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Full Length Research Paper

A Study on the use of carrot juice in the tissue culture of *Daucus carota*

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Effects of using carrot juice in the *in vitro* growth of *Daucus carota* were investigated. Callus was the only form of growth obtained after five weeks in culture. The increase in fresh weight, dry weight and moisture content of the explants with increasing concentration of carrot juice in the medium was much lower when compared to either the cytokinin benzyladenine (BA) and/or the auxin picloram. Spectrophotometric and chromatographic analyses revealed the presence of both indole-3-acetic acid (IAA) and abscissic acid (ABA) in the carrot juice, while gibberellic acid was absent.

Key words: Carrot juice, auxin, abscissic acid.

INTRODUCTION

Most of the current tissue culture methods are based on technologies developed over twenty years ago. The commercial use of plant tissue culture primarily involves the production of large number of plants with minimum input expenses. The main factors which ultimately influence the commercial propagation of plants *in vitro* are: the selection of plant species, the physical environment and the chemical media for *in vitro* culture. Research efforts have addressed growth regulating compounds and salt mixes and there seems to be good scope for substituting the expensive chemical nutrient media with low cost natural extracts.

The effects of using yeast and plant extracts in *in vitro* culture have been investigated by a number of workers. One of the earliest report is that of Overbeek et al. (1941), who succeeded in growing immature *Datura* embryos in culture by including the liquid endosperm of *Cocos nucifera* (coconut milk) in their culture medium. Coconut milk was also shown to stimulate cell division in other cultured tissues and its use as a supplement was adopted in many laboratories (Duhamet and Gautheret, 1950; Morel, 1950; Nickell, 1950; Duhamet, 1951; Henderson et al., 1952; De Ropp et al., 1952; Archibald, 1954; Wiggans, 1954). Other complex plant juices and liquid endosperms have been shown to possess stimulatory properties more or less similar to those of coconut milk. These include liquid endosperm from immature corn (Nétien et al., 1951), tomato juice (Nitsch, 1951; Straus and La Rue, 1954), immature fruits and seeds (Steward and Caplin, 1952; Steward and Shantz, 1959), orange juice, malt extract, yeast extract, casein hydrolysate, leaf extracts, sap from a number of plants and tumour extracts (Butenko, 1968).

Great interest has also been attached to the identification of the active constituents of the natural fluids used in tissue culture medium. Thus, the active components of coconut milk have been identified by Pollard et al. (1961) to include a nitrogenous component (consisting of reduced nitrogen compounds, particularly amino acids and their amides), a neutral component (most prominent constituents being m-inositol, scyllo-inositol and sorbitol) and an active component which has been identified to contain auxin and gibberellin-like substances (Paris et al., 1954; Radley and Dear, 1958; Dix and Van Staden, 1982). Similarly, Straus (1960) has shown that tomato juice, yeast extract or casein hydrolysate function by supplying a form of organic nitrogen (a mixture of amino acids) while malt extract provide an auxin, kinetin, m-inositol, urea and arginine to *in vitro* cultured explants (Steinhart et al., 1961).

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This study presents the effects of using carrot juice in the *in vitro* culture of *Daucus carota*. Spectrophotometric and chromatographic methods used in an attempt to identify the presence of growth promoting substances in carrot juice is also presented.

MATERIALS AND METHODS

Plant material

Carrot taproots purchased from the local market were used as plant materials for this study. The carrots were chosen according to their tenderness, smoothness and freshness. After removing 1 cm sections from both ends, the carrots, were peeled and washed followed by immersion in 70% ethanol for 20 min. Sterilization was carried out in 3.5% sodium hypochlorite solution for 15 min, to which two drops of Tween 20 were added. This was followed by rinsing three times in sterile distilled water (10 min in each rinse). The concentration of sodium hypochlorite and sterilization period was chosen from a preliminary experiment and based on percentage contamination and necrosis of explants. About 8 mm thick discs were cut from the carrots and out of these, cubes (about 8 mm^3) were cut in such a way so as to include the xylem, phloem and the cambium. The cubes were inoculated onto the culture medium, maintaining polarity.

Culture medium and culture conditions

The culture medium used for all experiments was based on Murashige and Skoog's (1962) medium to which 8 gL^{-1} Difco Bacto agar and different amount of carrot juice was added (0, 20, 50 or 70 %), but no plant growth regulators. The control consisted of basal Murashige and Skoog's medium supplemented with either Picloram (0, 0.05 or 1.0 mgL^{-1}) and/or BA (0, 1.0 or 5.0 mgL^{-1}). Medium pH was adjusted to 5.8 with KOH before adding the agar. All media were sterilized by autoclaving at 121^{0}C and 101 KPa for 20 min. Cultures were incubated at 24±2^{0}C and exposed for 16 h per day to an illumination of 15.7 $\mu molm^{-2}s^{-1}$ provided by daylight-type fluorescent lamps.

Experimental design

A completely randomized design was used with the only recognisable differences between explants being the treatments applied to them. All treatments consisted of ten replicates and each replicate contained two explants. The fresh weight, dry weight and the moisture content were recorded after five weeks in culture. The mean for each treatment was calculated. A two-way Anova with replication was done using Microsoft Excel 97. Least significant differences between means were calculated to identify treatment means which differed significantly at the 5% level of significance.

Extraction and bioassay for auxins

Considerable losses may occur during extraction and bioassay of auxins from plant materials due to the presence of peroxidases, sublimation during drying of the extracts or through oxidation if not assayed quickly (Mann and Jaworski, 1970). Consequently, the auxins were extracted using the procedure of Mann and Jaworski (1970). Extracts were assayed using a modified Salkowski technique (Gordon and Paleg, 1957), which is specific for indole-3-acetic acid (IAA).

Extraction and bioassay for abscissic acid

Abscissic acid was extracted using the procedure of Saunders (1978). The acidic-diethylether-soluble extracts were dried over a water bath at 35^{0}C and the residues redissolved in 5 ml ethanol prior to chromatography. The resuspended extracts were loaded onto thin layer silica gel plates and separated by ascending chromatography with toluene:ethyl acetate:acetic acid (40:5:2 vol/vol). The chromatograms were dried and viewed under uv light. The retardation factor (Rf) values were determined.

Extraction and bioassay for gibberellins

Gibberellin-like substances were extracted from carrot juice using the procedure of Reeve and Crozier (1978). The dried residues were dissolved in 5 ml ethanol and loaded onto chromatography paper and separated by ascending chromatography with iso-propanol:25% ammonium hydroxide:water (10:1:1 v/v). The chromatograms were stained with iodine.

RESULTS

Calli of different sizes were produced by the explants in all the experiments. These occurred both on the surface in contact with air and the surface touching the solidified medium. The colour of the calli varied between green, cream and purple and they were much harder compared to the uncultured explants. Figure 1 shows the fresh weight and dry weight of the callus producing carrot explants after five weeks in culture at different concentrations of carrot juice. There was a general increase in the fresh weight and dry weight of the explants, although a slight decrease in dry weight was observed in the medium containing 50% of carrot juice.

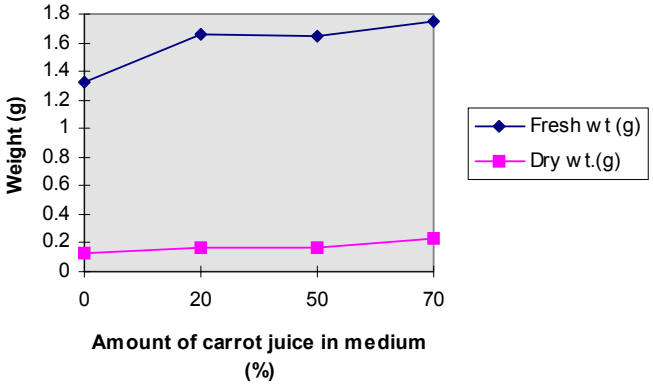

Figure 1. Effect of different concentrations of carrot juice on fresh weight and dry weight of carrot explants after five weeks in culture.

The effects of using either the auxin picloram or the cytokinin benzyladenine (BA) are shown in Figures 2 and 3 respectively. A linear increase in both fresh weight and dry weight was observed in both cases. The highest fresh weight and dry weight being obtained when picloram (1.0

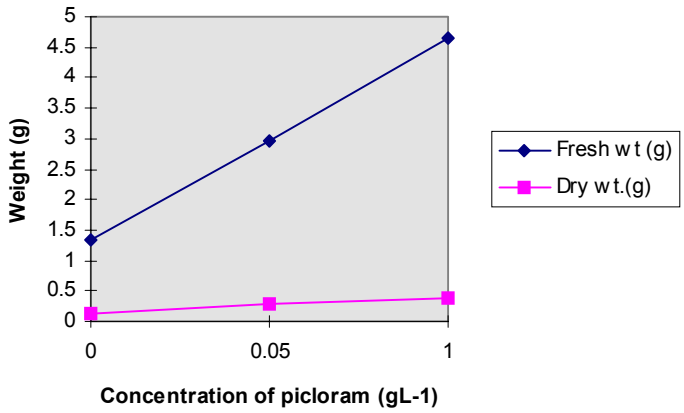

Figure 2. Effect of different concentrations of Picloram on fresh weight and dry weight of carrot explants after five weeks in culture.

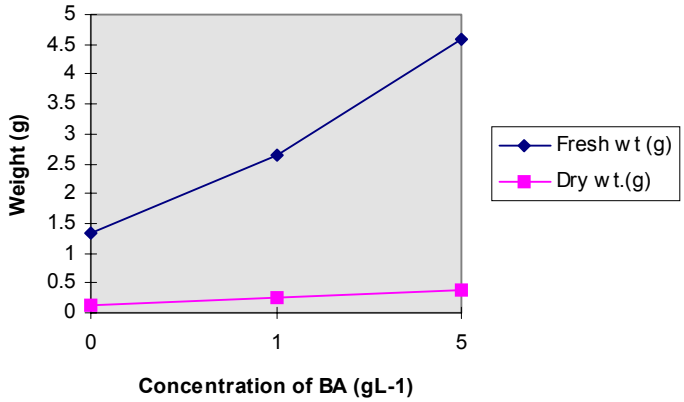

Figure 3. Effect of different concentrations of benzyladenine on fresh weight and dry weight of carrot explants after five weeks in culture.

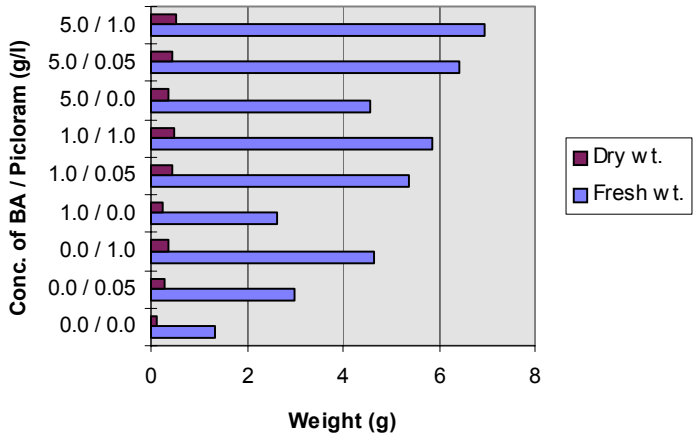

Figure 4. Effect of using combinations of BA and Picloram on fresh weight and dry weight of carrot explants after five weeks in culture.

Table 1. Moisture content (%) of carrot explants at different concentration combinations of BA and Picloram after five weeks in culture.

Picloram (mgL^{-1})	BA (mgL^{-1})		
	0.0	**1.0**	**5.0**
0.0	90.5342 [a]	90.6261 [ab]	91.8363 [c]
0.05	90.8560 [b]	91.9538 [c]	92.7681 [d]
1.0	91.8739 [c]	92.0018 [c]	92.6797 [d]

Means followed by the same letter do not differ significantly at the 5% level of significance.

Table 2. Moisture content of carrot explants after five weeks in culture.

Treatment	Amount	Moisture content (%)
Carrot juice (%)	0	90.5342 [c]
	20	89.7189 [b]
	50	89.9164 [b]
	70	86.5097 [a]
Picloram ($g\ L^{-1}$)	0.0	90.5342 [c]
	0.05	90.8544 [c]
	1.0	91.8730 [d]
Benzyladenine ($g\ L^{-1}$)	0.0	90.5311 [c]
	1.0	90.6261 [c]
	5.0	91.8363 [d]

Means followed by the same letter do not differ significantly at the 5% level of significance.

mgL^{-1}) was used. The result of using combinations of BA and picloram is shown in Figure 4.

Table 1 shows the moisture content of the explants at different concentration combinations of BA and picloram. Table 2 shows the moisture content of carrot explants after five weeks in culture at different concentrations of carrot juice, picloram or BA. Whereas a general increase in the moisture content of the carrot explants were observed with increasing concentration of Picloram and/or BA, a decrease in this parameter was observed with increasing concentration of carrot juice. The absorbance (at 529 nm) of the carrot juice was 0.09 which corresponds to 37.6 ng of IAA/g of carrot.

Figure 5 illustrates the sketch of the TLC plate for abscissic acid detection as viewed under U.V light. Both spots had a Rf value of 0.18. This indicates that the carrot juice contained abscissic acid. Paper chromatogram of carrot juice and pure gibberellic acid showed that the latter was absent in carrot juice (Figure 6). The Rf value for pure GA is 0.60.

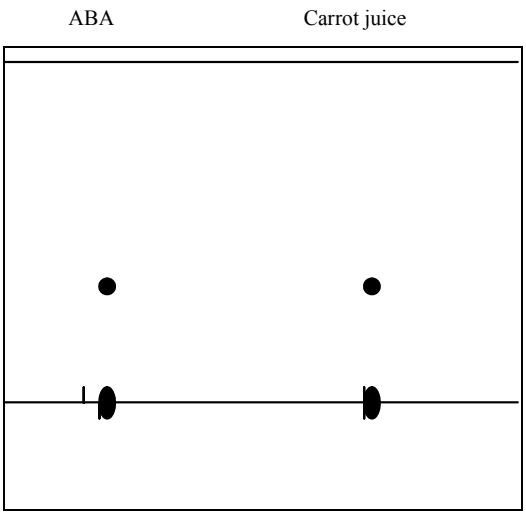

Figure 5. Sketch of the TLC plate as viewed under U.V light (not to scale).

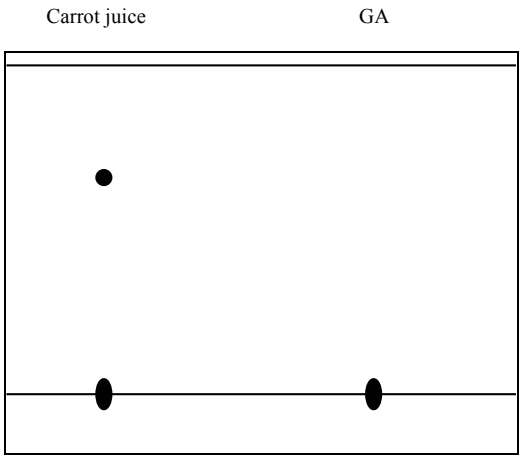

Figure 6. Sketch of the paper chromatogram of pure GA and carrot juice after staining with iodine (not to scale).

DISCUSSION

Results obtained indicate that using carrot juice in the culture medium does not enhance growth of carrot root explants when compared to BA and/or picloram. This is most probably due to the presence of abscissic acid (ABA) in carrot juice. ABA is known to slow down growth and to moderate the effects of auxins and cytokinins in *in vitro* culture (Abe et al., 1991). Other similar inhibitory substances in the carrot juice may also account for this finding. For example, presence of jasmonates, tuberonic acid and their derivatives are known to be inhibitors of growth in tissue culture (Parthier, 1991). Inclusion of carrot juice in the medium might have also caused an increase in the level of a number of substances such as vitamins, amino acids, fatty acids, purines, pyrimidines and carbohydrates, all known to be present in carrot juice (Souci et al., 1994), above the optimum normally required in tissue culture, resulting in adverse effects.

The decrease in moisture content with increasing concentration of carrot juice could be a result of an increase in the osmotic potential of the medium by mannitol, which can be present in carrot juice at concentration of up to 160 mg/100 g of carrot tissue (Souci et al., 1994). The osmotic potential of Murashige and Skoog's (1962) medium is in the range of 2.20 - 2.27 bar and addition of 1 M mannitol to this medium can raise the osmotic potential by 22.4 bar (Pierik, 1987). This can lead to cessation of water uptake by the tissues and as a result, growth and organ formation can stop.

The greater effectiveness of medium supplemented with carrot juice than unsupplemented medium in promoting callus formation may be explained by the presence of the auxin IAA in the carrot juice. Auxins often need to be supplied to culture media in order to elicit a response in cultured tissues (Halperin and Wetherell, 1964). Paper chromatography did not reveal the presence of gibberellic acid in either autoclaved or non-autoclaved carrot juice. This is in agreement to the findings of Koshioka et al. (1988).

Analysis of other constituents of carrot juice such as cytokinins, vitamins, amino acids and endogenous sterols is contemplated for further studies in order to define a specific culture medium based on carrot juice. It is believed that success of such experiments would pave way for reducing the cost of *in vitro* culture of plants to a fraction of what it is now.

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REFERENCES

Abe J, Nakashima H, Mitsui K, Mikami T, Shimamoto Y (1991). Tissue culture response of Beta germplasm: Callus induction and plant regeneration. Plant Cell Tissue Organ Cult. 27: 123-127.

Archibald JF (1954). Culture *in vitro* of cambial tissues of cacao. Nature 173: 351-353.

Butenko RG (1968). In: Plant Tissue Culture and Plant Morphogenesis, Israel Program for Scientific Translations, Jerusalem, pp. 40-45.

De Ropp RS, Vitucci JC, Hutchings BL, Williams JM (1952). Effect of coconut fractions on growth of carrot tissues. Proceedings of the Society of Experimental Biology 81: 704-709.

Dix L, Van Staden J (1982). Auxin and gibberellin-like substances in coconut milk and malt extract. Plant Cell Tissue Organ Cult. 1: 239-245.

Duhamet L (1951). Action du lait de coco sur la croissance des cultivés de tissus de crown-gall de vigne, de tabac, de topinambour et de scorsonère. Comp. Rendus de la Soc. de Biol. 145: 1781-1785.

Duhamet L, Gautheret RJ (1950). Structure anatomique de fragments de tubercules de topinambour cultivés en presence de lait de coco. Comp. Rendus de la Soc. de Biol. 144: 177-184.

Gordon SA, Paleg LG (1957). Observations on the quantitative determination of indole acetic acid. Physiol. Plant. 10: 39-47.

Halperin W, Wetherell DR (1964). Adventive embryony in tissue cultures of wild carrot. Am. J. Bot. 51: 274-283.
Henderson JHM, Durrell ME, Bonner J (1952). The cultures of normal sunflower callus. Am. J. Bot. 39: 467-472.
Koshioka M, Jones A, Pharis RP (1988). The potential of cell suspension cultures of *Daucus carota*. L. as a source of [^{3}H] GA. Agric. Biol. Chem. 52(1): 55-61.
Mann JD, Jaworski, EG (1970). Minimising loss of IAA during purification of plant extracts. Planta 92: 285-291.
Morel G (1950). Sur la culture des tissus de deux monocotylédones. Comp. Rendus de L' Acad. des Sci. 230: 1099-1105.
Murashige T, Skoog F (1962). A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol. Plant. 15: 473-497.
Nétien G, Beauchesne G, Mentzer C (1951). Influence du "lait de maïs" sur la croissance des tissus de carotte *in vitro*. Comp. Rendus de L' Acad. des Sci. 233: 92-98.
Nickell LG (1950). Effect of coconut milk on the growth *in vitro* of plant virus tumour tissue. Botanical Gazette 112: 225-229.
Nitsch JP (1951). Growth and development *in vitro* of excises ovaries. Am. J. Bot. 38: 566-571.
Overbeek JV, Conklin ME, Blakeslee AF (1941). Factors in coconut milk essential for growth and development of very young *Datura* embryos. Science 94: 350-352.
Paris D, Duhamet L, Goris A (1954). Action des vitamines et des acides aminés contenus dans le lait de coco sur la proliferation d'une souche de tissus de carotte. Comp. Rendus de la Soc. de Biol. 148: 296-302.
Parthier B (1991). Jasmonates, new regulators of plant growth and development. Many facts and few hypotheses on their actions. Acta Bot. 104: 405-464.
Pierik RLM (1987). In: *In vitro* culture of higher plants. Martinus Nijhoff publishers, Kluwer academic press, The Netherlands, pp. 66-67.
Pollard JK, Shantz EM, Steward FC (1961). Hexitols in coconut milk: their role in nurture of dividing cells. Plant. Physiol. 36: 492-498.
Radley M, Dear E (1958). Occurrence of gibberellin-like substances in coconut. Nature 182: 1098-1100.
Reeve DR, Crozier A (1978). The analysis of gibberellins by HPLC. In: Hillman JR. (ed), Isolation of plant growth substances, Cambridge University Press, U.K., pp 41-77.
Saunders PF (1978). The identification and quantitative analysis of abscissic acid in plant extracts. In: Hillman JR (ed) Isolation of plant growth substances, Cambridge University Press, U.K., pp. 115-131.
Souci SW, Fachmann W, Kraut H (1994). In: Food composition and nutrition tables. Medpharm Scientific publishers, Stuttgart, pp. 627-629.
Steinhart CE, Standifer LG, Skoog F (1961). Nutrient requirements for *in vitro* growth of spruce tissue. Am. J. Bot. 48: 465-472.
Steward FC, Caplin SM (1952). Investigation on growth and metabolism of plant cells: Evidence on the role of coconut milk factor in development. Ann. Bot. 16: 491-498.
Steward FC, Shantz EM (1959). The chemical regulation of growth (some substances and extracts which induce growth and morphogenesis). Ann. Rev. Plant. Physiol. 10: 379-386.
Straus J (1960). Maize endosperm tissue grown *in vitro* : Development of a synthetic medium. Am. J Bot. 47: 641-646.
Straus J, La Rue CD (1954). Maize endosperm tissue grown *in vitro* : Cultural requirements. Am. J. Bot. 41: 687-692.
Wiggans SC (1954). Growth and organ formation on callus tissues derived from *Daucus carota*. Am. J. Bot. 41: 321-326.