughout the year if adequate moisture is available (http://www.agridept.gov.lk). There are three main Radish varieties recommended for Sri Lankan conditions as Japan Ball, Beeralu Rabu and Table Radish. Beeralu Rabu is the recommended variety for low country, while Japan ball recommended for up country, Sri Lanka. Sri Lankan radish varieties contain small sizes of tubers. Therefore those varieties have to obtain larger tuber to develop the appearance and quality of the tuber to achieve the export market by genetic improvements. Major genetic improvement of radish has been achieved by conventional plant breeding methods, such as crossing but these methods are time and labour consuming. In recent years, advances in plant genetic engineering have opened a new avenue for crop improvement and various transgenic plants with novel agronomic characteristics have been produced (Singh *et al*., 2008). The success in plant genetic engineering is dependent upon several factors, from which an efficient tissue culture system, with high plant regeneration potential, is a fundamental option Growth and *in vitro* shoot multiplication are affected by many factors (Anwar *et al*., 2005), one of which is the concentration and type of exogenous carbon sources added to medium as the energy source, and to maintain the osmotic potential (Lipavska *et al*., 2004). There have been various opinions on the beneficial effects of various carbon sources (sucrose, fructose, glucose, dextrose table sugar etc.) on the growth of plants *in vitro* (Mohammad and Mohammad, 2009) Sucrose (2-5%) is the most popular

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carbohydrate used in tissue culture (Bridgen, 1994). In general most of the tissue culture studies are performed using sucrose as the sole carbon source due to its efficient uptake across the plasma membrane. Glucose also has been reported to have various effects on the *in vitro* growth of plants. Cunha and Ferreira (1999) on *Linum usitatissium* showed that medium supplemented with monosaccharide (glucose or fructose) at concentrations of 4%gave consistently highly embryonic culture with higher somatic embryo frequencies and higher growth rate compared with medium supplemented with either sucrose or maltose (Cunha and Ferreira, 1999). The use of fructose is considered as an excellent source of carbohydrate for embryo culture (Mauney *et al*., 1961). Kaufman *et al.* (1962) and Dickinson (1996) used fructose as a good source for the culture of stem segments and pollen. According to these reports various carbon sources affect in various way for the growth of *in vitro* cultured plants. 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 hypocotyl explants of Radish (*Raphanus sativus*).

MATERIALS AND METHODS

Plant source

Seeds of Radish were purchased from the Seed and Planting Material Division, Department of Agriculture, Sri Lanka.

Aseptic plantlets

Seeds were surface-sterilized by washing tap water, soapy water, immersing in 70% ethanol for 3 minutes, three times from distilled water and soaking in a 20% Clorox for 20 minutes respectively. Sterilized seeds were then rinsed three times in sterilized distilled water (Dahanayake *et al*., 2010).

Culture medium

Hypocotyl explants (0.5 cm) of 15 days old aseptic plantlets of Radish (*Raphanus sativus*) var.'Beeralu' were inoculated on MS medium supplemented with different carbon sources such as sucrose, glucose, maltose and maltose at (1-5%) and gelled with 0.8%agar supplemented with cytokinin BAP (2.5 mg/L) and NAA (0.1 mgL^{-1}). The pH of the medium was adjusted to 5.8-6.00 before gelling with agar and autoclaved at 121°C and 1.4$kgcm^{-2}$ for 20 minutes.

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 with 25 replicates per treatment.

Data collection and analysis

Experiment was arranged according to the Complete Randomized Design (CRD). Shoot regeneration were evaluated 30 days after initiation. Number of explants with shoots in different treatment, number of shoots per explants and length of shoots in different treatments were recorded. Statistical analysis was carried out using the Student Newman-Kuells Means Sepa-ration Test of SAS program (9.1.3).

RESULTS AND DISCUSSION

The growth, multiplication rate and other physiological parameters were affected 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, dextrose performed well followed by sucrose, and maltose in terms of inducing multiple shoot number. The results were depicted in (Table 1 and Fig. 1) respectively. The maximum shoot number (6 shoots/explant) was recorded at 2% dextrose. The next best concentration for obtaining maximum number of shoots was at 3% sucrose (5shoot/explant). It is the recommended dose of sucrose using in MS medium. Least number of shoots (0 shoot/explant) was obtained in MS medium supplemented with 1%, 2% sucrose, 1%, 2%, 3% maltose, 1%, 2%, 3%, 4%, 5% glucose and 4%, 5% dextrose. High frequency of shoot regeneration was observed at 3%, 4% glucose, 4%, 5% maltose and 3% dextrose, but maximum number of shoots was obtained at 2% dextrose only. When consider 4% dextrose 2nd highest number of shoots (5shoots/explant) and maximum shoot length was given and regeneration frequency was 100% in 4% dextrose. That result was revealed 4% dextrose was best for shoot regeneration and gain maximum shoot length. Maximum negative effect was given by glucose. Shoots were not regenerated in most of concentration of glucose except 4%.

Effect of different carbon sources on length of shoots

Maximum shoot length was obtained by 3% dextrose (4 cm) and 2nd highest shoot length (2 cm) was obtained by 5%

glucose and 3rd highest shoot length (1.5 cm) was gained by 2% dextrose and 5% maltose. Least length of shoots was attained by 4% glucose. The growth, multiplication rate and other physiological parameters were affected by type and concentration of carbon source used (Kumara Swamy *et al.*, 2010). In the present study also, growth of *Raphanus sativus* 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 Pogostemoncablin Berth (Kumara Swamy *et al.*, 2010), (Anwar *et al.*,2005), Peach root (Tauquer *et al.*, 2007). But in the present study high frequency, maximum number of shoots was induced on dextrose supplemented medium. Dextrose was not much practiced as a carbon sources in micropropagation. However dextrose was the best in the present study. It increased number of shoots per explant and also length of shoots.

As reported by (Sridhar *et al.*, 2011) fructose was the best for shoot regeneration and to increase the shoot length of *Solanum nigrum* (L.) and as well Ilczuk *et al.* (2013) stated that fructose was the best carbon sources for micropropagation of *Physocarpus opulifolius* (L.) Maxim.). Nevertheless 4% dextrose was the best in the present study and fructose was not used. As conveyed by Anwar *et al.* (2005) sucrose was the best for shoot regeneration in *Cenetella asiatica* but 4% dextrose was the best in the present study.

As said by Preethi *et al.* (2011) least number of shoots was given by glucose in *Solanum nigrum* (L.) and *Stevia rebaudiana*. It was in line with results of the present study.

Table 1: Effect of different carbohydrate sources on multiple shoot regeneration from sterilized hypocotyl explants of *Raphanus sativus* supplemented with 2.0 mgL^{-1} BAP and 0.1 mgL^{-1} NAA.

Number of shoots per explant (shoots/explant)

Concentrations (%)	Sucrose	Maltose	Glucose	Dextrose
1	0c	0c	0b	1c
2	0c	0c	0b	6a
3	5a	0c	0b	5b
4	1b	2b	1a	0d
5	1b	5a	0b	0d
Shoot length (cm)				
1	0b	0c	0c	1c
2	0b	0c	0c	1.5b
3	1a	0c	0c	4a
4	1a	0.5b	0.2b	0d
5	1a	1.5a	0c	0d
Regeneration frequency (%)				
1	0	0	0	68
2	0	0	0	80
3	100	0	0	100
4	100	100	68	0
5	80	100	0	0

Means fallowed by the same lower case letters in each column are not significantly different at 5% level in Duncan's Multiple Range Test

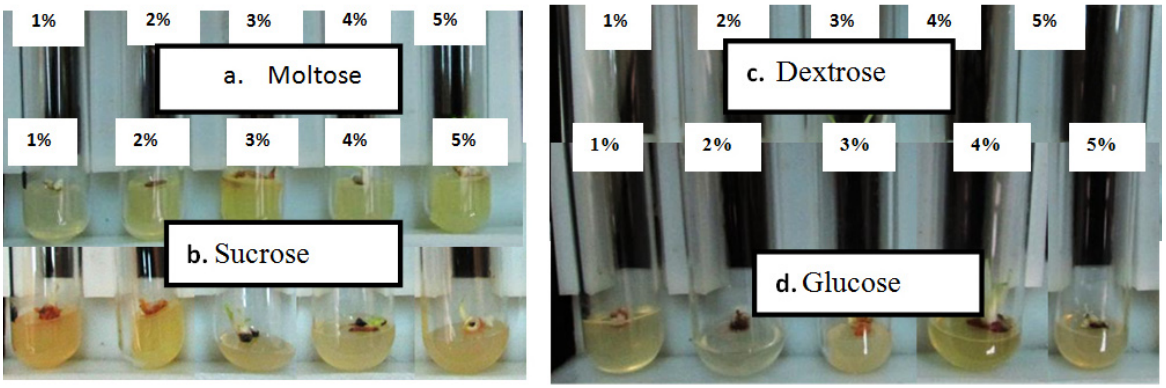

Fig 1: Multiple shoot initiation from hypocotyl explants supplemented with a.) Maltose b.) Sucrose c.) Dextrose and d.) Glucose in 1%-5% concentrations

CONCLUSION

It can be concluded that among the different carbon sources used, dextrose performed well followed by sucrose, maltose and glucose in terms of multiple shoot induction. Among the dextrose concentrations 4% was the best for enhance shoot regeneration of Radish. However, further research is highly required to explore the effect of different variety of carbon sources on *in vitro* plant regeneration of (*Raphanus sativus* L.) Var. Beeralu Rabu.

REFERENCES

Anwar H, Hossain T, Ali R and Mahbubur Rahman SM . 2005. Effect of different carbonsources on *in vitro* regeneration of Indian Penny wort (*Centella asiatica* L.). *Pak. J. Biol*. Sci. **8** (7):963 – 965.

Bridgen MP. 1994. A review of plant embryo culture. *Hort Science.* **29**:1243-1245.

Cho M A, Min S R, Ko SM, Liu J R, Choi PS. 2008. Agrobacterium-mediated genetic transformation of radish (*Raphanus sativus* L.). *Plant Biotechnology* **25**:205–208.

Cunha A and Ferreira F.1999. Influence of medium parameters on somatic embryogenesis from hypocotyls explants and flx (*Linum usitatissium* L.). *J. Plant Physiol*.**155**:591-597.

Dahanayake N, Chen XL, Zhao FC, Yang YS, Wu H. 2010, An Efficient *In Vitro* Propagation System for Purple Cone-Flower (*Echinacea Purpurea* L.). *Journal of Tropical Agricultural Research & Extension* **13**(2): 29.

Dickinson DB. 1996. Relation between external sugars and respiration of germinating lilly pollen. *Proc. Am. Soc. Hort*. **88**: 651- 656.

Ilczuk A, Jagiełło-Kubiec K, Jacygrad E. 2013. The effect of carbon source in culture Medium on micropropagation of common Ninebark (*Physocarpus opulifolius* (L.) Maxim.). *Acta Sci. Pol., Hortorum Cultus* **12**(3): 23-33.

Kaufman PB, Katz JM., Yoder ME.1962. Growth responses of Avena stem segments to various sugars. *Nature.* **196**:1332- 1333.

Kumaraswamy, Balasubramanya MS, Anuradha M.2010. *In vitro* multiplication of patchouli through direct organogenesis. *Afr. J. Biotechnol*. **9**(14):2069 – 2075.

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

Mauney JR. 1961. The culture *in vitro* of immature cotton embryos. *Bot. Gaz*. **122**:205- 209.

Mohammad A and Mohammad BH. 2009. The Effects of Different Culture Media on the Callus Production of Radish (*Raphanus sativus* L.), *Romanian Biotechnological Letters* **14** (4):4519-4523.

Preethi D, Sridhar TM and Naidu CV. 2011. Carbohydrate Concentration Influences on In Vitro Plant Regeneration in Stevia rebaudiana. *Journal of Phytology* **3** (5): 61-64.

Sridhar TM and Naidu CV. 2011.Effect of Different Carbon Sources on In Vitro Shoot Regeneration of *Solanum nigrum* (Linn.)-An Important Antiulcer Medicinal Plant. *Journal of Phytology* **3**(2): 78-82.

Tauquer AN, Abbasi A, Hafiz I and Ali A. 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.

Singh AK, Manibhushan, Chandra N and Bharati RC.2008. Suitable crop varieties for limited irrigated conditions in different agro climatic zones of India. *Int. J. Trop Agr.* **26** (3-4): 491-496.

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