Seasonal changes in carrot (*Daucus carota* L.) tissues cultured *in vitro*

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Studies on seasonal changes in plant tissues cultured *in vitro* are of a practical and theoretical value. In practice it is very useful to know whether cultured tissues, serving very often as the basic material for investigating numerous physiological problems, undergo changes related to the time of the year. In respect to plant tissues cultured *in vitro* there exists already a widespread knowledge that they are not always a fixed and stable biological material, often subject to reversible or irreversible modifications of their morphological, anatomical or physiological character (Gautheret 1955).

As regards periodical changes in cultured tissues an endogenous, diurnal growth and turgor rhythm was revealed in carrot tissue (Enderle 1951). An annual rhythm has as yet only been suggested by some of the authors, but close investigations of tissues, cultured over a period of a whole year under unvarying conditions of light and temperature, are lacking.

From the theoretical aspect, if an endogenous annual rhythm were to be found in isolated plant tissues, previously cultured *in vitro* for several years, the hypothesis according to which a biological rhythm is a fundamental phenomenon in cells and tissues would thus be supported (Bünning 1956, 1958).

Following indexes of seasonal changes in carrot tissues cultured *in vitro* were established: tissue growth expressed in terms of fresh and dry matter, level of soluble sugars, rate of respiration and osmotic value of the tissues.

Research was carried out on tissues cultured under various conditions, possibly approaching the optimum, and decidedly suboptimal for the development of carrot tissue. Certain rhythmical phenomena, e.g. movements of bean leaves (Leinweber 1956) are always closely related to a given regime of temperature, wherein there is a thermal optimum under which these movements are most intense. Henssen (1954) investigated the development of turions in *Spirodela polyrrhiza* under stable laboratory conditions, and found the annual rhythm of this physiological process to be detectable solely on media abundant in

mineral salts; should these salts be deficient, the formation of these shoots could occur at any time of the year.

The above given data show that an endogenous rhythm may become expressed under specified conditions, whereas under different conditions it may remain latent. In the present work differentiated conditions for the tissue culture were obtained by establishing different temperatures, applying light or darkness and by supplying the media with various sugars.

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MATERIAL AND METHODS

Tissue culture

Research was carried out on carrot (*Daucus carota* L.) tissue cultured *in vitro*. The tested strain was isolated by Gautheret (1939). At first it represented a normal tissue, requiring for its growth a supply of β -indolilacetic acid in a concentration of 0.05 mg/l to the medium. In 1948, this strain was brought to the Department of General Botany of the Poznań University where, after a few years culture it became anergized to auxin. Continuing to grow in an auxinfree medium it maintained a morphological structure of normal tissue, while Gautheret's anergized carrot tissue revealed a pattern of growth peculiar by its breakup into small clusters.

Until the beginning of the present research the tissues were cultured under room conditions. In order to examine seasonal variation tissues were cultured in two thermostats, at 18 and 24°C. Each of the thermostats was divided into two compartments, one of which was shaded and the other exposed to continuous, fluorescent light (600 lux). Within the thermostats the moisture level was maintained at 85—90 per cent of relative humidity.

Tissues were cultured on four kinds of media, containing:

1. Heller's mineral medium (1952),
2. 0.6 per cent agar,
3. one of the following sugars: a 1 per cent solution of glucose, a 1 per cent solution of fructose, a 2 per cent solution of sucrose and a 3 per cent solution of raffinose. The concentrations of sugars were matched with the osmotic concentration of the media. The theoretical osmotic concentration of the media was 0.1 osmM.

Tissues were cultured in test-tubes 23 by 120 mm in size. Tubes containing the media were inoculated with 100 mg particles of tissues sectioned by means of a scalpel; sizes of these inocula were checked

against established standards. Differences in weight of the individual inocula remained under 5 mg. In the case of carrot tissue the initial weight of 100 mg is much above the minimum initial size of 10 mg (Gautheret 1959).

All of the four media were each time inoculated with 192 particles of tissue, equal amounts of which (48 particles) were placed under the different thermal and light conditions. Hence the tissues were cultured simultaneously under following conditions for a whole year:

1.		{ 24°C, light
2.		{ 24°C, darkness
3.	2 per cent solution of sucrose,	{ 18°C, light
4.		{ 18°C, darkness
5.		{ 24°C, light
6.	3 per cent solution of raffinose,	{ 24°C, darkness
7.		{ 18°C, light
8.		{ 18°C, darkness
9.		{ 24°C, light
10.		{ 24°C, darkness
11.	1 per cent solution of glucose,	{ 18°C, light
12.		{ 18°C, darkness
13.		{ 24°C, light
14.		{ 24°C, darkness
15.	1 per cent solution of fructose,	{ 18°C, light
16.		{ 18°C, darkness

The growth period of the transplants lasted each time 52 days. Transplantations were performed for 2 years. Prior to this period, in order to adapt the tissues to the various culture conditions, they were precultured under the same conditions for 6 months.

Determination of fresh and dry matter of tissues

After 52 days of culturing the tissues were taken out of the test-tubes; the bottom portions were then precisely cleaned with filter-paper and the entire tissues weighed with an approximation of 1 mg. Subsequently each one was divided into two parts, by cutting off the bottom portion, which might have been contaminated by the medium; both parts were dried and weighed together. Top portions of the tissues were utilized for determining saccharides and total nitrogen.

Preliminary drying was performed at 100°C for 45 minutes; drying at 75°C to a constant weight succeeded. The dried material was kept in a vacuum desiccator over calcium chloride.

Each series of measurements included determinations of fresh and dry matter of all 12 tissues.

Determination of total and protein nitrogen

Total and protein nitrogen were determined in dried material acc. to the micro-Kjeldahl method. The determination of protein nitrogen was carried out after Pirschle (1941): the plant material was extracted for 1½ hr on a boiling water bath and proteins precipitated with a 20 per cent solution of trichloroacetic acid.

Both total and protein nitrogen were determined in two samples taken from 12 ground and mixed tissues. In order to detect the differences between individual tissues 12 determinations were carried out on 12 different tissues.

Determination of soluble sugars

10 mg of dried and pulverized tissue material was extracted with 50 ml of water at 75°C for 45 min. The extract was filtered into a 100 ml measuring flask, and 2 ml of it was subjected to a colour reaction with anthrone (Peach, Tracey 1955). The extinction of the coloured solution was determined with the use of spectrophotometry, at a wavelength 620 mμ, against glucose.

This means provided a satisfactory reproductivity of the colour reaction and an intensity of coloration, proportionate to the amount of added sugar. It was only essential to check strictly the timing of the reaction with anthrone. This was provided for as follows: all the components of the reaction were mixed in test-tubes of Jena glass, 18 mm in diameter, immersed in an ice bath; subsequently the test tubes were placed in a boiling water bath for 9 min. where the reaction took place; once again were they removed, back to the ice bath, in order to put an end to the reaction after a given space of time. Each time the sugar determinations were replicated on a mixture of 12 tissues. For the purpose of revealing individual differences in the sugar content, determinations were carried out in all 12 different tissues.

Measurements of the respiration rate

The respiration rate was measured by means of the manometric method, in Warburg's vessels of a 15—20 ml total capacity, at a 2 ml volume of the liquid phase. The vessels contained 0.2 ml of 1 N KOH. Measurements were carried out at 25°C and the thermal equalization was completed after 15 min. The deflection amplitude of the manometers was 50 mm, while the oscillation — 110/min.

Tissues cleaned from agar were sliced at 0.5 mm. in thickness, 0.5 gm portions were weighed and placed in 10 ml of 1/15 M phosphate buffer

of a pH 6.0 for 2 hours; from time to time these portions were mixed. Rinsed slices were strained on filter paper and placed in Warburg's vessels containing the same buffer that was used for rinsing the slices. Respiration followed a linear pattern for at least 3 hours. Respiration measurements were thrice replicated and the results averaged.

Determination of the osmotic value of tissues

Osmotic value of the tissues was determined acc. to the micro-cryoscopic method, with the use of a micro-cryoscope constructed by Obuchowicz (1956). A comparison of the values obtained from material fixed at high temperatures, and from fresh material, failed to reveal any significant differences, so that the subsequent determinations were performed on fresh tissues.

Tissues were crushed between two slides and the juice thus obtained was poured into capillary tubes of Jena glass, 1.5 mm in diameter, 30—40 mm in length, the reduced ends of which were sealed over a micro-burner, avoiding an overheating of the liquid. Subsequently the capillary tubes were centrifuged in appropriate holders at 8.000 g for 10 minutes. Centrifugation of the samples reduced the dispersion of results. The maximum differences between 10 measurements of the same juice were 0.03°C without centrifugation, while 0.02°C when centrifugating took place.

The supernatant was collected into suitable gauged capillary tubes 0.2 mm in diameter, and separated into columns by shaking. Measurements were carried out only on columns with a 1:1 or 2:1 height:diameter ratio.

Each series of measurements included 3 tissues, each of which was represented by 2 gauged capillary tubes. Thus 6 c. tubes were measured and the sum of results was each time multiplied by the number of columns of the different c. tubes. The average of columns in a single capillary tube was regarded as an elementary measurement.

The method accuracy of the micro-cryoscopic measurements remained at 0.02°C . It was maintained both in respects to the NaCl solution and to the juice pressed out of the tissue, centrifuged and sampled in a series of gauged capillary tubes. In order to establish the sampling error — the tissue was divided into 6 portions, each of which was crushed separately. The maximum differences between the six measurements amounted to 0.04°C when the juice was centrifuged, and to 0.06°C when it was not (larger numbers of measurements from a single tissue could not be made because of technical difficulties).

The capillary tubes were freezed with ethyl chloride.

Reagents

All the used reagents possessed the degree of purity "pro analysi". Sugars in the media were supplied by: Hoffmann-La Roche, Sigma and Light. Redistilled water from a glass apparatus was used.

Statistical methods

Statistical methods were an aid in the interpretation of results Ruszczyc (1955), Koller (1955) and Gautheret's (1959).

In order to determine and compare the rate of growth changes of tissues, cultured in various conditions, a coefficient of variation was calculated as follows: $C_v = \frac{100 \cdot \sigma}{\bar{x}}$, where $\bar{x}$ = the calculated mean, σ = the mean deviation. Test t and F (analysis of variance) were applied to determine the accuracy of means and the significant differences between means. The smallest significant difference (d) between mean values of tissue growth in various seasons was calculated:

$$d = t \sqrt{\frac{2\sigma^2}{n}}$$

These particular statistical tests could be applied as carrot tissue growth follows a distribution which is in accordance with the law of Gauss (Delaporte, Heller 1950).

EXPERIMENTAL RESULTS

Tissue growth and dry matter content

Tissue growth during different seasons expressed in terms of fresh and dry matter after 52 days of culture, and the percentage dry matter levels in tissues, are listed in Table 1. The numerical values represent means of measurements, whose sampling terms coincide over a period of two years; and of additional data listed in columns 1 and 3, referring only to the second year and whose sampling terms fall between analytical points of the previous year. These additional measurements were carried out in order to determine with a greater degree of accuracy the spring period of intensified growth which was noticed in the first year.

The tabulated results of the differences in growth are summarized graphically to obtain a better ocular demonstration (1 — 4). The smallest significant difference (d) between the means calculated for tissues cultured under various conditions was indicated in applying test F with an assumed probability degree of 99 per cent.

Table 1 and the graphs show that in all cases there is a tendency

Table 1

Carrot tissue growth in different months in mg of fresh and dry matter and the percentages of the tissue dry matter — after 52 days of culture

Tissue culture conditions	1		2		3		4		5		6		7		8		9	10	11								
	16.I—9.III		16.II—9.IV		20.III—12.V		25.IV—15.VI		29.V—20.VII		1.VII—20.VIII		4.IX—26.X		11.XI—2.I												
	f.m.	d.m. %	f.m.	d.m. %	f.m.	d.m. %	f.m.	d.m. %	f.m.	d.m. %	f.m.	d.m. %	f.m.	d.m. %	f.m.	d.m. %	r	r—d	Cv								
Sucrose, light	1039	61	5.9	1134	65	5.7	943	52	5.5	893	46	5.2	967	57	5.9	851	45	5.3	952	52	5.5	1030	52	5.0	283	105	17
Sucrose, darkness	665	45	6.8	690	44	6.4	552	39	7.1	647	39	6.0	540	37	6.8	601	35	5.8	583	38	6.5	657	41	6.2	138	4	18
Sucrose, light	342	16	4.7	554	25	4.5	337	15	4.5	381	18	4.7	400	18	4.5	321	16	5.0	346	16	4.6	375	16	4.3	233	132	21
Sucrose, darkness	340	18	5.3	416	19	4.6	354	18	5.1	315	16	5.1	284	14	5.0	238	11	4.6	310	14	4.5	323	16	4.8	178	99	20
Raffinose, light	844	49	5.8	877	48	5.5	880	48	5.5	835	43	5.1	755	44	5.8	805	46	5.7	782	45	5.8	880	54	6.1	124	—14	16
Raffinose, darkness	579	34	5.9	700	45	6.4	633	39	6.2	563	39	7.0	662	40	6.0	652	38	5.9	589	38	6.4	598	37	6.2	137	17	17
Raffinose, light	497	22	4.4	545	22	4.0	528	24	4.5	480	21	4.3	500	23	4.5	503	22	4.4	473	22	4.6	529	24	4.5	72	—8	13
Raffinose, darkness	381	18	4.7	395	18	4.6	410	19	4.7	402	20	5.0	377	18	4.7	322	16	4.9	350	17	4.9	319	16	5.0	91	15	17
Glucose, light	688	32	4.6	853	35	4.1	690	32	4.6	572	25	4.4	636	32	5.0	375	20	5.3	525	26	5.0	651	30	4.6	478	325	19
Glucose, darkness	350	18	5.1	658	28	4.3	475	24	5.1	500	26	5.2	454	25	5.5	413	22	5.3	390	20	5.0	340	18	5.3	318	232	16
Glucose, light	638	25	3.9	912	29	3.2	616	24	3.9	562	20	3.6	502	19	3.8	393	16	4.1	511	19	3.7	604	21	3.5	519	403	15
Glucose, darkness	327	13	4.0	414	18	4.3	326	17	5.2	290	13	4.5	310	15	4.7	300	14	4.7	286	13	4.5	279	13	4.7	135	76	17
Fructose, light	383	25	6.5	592	30	5.1	517	29	5.6	462	24	5.3	530	33	6.2	333	19	5.7	376	23	6.0	509	29	5.7	259	127	24
Fructose, darkness	356	25	7.0	663	43	6.5	500	33	6.6	305	21	6.9	296	21	6.7	390	26	6.7	420	27	6.5	416	27	6.5	367	277	20
Fructose, light	680	31	4.6	769	32	4.2	550	27	4.9	505	23	4.5	462	21	4.5	453	21	4.6	626	25	4.0	688	30	4.4	317	172	21
Fructose, darkness	476	25	5.2	486	26	5.4	319	20	6.3	300	18	6.1	330	19	5.7	405	22	5.4	359	19	5.3	306	19	6.1	186	103	19

Abbreviations: f. m. — fresh matter; d. m. — dry matter; r — difference between the extreme values of fresh matter; d — the smallest significant difference of fresh matter; C — coefficient of variation of the fresh matter (average annual).

Graph 1. Fresh matter of carrot tissue cultured in different months and weighed after 52 days of culture. Tissue cultured on raffinose under varying conditions of temperature and light. The shaded area indicates the smallest significant difference between weights represented in each graph. The last point of each graph is a repetition of the first point.

Graph 2. Fresh matter of carrot tissue cultured in different months and weighed after 52 days of culture. Tissue cultured on sucrose, under varying conditions of temperature and light. For further expl. see Graph 1.

Graph 3. Fresh matter of carrot tissue cultured in different months and weighed after 52 days of culture. Tissue cultured on glucose under varying conditions of temperature and light. For further expl. see Graph 1.

Graph 4. Fresh matter of carrot tissue cultured in different months and weighed after 52 days of culture. Tissue cultured on fructose under varying conditions of temperature and light. For further expl. see Graph 1.

of increasing the growth rate in February — March, and a reversed tendency in July — August; the rate of expression however of these tendencies differs in relation to the various sugars in culture media. In Table 1 (columns 9 and 10) are listed: calculated differences between the extreme values of the tissue fresh matter in various months, and the same differences diminished by the smallest significant difference (d). These data show that the greatest seasonal differences in growth occur in tissues cultured on glucose, lesser on fructose and sucrose, smallest on raffinose, in the case of which they remain below or just above the smallest significant difference (d).

The kind of sugar in the medium influences also the dispersion of the tissue growth values within the given series. Coefficients of variation reveal the largest variation in tissues cultured on fructose, whereas on raffinose the growth is most equalized.

The percentage dry matter in tissues remains in general on the same level throughout the whole year. The growth differences in question are therefore not related to the differences in tissue water content.

Total and protein nitrogen

In Table 2 are listed the contents of total and protein nitrogen in tissues in different seasons. Prior to assuming an occurrence of seasonal differences, 12 measurements carried out on a mixture of 12 tissues, and 12 — on separate tissues, were interpreted statistically. Thus, both the experimental error and the individual variation of the tissues were brought to light. In determining total nitrogen, the product $t \cdot \sigma_m$ (at 99 per cent of probability) in the case of analysis of tissue mixtures amounted to 0.012, and in the case of separate tissues — to 0.11 (mg/100 mg of dry matter). In determining protein nitrogen the product $t \cdot \sigma_m$ worked out at respectively 0.004 and 0.104.

It follows that individual differences in levels of the examined nitrogen fractions, possible to detect by the applied method, occur between tissues. Taking into account individual variation, significant differences between the means, calculated according to test t , would amount to: 0.2 mg/100 mg of dry matter for total nitrogen, and 0.13 mg/100 mg of dry matter for protein nitrogen. In considering these data, a lack of significant differences between values standing in Table 2 for the contents of total and protein nitrogen in tissues cultured under various conditions, depending on the season, must be accounted for.

Respiration of tissues

Measurements of the respiration rate of slices of a series of tissues growing simultaneously under the same conditions disclose a rather considerable dispersion of results. E.g. the mean result of respiration

Table 2

Total and protein nitrogen in carrot tissue in different months (in mg/100 mg of tissue dry matter). In each case the two dates indicate the period between inoculation and weighing of the tissues

Tissue culture conditions	1		2		3		4		5		6		7		8	
	16.I—9.III		16.II—9.IV		20.III—12.V		25.IV—15.VI		29.V—20.VII		1.VII—20.VIII		4.IX—26.X		11.XI—2.I	
	Total N	Protein N	Total N	Protein N	Total N	Protein N	Total N	Protein N	Total N	Protein N	Total N	Protein N	Total N	Protein N	Total N	Protein N
Sucrose, light	1.43	0.96	1.43	1.01	1.45	0.97	1.53	0.99	1.45	0.91	1.62	0.90	1.50	0.96	1.38	0.90
Sucrose, darkness	1.46	0.95	1.45	0.94	1.40	0.99	1.58	0.93	1.42	0.97	1.56	0.90	1.52	0.95	1.41	0.96
Sucrose, light	2.03	0.86	2.07	0.86	2.21	0.93	2.00	0.93	2.10	0.84	2.09	0.87	2.01	0.87	2.08	0.91
Sucrose, darkness	1.82	0.84	1.86	0.88	2.02	0.86	1.92	0.86	1.90	0.89	1.94	0.92	1.83	0.94	1.97	0.90
Raffinose, light	1.57	1.05	1.51	1.03	1.45	1.03	1.55	0.98	1.50	1.02	1.52	0.96	1.49	1.00	1.44	1.06
Raffinose, darkness	1.59	1.03	1.47	0.95	1.49	1.04	1.52	0.95	1.50	1.04	1.58	1.04	1.55	1.02	1.46	0.98
Raffinose, light	2.02	0.87	1.87	0.86	1.92	0.92	1.98	0.86	1.90	0.90	1.92	0.89	1.89	0.89	1.82	0.87
Raffinose, darkness	1.84	0.88	1.79	0.81	1.86	0.88	1.84	0.80	1.88	0.82	1.83	0.73	1.88	0.80	1.95	0.85
Glucose, light	2.13	1.23	2.11	1.17	2.19	1.29	2.13	1.27	2.05	1.23	2.14	1.37	2.10	1.25	2.22	1.29
Glucose, darkness	2.10	1.15	2.00	1.12	2.15	1.15	2.08	1.14	2.00	1.19	2.07	1.16	2.20	1.18	2.11	1.22
Glucose, light	2.27	1.01	2.16	1.00	2.27	1.06	2.20	0.95	2.25	0.95	2.28	1.03	2.28	1.01	2.31	0.93
Glucose, darkness	2.37	1.12	2.39	1.15	2.36	1.07	2.25	1.16	2.30	1.11	2.34	1.15	2.21	1.13	2.26	1.15
Fructose, light	2.18	1.16	2.05	1.24	2.24	1.17	2.22	1.11	2.27	1.17	2.24	1.22	2.15	1.17	2.13	1.19
Fructose, darkness	2.16	1.18	2.07	1.04	2.05	1.14	2.13	1.11	2.08	1.17	2.00	1.11	2.05	1.12	2.11	1.14
Fructose, light	2.16	1.11	2.22	1.09	2.24	1.06	2.28	1.07	2.37	1.03	2.30	1.12	2.28	1.14	2.15	1.04
Fructose, darkness	2.02	0.99	2.05	1.04	2.10	1.04	2.11	0.98	2.05	1.00	2.04	1.03	2.04	1.03	2.06	0.90

measurements in 10 tissues equals $47 \mu\text{l O}_2/\text{gm}$ of fresh matter/hr, while the product $t \cdot \sigma_m = 5.7$. For this reason, the results given in Table 3 and representing means of 3 parallel measurements of respiration in 3 different tissues do not permit to draw conclusions of an existence of seasonal differences in the respiration of tissues.

Table 3

Respiration rate of carrot tissue in different months expressed in $\mu\text{l O}_2/\text{g}$ of tissue fresh matter/h. Each numerical value is a mean of three parallel measurements. In each case the two dates indicate the period between inoculation and the day measurements of the respiration rate

Tissue culture conditions		1	2	3	4	5	6	7	8
		16.I— 9.III	16.II— 9.IV	20.III— 12.V	25.IV— 15.VI	29.V— 20.VII	1.VII— 20.VIII	4.IX— 26.X	11.XI— 2.I
Sucrose	24°								
light		60	58	63	65	59	62	62	61
Sucrose	24°								
darkness		56	63	59	56	59	61	60	63
Sucrose	18°								
light		30	29	28	35	28	29	35	35
Sucrose	18°								
darkness		22	25	24	22	25	23	29	28
Raffinose	24°								
light		55	56	57	59	57	60	53	60
Raffinose	24°								
darkness		59	57	60	60	58	60	62	60
Raffinose	18°								
light		31	29	32	29	34	34	33	32
Raffinose	18°								
darkness		30	31	29	33	32	28	28	35
Glucose	24°								
light		44	43	46	50	47	45	46	48
Glucose	24°								
darkness		37	38	37	38	40	37	40	39
Glucose	18°								
light		26	27	27	30	30	32	29	29
Glucose	18°								
darkness		47	49	45	47	45	49	50	45
Fructose	24°								
light		55	51	55	56	56	56	54	58
Fructose	24°								
darkness		54	55	51	54	50	57	51	54
Fructose	18°								
light		50	51	52	55	52	50	54	53
Fructose	18°								
darkness		50	53	55	55	56	50	50	53

The level of soluble sugars in tissues

As in case of nitrogen analyses, 12 determinations of soluble sugars were carried out on a mixture of 12 tissues, and 12 — on separate tissues, in order to establish the experimental error and the individual differences between tissues in respect to the sugar level.

Table 4

The content of soluble sugars in carrot tissue cultured in various months. Each numerical value is a mean of two parallel determinations. Results are expressed in mg of sugar/100 mg of the tissue dry matter. In each case the two dates indicate the period from inoculation to the weighing of the tissue

Tissue culture conditions		1	2	3	4	5	6	7	8
		16.I—9.III	16.II—9.IV	20.III—12.V	25.IV—15.VI	29.V—20.VII	1.VII—20.VIII	5.IX—26.X	11.IX—2.I
Sucrose	24°C,								
light		36.7	38.2	38.8	34.0	36.3	33.8	35.5	33.6
Sucrose	24°C,								
darkness		35.4	35.3	34.6	39.1	36.4	38.8	37.0	36.3
Sucrose	18°C,								
light		28.8	28.1	27.4	29.2	27.5	29.7	26.3	28.5
Sucrose	18°C,								
darkness		36.3	35.3	35.2	34.9	35.2	37.9	33.3	35.7
Raffinose	24°C,								
light		32.8	36.6	37.0	36.1	34.6	34.3	37.5	35.2
Raffinose	24°C,								
darkness		36.4	34.0	35.5	35.0	38.1	40.0	37.2	36.0
Raffinose	18°C,								
light		26.3	30.5	29.1	30.2	29.0	27.4	29.4	30.1
Raffinose	18°C,								
darkness		35.0	37.9	34.4	35.0	36.1	34.6	35.7	36.4
Glucose	24°C,								
light		12.3	14.1	14.6	13.9	12.1	13.4	12.7	14.0
Glucose	24°C,								
darkness		19.5	19.3	17.2	19.6	19.1	18.8	19.0	19.6
Glucose	18°C,								
light		18.5	17.8	16.1	16.8	19.1	20.8	20.8	15.3
Glucose	18°C,								
darkness		20.0	19.2	18.0	18.0	17.9	18.4	19.4	17.3
Fructose	24°C,								
light		19.2	18.7	18.5	21.8	19.6	21.4	19.9	20.2
Fructose	24°C,								
darkness		20.6	19.0	16.3	19.6	18.3	18.8	17.9	17.1
Fructose	18°C,								
light		19.7	19.0	17.1	21.7	19.4	19.4	19.5	18.3
Fructose	18°C,								
darkness		25.6	26.1	22.7	24.1	27.0	28.9	28.4	27.3

The product $t \cdot \sigma_m$ in the case of analyses of the tissue mixture was equal to 1.4 mg/100 mg of tissue dry matter; while in the case of separate tissues — 5.9. In considering the individual variability, the significant difference between the values listed in Table 4 amounts to 8.5 mg/100 mg of the tissue dry matter. For this reason, a lack of seasonal differences in the level of soluble sugars in tissues must be assumed.

Table 5

The osmotic value of tissues cultured in different months, expressed in terms of osmo-moles (osmM)

Tissue culture conditions		1	2	3	4	5
		16.I—9.III	16.II—9.IV	25.IV—15.VI	1.VII—20.VIII	11.XI—2.I
Sucrose	24°C,					
light		0.36	0.30	0.38	0.34	0.30
Sucrose	24°C,					
darkness		0.38	0.35	0.38	0.35	0.36
Sucrose	18°C,					
light		0.33	0.30	0.40	0.33	0.32
Sucrose	18°C,					
darkness		0.37	0.35	0.33	0.32	0.32
Raffinose	24°C,					
light		0.34	0.31	0.31	0.34	0.33
Raffinose	24°C,					
darkness		0.37	0.35	0.38	0.36	0.36
Raffinose	18°C,					
light		0.36	0.30	0.31	0.35	0.37
Raffinose	18°C,					
darkness		0.38	0.31	0.35	0.35	0.34
Glucose	24°C,					
light		0.33	0.26	0.32	0.30	0.28
Glucose	24°C,					
darkness		0.32	0.31	0.30	0.31	0.31
Glucose	18°C,					
light		0.31	0.24	0.24	0.30	0.28
Glucose	18°C,					
darkness		0.33	0.28	0.23	0.31	0.29
Fructose	24°C,					
light		0.37	0.28	0.28	0.29	0.31
Fructose	24°C,					
darkness		0.38	0.29	0.30	0.31	0.34
Fructose	18°C,					
light		0.39	0.29	0.25	0.30	0.31
Fructose	18°C,					
darkness		0.35	0.28	0.30	0.33	0.35

Osmotic value of tissues

In describing the applied methods it was reported that 6 measurements of the osmotic value were carried out in 6 parts of the same tissue. The product $t \cdot \sigma_m$ for the 6 measurements equalled 0.04 (centrifugated juice). In order to compare the experimental error with the possible individual differences of tissues, 6 determinations of the osmotic value were carried out in 6 separate tissues. The product $t \cdot \sigma_m$ was equal in this case to 0.08°C thus indicating an occurrence of differences, detectable by the used method, in the osmotic value of individual tissues.

Results of cryoscopic measurements of the osmotic value were converted to osmo-moles ($1 \text{ osmM} = \frac{t^0}{1.85}$; Netter, 1959). They are given as such in Table 5. In view of the rather large individual differences, the significant difference between results represented in Table 5, which are the means of measurements of the osmotic value in three tissues, amounts to 0.23°C, or 0.12 osmM.

Considering the above, a lack of differences in the osmotic value of tissues throughout the whole year must be assumed.

DISCUSSION

Apart from differences in the growth rate of carrot tissues, no seasonal fluctuations in any of the examined parameters were found. The level of sugars, of protein and total nitrogen, respiration rate and the osmotic value in tissues revealed throughout the whole year no greater changes; solely small and irregular deviations from the level, probably due to the individual differences between parts of tissues cultured in various test-tubes, were found.

It is difficult to account for this pattern as it was rather expected that a growth rate increase will be accompanied by an increase in the respiration rate. It should however be remembered that measurements of the respiration rate were carried out on slices, the respiration of which represents the plant potentialities in this respect, but fails to provide an accurate picture of the actual gaseous exchange between tissue and environment (Laties 1959).

As regards the stability in the level of the examined fractions of nitrogenous compounds, considered against the differences in the growth rate of tissues throughout the whole year, one might perform some comparative analyses with the results of research after metabolic changes during the synchronous growth of *Chlorella pyrenoidosa* (Schmidt, King 1961).

A stepwise increase in the number of cells in cultures was not followed by a simultaneous rise in the level of total phosphorus, total

nitrogen or even total phosphorus of the nucleic acids. Only the levels of more specified chemical compounds, e.g. of phosphoric compounds soluble in cold trichloroacetic acid, or of phosphorus in phospholipids were found to be related with the growth in media. It is very probable that seasonal responses to changes in growth of carrot tissues occur in the fluctuations of the more refined fractions of metabolites.

The annual growth rhythm of the tissue exhibited a larger or smaller range of fluctuations in dependence on the kind of sugar added to the cultural media. In the case of sugars producing in general a slower rate of growth and lower rate of respiration (glucose, fructose) the fluctuations were more expressed than in the case of the other two sugars (sucrose, raffinose). The facts explained so far do not account for this phenomenon. It is only possible to learn that marked annual fluctuations are in general not related to lowered growth and respiration rates. All the varying conditions of light and temperature, which brought about larger differences in growth and respiration than the mentioned sugars, bore no effect on the rhythm in itself.

SUMMARY

1. The tissue of carrot (*Daucus carota*) was cultured for 2 years under the following, stable conditions: temperature 18°C and 24°C, light (600 lux) or darkness, a medium containing one of the sugars; 1% glucose, 1% fructose, 2% sucrose, 3% raffinose.

2. Annual fluctuations in the level of both fresh and dry matter, with a maximum in springtime and minimum in July—September (Table 1, graphs 1—4) occurred in the tissues; the range of these fluctuations was related to the kind of sugar in the medium (largest on glucose, smallest on raffinose).

3. Temperature and light bore no influence on the seasonal fluctuations in the tissue growth.

4. No seasonal fluctuations were found in tissues in the percentage of dry matter, respiration rate, levels of soluble sugars, total and protein nitrogen, and osmotic value (Tables 2—5).

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Badania nad zmiennością sezonową tkanki marchwi (Daucus carota L.) hodowanej in vitro

Streszczenie

1. Tkanka marchwi (*Daucus carota*) hodowana była przez 2 lata w stałych następujących warunkach: temperatura 18°C i 24°C, światło (600 lux) lub ciemność oraz pożywka z dodatkiem jednego z cukrów: 1% glukozy, 1% fruktozy, 2% sacharozy i 3% rafinozy.

2. Tkanka wykazywała roczne wahania zarówno świeżej, jak i suchej masy z maksimum na wiosnę i minimum w okresie: lipiec—wrzesień (tabela 1, wykresy 1—4), przy czym rozpiętość tych wahań była uzależniona od rodzaju cukru w pożywce (największa na glukozie, najmniejsza na rafinozie).

3. Temperatura i światło nie miały wpływu na występowanie sezonowych różnic wzrostu tkanki.

4. Nie stwierdzono sezonowych różnic w tkance pod względem procentowej zawartości suchej masy, intensywności oddychania, zawartości cukrów rozpuszczalnych, azotu całkowitego i białkowego oraz wartości osmotycznej (tabele 2—5).