The complex effect of external conditions on growth and respiration of carrot (*Daucus carota* L.) tissues cultured *in vitro*

J. MICHEJDA

Chemical composition of the medium, temperature and light are the most important external factors influencing the growth of plant tissues cultured *in vitro*. The problem of the appropriate choice of these factors arose simultaneously with the problem of inducing tissues to grow *in vitro*. In the first place a suitable medium, containing mineral components and a source of carbon available for tissues (Gautheret 1942; White 1943) had to be prepared. The more detailed investigations, e.g. of Hildebrandt and Riker (1953) led to a better knowledge of sugar requirements of tissues.

The effect of light and temperature on plant tissue growth *in vitro* were studied by Gioelli already in 1938. The systematical investigations were started much later (Hildebrandt, Riker a. Duggar 1945; Nickell a. Burkholder 1950; Rier a. Henderson 1957; De Capite 1955) yet many investigators applied only simple room conditions.

In examining the influence of external conditions on plant tissue growth individual factors were usually considered separately. Exclusively De Capite's work (1955) treated light in conjunction with temperature, while Rier and Henderson (1957) on the other hand tested the influence of temperature in relation to the nutrients.

The present paper examines the simultaneous influence of three factors, viz. sugar in medium, temperature and light.

MATERIAL AND METHODS

Carrot (*Daucus carota* L.) tissue cultured *in vitro* was the tested plant material. The tissue was cultured under 16 different variants of environmental conditions. The following factors i.e. two temperatures of 18°C and 24°C, light (600 lux) and darkness, four kinds of sugars supplied to the media, viz. 1% glucose, 1% fructose, 2% sucrose and 3% raffinose were combined as follows:

1.	{	24°C, light
2.		24°C, darkness
3.		18°C, light
4.		18°C, darkness
- 1% glucose,

5.	1% fructose,	24°C, light
6.		24°C, darkness
7.		18°C, light
8.		18°C, darkness
9.	2% sucrose,	24°C, light
10.		24°C, darkness
11.		18°C, light
12.		18°C, darkness
13.	3% raffinose,	24°C, light
14.		24°C, darkness
15.		18°C, light
16.		18°C, darkness

Determinations of fresh and dry matter and of the respiration rate were described in the previous paper (Michejda 1963). The experimental data reported below are the averages of 8 trials conducted at various times of the year; fresh and dry matter determinations were replicated each time on 12 tissues, respiration rate determinations — on 3 tissues.

EXPERIMENTAL RESULTS

Fresh and dry matter of tissues (Fig. 1.)

A temperature of 24°C is more favourable for tissue growth (expressed both in terms of fresh and dry matter) than 18°C in cases of media containing sucrose, raffinose and glucose. Differences in growth

Fig. 1. Fresh and dry matter of carrot tissues after 50 days of culturing under different external conditions. Diagrams of the same column correspond to tissues cultured on the same sugar; light and darkness are indicated by clear and shaded areas, respectively; the first two columns of each group represent the temperature of 24°C, the two next columns — the temperature of 18°C.

at these two temperatures are most evident on sucrose, slightly less marked on raffinose and smallest on glucose. In culturing on fructose on the other hand completely different relations are observed: in the

light, growth (fresh matter) is more intense at 18°C than at 24°C; while in darkness the temperature of 24°C is slightly more favourable.

As regards the dry matter of tissues cultured on fructose it remains on a very like level — whatever the temperature and light conditions.

Light bears a positive influence on tissue growth, both at the higher and lower temperature; on sucrose media, however, this influence at 18°C is very slight, while on media with other sugars it is greater; at 24°C the influence of light is evident on sucrose but very small on fructose media.

Least favourable conditions for tissue growth, whichever of the sugars was added, occur beyond doubt at 18°C, in darkness.

Tissue respiration (Fig. 2.)

Temperature bears a marked effect on the respiration of tissues cultured on sucrose and raffinose media. In considering the respiration rate in terms of tissue fresh matter, in the higher temperature respiration is found to be twice as intense as in the lower temperature. If the respiration rate is referred to the tissue dry matter or to the protein nitrogen, although the differences are not so marked, the temperature of 24°C bears a more favourable effect than that of 18°C.

The respiration rate of tissues cultured on media containing sucrose and raffinose is much the same, whichever the temperature and light conditions. Tissues cultured on media containing fructose and glucose exhibit a respiration rate which behaves quite differently. In the case

Fig. 2. Respiration rate of sections of carrot tissue cultured for 50 days under various external conditions, given in terms of fresh matter, dry matter and protein nitrogen of the tissue (For the presentation of results see Fig. 1).

of fructose, the intensity of respiration calculated on a fresh matter basis is the same in both temperatures. Calculated on a dry matter, or protein nitrogen basis the oxygen intake is found to be higher at 18°C.

A similar pattern occurs in tissues cultured on glucose — especially reckoned in terms of protein nitrogen, the respiration rate is higher at 18°C. In general, tissues cultured on glucose reveal low oxygen requirements, particularly at 24°C, and when these requirements are referred to the fresh matter.

Table 1

Carrot tissue cultured on different percentage concentrations of various sugars

Sugar	Glucose		Fructose		Sucrose		Raffinose
Percentage concentration	1%	3%	1%	3%	2%	3%	3%
Approximate osmotic value in osmM	0.1	0.2	0.1	0.2	0.1	0.13	0.1
Tissue growth mg	853	893	592	324	1134	1146	877
Dry matter mg	35	56	30	30	65	71	48
Percentage dry matter	4.1	6.2	5.1	9.2	5.7	6.2	5.5
Total N mg/100 mg dry matter	2.11	1.64	2.05	1.90	1.43	1.33	1.51
Protein N mg/100 mg dry matter	1.17	0.94	1.24	0.83	1.01	1.00	1.03
Respiration μ l O ₂ /g fresh matter/hr	43	45	51	46	58	48	56
Soluble sugars mg/100 mg dry matter	14.1	33.3	18.7	29.0	38.2	48.8	36.6
Osmotic value osmM	0.26	0.36	0.28	0.51	0.32	0.36	0.33

The experimental data show that out of the three examined factors, i.e. sugar, temperature and light, the kind of sugar added to the medium was most effective. In the research work equimolar solutions of sugar were utilized. In order to compare the effect of the same sugars, supplied in equal percentage concentrations, on tissues, an analysis was carried out wherein beside the usual concentrations, 3 per cent concentrations of all three kinds of sugar were simultaneously applied. Table 1 gives the results. As shown above, the tissue growth on a 3% sugar concentration is more or less the same as on a 1% concentration (in the case of glucose), or a 2% concentration (in the case of sucrose). In this respect tissue cultured on fructose is an exception; as the concentration of fructose rises from 1 per cent to 3 per cent the tissue growth rate falls. The

percentage of dry matter is higher on 3% sugar concentrations, and coincides with the increased level of soluble sugars in tissues and the raised osmotic value which, particularly in the case of fructose, increases very greatly. Water uptake by tissues on 3% fructose is clearly hindered. On 3% sugar concentrations the respiration rate falls and the level of total and protein nitrogen in tissues is lowered.

In general, changes effected in tissues by a rise in the sugar concentration of media are more visible in the cases of glucose and fructose than in the case of sucrose. This is quite obvious when the differences in molar concentrations between 1% and 3% glucose or fructose and 2% and 3% sucrose are considered. In the first case the difference in the osmotic value is around 0.1 osmM while in the second — around 0.03 osmM. Here the effective influence of osmotic value of the medium on the tested properties of tissues is clearly evident. In order to eliminate this additional factor it seems more reasonable to study the effect of sugars on their equimolar concentrations.

The obtained effects enable to observe a similar behaviour of tissues on pairs of sugars, viz. on raffinose and sucrose as well as on glucose and fructose. Raffinose and sucrose are more suitable sugars for tissue growth than glucose and fructose. Not only the fresh and dry matter increases in amount, but also the outward aspect of tissues cultured on sucrose and raffinose lacks necrotic areas which often appear on tissues grown on glucose, and even more often on tissues cultured at 24°C on fructose. In addition, tissue growth on fructose is least uniform.

Tissues respond to applied temperatures differently, in dependence on the sugar in media. A temperature of 24°C is much more appropriate than 18°C for the growth and respiration of tissues cultured on sucrose and raffinose. When the latter are cultured on glucose and fructose, growth and respiration (mostly expressed in terms of protein nitrogen of tissues) at 18°C reveal a tendency to approximate, or even to surpass the relative values at 24°C.

DISCUSSION

The present results lead to a general conclusion of a complex nature of the effect of external factors on growth and metabolic processes of the tested tissue. Among these elements of the environment influencing the cultured tissues sugar, a nutrient, was found to be most effective.

Plant tissues develop within the range of 10 to 30°C, at an optimum of 25°C for most of the tissues (Gautheret 1959). Present results seem to indicate the following: tissue growth curves plotted against e.g. light and temperature may represent different patterns, with extremities on different levels, in dependence on the kind of sugar used as the source of carbon for the tissue development.

De Capite's investigations (1955) carried out among others on carrot tissues cultured *in vitro* showed (1) that the optimum for tissue growth in darkness falls between 23 and 26°C; (2) that above 26°C growth is a function of temperature and light (which bears a stimulating action), whereas below 26°C it seems only to be a function of temperature. The present results differ from De Capite's findings in that a stimulating effect of light, both at 18°C and 24°C, on the production of fresh and dry matter was found.

A similar response of tissues to the pairs of sugars: sucrose and raffinose, glucose and fructose, may perhaps be explained on grounds of metabolic processes of these sugars within the tissues. Sucrose is the most appropriate sugar for the development of the majority of tissues (Gautheret 1959) which may accumulate large quantities of this sugar. In carrot tissue (Goris 1954) sucrose amounts to 50 per cent of the total content of sugars. Sucrose is found in the tissues always, whichever sugar was supplied to the medium (Ball 1955). The availability of other sugars is related to the easiness of their conversion to sucrose.

The breakdown of raffinose may run along two different lines (Shafizadeh a. Wolfrom 1958): invertase may convert it to melibiose and d-fructose, α -d-galactopyranosidase — to sucrose and d-galactose. The accumulation of, among others, galactose in tissues cultured on raffinose (Ball 1955) indicates a decomposition of the latter type. This way raffinose may be a direct source of sucrose for the growing tissue.

Carrot tissue cultured *in vitro* synthesizes and accumulates considerable amounts of sucrose, whether cultured on glucose or on fructose; yet fructose appeared to be more effective (Goris 1954). The synthesis of sucrose from hexoses is an oxygen-requiring process, involving phosphorylation and therefore consuming energy (James 1957). This may account for the slower rate of tissue growth on hexoses in comparison with sucrose or raffinose.

In view of the fact that sucrose, raffinose, glucose and fructose become involved in the metabolic processes following different initial transformations, differences in the thermal optimum for tissue development on the particular sugars may be related to these initial processes.

SUMMARY

1. Growth (in terms of fresh and dry matter) and respiration of carrot tissue, cultured under 16 different variants of external conditions, arranged in combinations of the following elements: 1% glucose, 1% fructose, 2% sucrose, 3% raffinose; light and darkness; temperatures of 18°C and of 24°C, were examined.

2. It was found that the effect of temperature and light on growth and respiration of tissues is dependent on the kind of sugar in the medium. For tissues cultured on sucrose and raffinose definitely the temperature of 24°C is more

favourable for growth and respiration. Tissues cultured on glucose, and even more on fructose, attain at 18°C the same or even higher levels.

3. A complex character of the influence of external conditions on growth and respiration of carrot tissue cultured *in vitro* has been assumed. The similar behaviour of tissues on two pairs of sugars, viz. glucose and fructose on one hand, and sucrose and raffinose on the other, has been discussed.

Department of Plant Physiology
A. Mickiewicz University
in Poznań, Poland

(Entered: 3.2.1963.)

REFERENCES

- Ball E., 1955, Studies on the nutrition of the callus culture of *Sequoia sempervirens*, Ann. Biol., 31:81.
- Capite de L., 1955, Action of light and temperature on growth of plant tissue cultures *in vitro*, Amer. Jour. Bot., 42:869.
- Gautheret R. J., 1942, Manuel technique de culture des tissus végétaux, Masson, Paris, 170 pp.
- Gautheret R. J., 1959, La culture de tissus vegetaux, Masson, Paris, 863 pp.
- Gioelli F., 1938, Morfologia, istologia e fisiopatologia di meristemi secondari *in vitro*, Att. Ac. Sc. Ferrara, 16:1.
- Goris A., 1954, Transformations glucidiques intratissulaires, Ann. Biol., 30:297.
- Hildebrandt A. C., Riker A. J., Duggar B. M., 1945, Growth *in vitro* of excised tobacco and sunflower tissue with different temperatures, hydrogen ion concentrations and amounts of sugar, Amer. Jour. Bot., 32:357.
- Hildebrandt A. C., Riker A. J., 1953, Influence of concentrations of sugars and polysaccharides on callus tissue growth *in vitro*, Amer. Jour. Bot., 40:66.
- James W. O., 1957, Reaction paths in the respiration of the higher plants, Advanc. Enzymol., 18:281.
- Nickel L. G., Burkholder P. R., 1950, Atypical growth of plants. II. Growth *in vitro* of virus tumors of *Rumex* in relation to temperature, pH, and various sources of nitrogen, carbon and sulfur, Amer. Jour. Bot., 37:538.
- Rier J. P., Henderson J. H. M., 1957, The relationship of temperature and nutrition to the growth of sunflower tumor tissues *in vitro*, Plant Physiol., 32:494.
- Shafizadah F., Wolfrom M. L., 1953, Structure, properties and occurrence of the oligosaccharides. Encyclopedia of Plant Physiology Vol. VI., Springer, Berlin, 1444 pp.
- White Ph. R., 1943, A handbook of plant tissue culture, The Jaques Cattell Press, Lancaster, 277 pp.

Kompleksowy wpływ warunków zewnętrznych na wzrost i oddychanie tkanki marchwi (Daucus carota L.) hodowanej in vitro

Streszczenie

Zbadano wzrost (świeżą i suchą masę) i oddychanie tkanki marchwi hodowanej w 16 wariantach warunków zewnętrznych, uzyskanych przez kombinację 4 cukrów: 1% glukozy, 1% fruktozy, 2% sacharozy i 3% rafinozy, światła i ciemności oraz dwóch temperatur: 18° i 24°C.

Stwierdzono, że wpływ temperatury i światła na wzrost i oddychanie tkanek uzależniony jest od rodzaju cukru w pożywce. Dla tkanek hodowanych na sacharozie i rafinozie temperaturą odpowiedniejszą dla wzrostu i oddychania jest zdecydowanie 24°C, podczas gdy dla tkanek hodowanych na glukozie, a w jeszcze większym stopniu na fruktozie, zaznacza się w 18°C tendencja do zrównania, a nawet przewyższenia odpowiednich wielkości w temperaturze 24°C.

Wysnuto wniosek o kompleksowym wpływie czynników zewnętrznych na wzrost i oddychanie tkanki marchwi hodowanej *in vitro* oraz przedyskutowano podobieństwo zachowania się tkanek na parach cukrów: glukozie i fruktozie oraz sacharozie i rafinozie.