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Effect of buffer and pH on growth and protein content of carrot (*Daucus carota* L.) in liquid shake culture

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Abstract. Phosphate, succinate, acetate and maleate buffers were tested for buffering B_5 liquid medium. Phosphate (0·02 M) and acetate (0·002 M) were the most suitable buffers as they promoted dry weight accumulation and nitrogen assimilation, they also exhibited less change in pH after 5 days cultivation of carrot cells. The pH increase from 5·5 up to 6·5 also increased the dry weight accumulation and cell size ; pH 7·0 was detrimental to dry weight accumulation.

Keywords. Tissue culture; buffer ; carrot ; pH.

1. Introduction

There is scanty data on the effect of buffers on plant tissue culture. Schenk and Hildebrandt (1972) reported that citrate and succinate were the best buffers between pH 5 and 6, and that growth of calli of several plant species on these buffers was as good as or better than that on the unbuffered medium. Singh *et al* (1978) observed significant increase in fresh weight of carrot (*Daucas carota* L.) calli in 0·01 M phosphate but dry weight increased significantly in acetate, phosphate, maleate and succinate buffers in liquid shake culture. They also reported that buffers markedly affected the cell width and length in both carrot callus and shake cultures. The present study was undertaken to investigate the effects of some buffers and pH on the growth, cell size and protein content of carrot in liquid shake culture.

2. Materials and methods

Carrot callus was initiated from storage root explants on B_5 medium (Gamborg *et al* 1968) containing 1 μg ml^{-1} 2, 4–D. A liquid shake culture was derived by transferring an actively growing carrot callus to liquid B_5 medium. It was regularly subcultured every 10 days. The shake cultures were agitated in a gyratory

shaker at 150 rpm and were kept in a culture room at 28 ± 2° C in diffuse light. The experiment described below were laid out according to a randomized block design with three replications and the data were analysed accordingly (Steel and Torrie 1960).

The B_5 medium was buffered with acetate, succinate, maleate (0·001, 0·002, 0·005 M) and phosphate (0·01, 0·02, 0·05 M) at pH 5·5 to determine a suitable concentration for growth of cultures (determined as dry weight after 5 days). To determine the effect of pH on the carrot suspension cultures, buffered (acetate, maleate and succinate at 0·002 M and phosphate 0·02 M) and unbuffered media with pH 5·5, 5·0, 6·5 and 7·0 were tested. The dry weight (mg ml^{-1}), the pH of the supernatant and the proportion of isodiametric, elongated and highly elongated cells were determined after 5 days. The cells classified as isodiametric were 50-100 μm in diameter and had comparable long and short axes. The elongated cells were 100-200 μm long, while the highly elongated group consisted of cells in excess of 200 μm in length. From each flask a total of 50 cells were scored. Since pH 6·5 is most suitable for growth, the dry weight accumulation in buffered and unbuffered media at this pH was studied by pippeting out 5 ml cell samples at 6, 12, 24, 48, 72 and 120 hr after subculturing. The nitrogen content was determined in 6-day old shake cultures grown in buffered and unbuffered media (pH 6·5) by the micro-Kjeldahl technique. The per cent protein and total protein per flask were computed. Appropriately buffered and unbuffered B_5 media (20 ml for 100 ml and 40 ml for 250 ml culture flask) were distributed in culture flask, which were stoppered with cotton plugs. The flasks were inoculated with 5 ml and 12 ml suspension cultures (10 ± 0·5 mg ml^{-1} dry weight) in 20 ml and 40 ml media, respectively.

3. Results and discussion

3.1. *Effect of buffers*

The analysis of variance revealed significant differences among treatment means due to buffers, concentration and interaction between buffers and concentrations. The dry weight of liquid shake cultures was generally higher in the buffered media than in the unbuffered control. Phosphate (0·02 M) was the most suitable buffer closely followed by acetate (0·002 M); succinate and maleate (0·002 M) also exhibited a significant increase in dry weight as compared to the control (table 1). These findings are in general agreement with those of Singh *et al* 1978 but they reported much higher dry wiehgts in the buffered media, particularly in acetate than those in the unbuffered media.

3.2. *Effect of pH*

Dry weights differed significantly due to buffers and pH but the interaction effect (buffers × pH) was non-significant. The cell dry weight showed general increase with pH up to 6·5 both in buffered and unbuffered media, but there was a sharp decline at pH 7·0. Phosphate (0·02 M) at pH 6·5 produced the highest dry weight, closely followed by acetate and succinate; maleate was comparable to

Table 1. Mean dry weight (mg ml^{-1}) of carrot shake cultures grown in different buffers (pH 5·5); each value is a mean of 3 replicates.

Buffer	Control	Concentrations (M)			
		0·001	0·002	0·005	Mean
Phosphate*	3·1	3·6	5·7	4·2	4·2
Acetate	3·3	4·3	5·0	4·1	4·1
Succinate	3·1	2·7	4·8	3·4	3·4
Maleate	3·3	2·9	4·0	3·9	3·5

C.D. ($P = 0·05$) buffer = 0·2 mg; Concentration = 0·2 mg; Interaction = 0·2 mg.
* 0·01, 0·02 and 0·05 *M*, respectively.

Table 2. Mean dry weight (mg ml^{-1}) of carrot shake cultures grown at different pH in buffered B_5 medium; each value is average of three replications.

Buffer	pH				Mean
	5·5	6·0	6·5	7·0	
Phosphate (0·02 M)	5·2	5·4	6·3	4·8	5·4
Acetate (0·002 M)	4·1	4·4	5·7	3·9	4·5
Succinate (0·002 M)	4·4	4·8	5·7	4·2	4·8
Maleate (0·002 M)	4·2	4·4	5·1	4·1	4·6
Control (unbuffered)	4·1	4·4	5·1	4·6	4·6
Mean	4·3	4·6	5·6	4·3	

C.D. buffers = 0·4 mg; C.D. pH = 0·3 mg.

the control (table 2). Thus an elevated pH (6·5) is more appropriate than the commonly used pH 5·5 for dry weight accumulation in carrot suspension cultures.

At pH 5·5 the proportion of isodiametric, elongated and highly elongated cells was comparable between unbuffered B_5 and maleate (0·002 M); phosphate, acetate and succinate had a considerably higher proportion of isodiametric cells than the control. The increase in pH resulted in an increase in the proportion of elongated cells at the expense of isodiametric cells; at pH 7·0 highly elongated cells were predominant in all the media (table 3). Thus the increased dry weight in the buffered media (pH 5·5) does not appear to be a result of increased cell size, since a greater proportion of cells was isodiametric and consequently smaller in the buffered media than in the control.

A study of the pattern of dry weight accumulation (figure 1) revealed that the buffered media particularly phosphate and acetate showed appreciably higher dry weight than the unbuffered control as early as 24 hr after subculture and this superiority was maintained till the end of the experiment (120 hr).

The pH of the supernatant from shake cultures in buffered and unbuffered media was monitored after 5 days (table 4). There was a general decline in pH in all the media particularly in the unbuffered control. At pH 5·5, acetate showed relatively less decline in pH, but at higher pH values both acetate and phosphate were comparable and superior to other buffers. These buffers also showed better growth of suspension cultures. Thus for buffering B_5 medium between pH 5·5 and 6·5 both phosphate and acetate are equally appropriate; acetate is effective at a much lower concentration (0·002 M) than the phosphate (0·02 M). Thus where a lower osmotic concentration is desirable, acetate would be preferable to phosphate.

Shake cultures maintained in phosphate, acetate and succinate showed appreciably higher total nitrogen assimilation by the cells (table 5). Conversion of total nitrogen to protein content revealed a higher protein content in cells cultured in phosphate followed by succinate and acetate. Thus increased dry weight of suspension cultures in the buffered media were associated with higher protein content as compared to the control. This indicated that the increase in dry

Table 3. Proportion (%) of round (50–100 μm), elongated (100–200 μm) and highly elongated (7200 μm) cells in carrot shake cultures grown in different buffers at various pH.

Buffer	Cell type	pH			
		5·5	6·0	6·5	7·0
Phosphate (0·02 M)	Isodiametric	42·6	35·3	29·7	22·1
	Elongated	22·6	33·6	33·4	35·0
	Highly elongated	34·8	31·2	37·9	43·0
Acetate (0·002 M)	Isodiametric	49·7	36·1	33·6	23·3
	Elongated	27·6	37·0	42·9	37·7
	Highly elongated	22·7	26·8	23·4	39·0
Succinate (0·002 M)	Isodiametric	50·0	42·5	41·4	26·9
	Elongated	31·5	31·5	29·3	28·1
	Highly elongated	17·6	26·1	29·7	44·9
Maleate (0·002 M)	Isodiametric	37·6	42·3	39·3	26·5
	Elongated	34·4	22·7	32·6	34·0
	Highly elongated	28·2	35·7	28·1	39·5
Unbuffered (Control)	Isodiametric	32·2	35·3	30·4	29·6
	Elongated	32·9	29·8	41·2	29·6
	Highly elongated	34·9	34·8	27·9	40·8

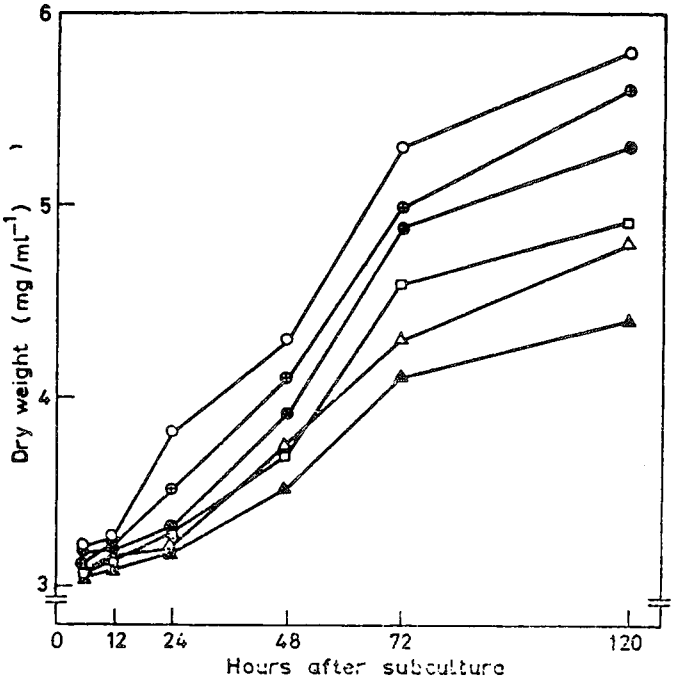

Figure 1. Dry weight accumulation in carrot shake cultures maintained in B_5 of pH 5·5 (▲), and 6·5 (□), and in B_5 buffered with 0·002 M maleate (△), succinate (●), acetate (⊕) and 0·02 M phosphate (○).

Table 4. Change in pH of supernatant from carrot shake cultures after 5 days growth ; each value is mean of 6 replicates.

Initial pH	Buffer				
	Phosphate (0·02 M)	Acetate (0·002 M)	Succinate (0·002 M)	Maleate (0·002 M)	Unbuffered control
5·5	5·26	5·40	5·30	5·26	5·18
6·0	5·80	5·85	5·76	5·70	5·55
6·5	6·32	6·33	6·30	6·20	6·15
7·0	6·80	6·70	6·70	6·70	6·60

Table 5. Total nitrogen (mg) and protein (mg) per flask and protein content (per cent) in carrot shake cultures grown in buffered and unbuffered media (pH 6·5)

Buffer	Dry weight (mg/flask)	Total nitrogen (mg/flask)	Total protein (mg/flask)	Protein content (%)
Phosphate (0·02 M)	232	3·18	19·88	8·6
Acetate (0·002 M)	224	2·98	18·53	8·3
Succinate (0·002 M)	212	2·96	18·40	8·7
Maleate (0·002 M)	192	2·47	15·44	8·0
Unbuffered (pH 6·5)	196	2·50	15·63	8·0
Unbuffered (pH 5·5)	176	2·30	14·38	8·1

weights was due to increase in the mass of cytoplasm and not due to accumulation of polysaccharides or some other storage products. These data along with those on the cell size, suggested that higher dry weight in the buffered media were associated with increase in cell number in the carrot shake cultures.

Thus B_5 may be suitably buffered with either 0·02 M phosphate or 0·002 M. acetate between pH 5·5–7·0 shake cultures exhibit higher dry weight, total nitrogen and protein content, and a lower frequency of elongated cells than those in the unbuffered control.

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