ACTA SOCIETATIS
BOTANICORUM POLONIAE
vol. XXXVII — nr 2
1968

Chlorophyll formation in callus tissues, cultured in vitro, of explants isolated from various root parts of four carrot varieties

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INTRODUCTION

The formation of chlorophyll in plant cultures, grown in vitro, was observed for the first time by Gautheret in 1934. According to Hildebrandt et al. (1963) as well as Wilmar, Hildebrandt, Riker (1964), the callus of carrot root has particular faculty to produce chlorophyll, since this pigment occurs here even on very poor nutritive media, where calluses of other species do not produce chlorophyll (Hildebrandt et al. 1963). As was ascertained in tobacco (Laetch, Stetler 1963), chlorophyll synthesized in stem calluses, is identical with that in the leaves. The course of photosynthesis is identical, but quantitatively lesser in callus tissues. The ratio between chlorophyll a and b is similar to that occurring in initial plants. Growing of plant cultures under suboptimal conditions causes the degradation of chlorophyll a (Wilmar, Hildebrandt, Riker 1964). The quantity of synthesized chlorophyll depends upon species, light (a positive correlation) (Earle, Torrey 1965; Hildebrandt et al. 1963) and on the kind of nutritive medium used (Hildebrandt et al. 1963; Vasil, Hildebrandt 1966; Venketeswaran 1966). In the experiment of Hildebrandt et al. (1963) 2,4-D restrained the chlorophyll synthesis, while in the majority of investigated species the presence of saccharose favored it. Chlorophyll has been produced even in the callus tissue of an albino line of tobacco under the influence of IAA and especially under that of combination of IAA with kinetin. On the nutritive medium, containing 2,4-D and IAA, chlorophyll has not been synthesized and even passaging, of the previously greening tissue, on 2,4-D restrained the synthesis of this pigment (Venketeswaran 1966). The carrot root callus synthesized the greater quantity of chlorophyll without sugar or at its high concentrations (4 and 8%) in the nutritive medium (Hildebrandt et al. 1963). Instead, the callus tissue, continuously cultured in vitro, synthesized most chlorophyll at the sugar concentration of 1%, while as this concentration increased, the quantity of chlorophyll decreased (Młodzianowski 1965). The kind

of sugar used is also not without influence on the chlorophyll formation (Hildebrandt et al. 1963). According to Wilmar, Hildebrandt and Riker (1964) the callus tissue of carrot is able to take up, in chlorophyll synthesis, even hardly available sources of iron, whereas the optimum of this element, required for growth, is close to that needed for the chlorophyll formation. In the chlorophyll synthesis various species exhibit a different response to the composition of the nutritive medium (Hildebrandt et al. 1963). According to Chen and Venketeswaran (1965) no interaction exists between growth of calluses and chloroplasts content in tobacco tissue.

Tricoloured (green, orange and neutral cells) small seedlings, obtained by Steward, Israel and Mapes (1966), deriving from single cell lines, have shown that the expression of genetically conditioned, biochemical possibilities is controlled by such factors, which affected also differentiation and morphology.

AIM OF WORK

The presented research work is a part of the investigations carried on at the Chair of Plant Breeding and Seed Production of the Agricultural College in Cracow on the ability for chlorophyll formation of roots and root calluses, of various carrot varieties, cultured in vitro.

From the theoretical aspect it is interesting to know, whether the chlorophyll formation in the callus of root explant is controlled either exclusively by environmental factors or depends also on the root segment, from which the explants, cultured in vitro, have been taken. We have been trying to solve the following questions:

1. Whether calluses, deriving form explants, taken from the same root segment, are greening in similar way.

2. How is established the chlorophyll content in calluses of explants, deriving from various root segments.

3. Whether the root shape (cylindric or coniform) does affect the chlorophyll formation in calluses of explants, isolated from various root segments.

4. Whether the greening grade of explants does influence the chlorophyll content of the callus which is grown on it.

METHOD

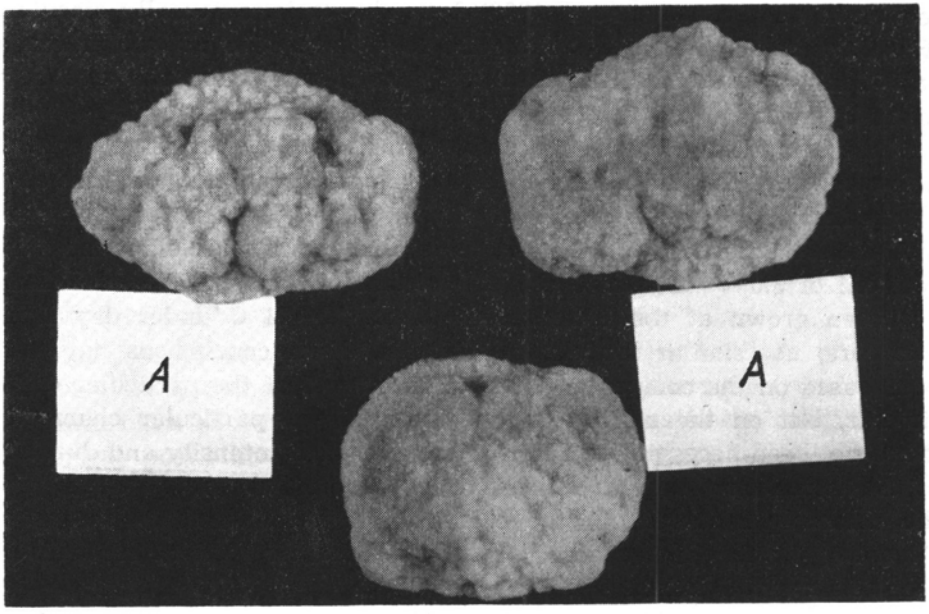
These investigations were carried out in the period of 1965—67 with four Polish carrot varieties: Nantejska, Amsterdamska, (cylindric root shape), Londyńska (coniform root shape) and Lenka (differentiated with respect to the root shape). Explants have been isolated by means of the

technique of Gautheret (1959) from the roots, externally sterilized with 0.2% solution of $HgCl_2$. Then the roots have been cut, under sterile conditions, into 2 cm. thick segments, from which explants have been taken by means of a corkborer (of 8 mm. diameter). These root segments have been marked with the successive letters of alphabet (A = segment below the top). Explants have been taken on the cambium line, in such a way that they contain cambium, pholem and xylem. Heller's nutritive medium have been used with the trace elements after Heller and with addition of 3.5% saccharose as well as that of 0.8% agar. The culture has been grown at the temperature of ca. 20—21°C under day light, therefore, not similar for all experiments. Since conclusions have not been based on the comparison of absolute values of the particular experiments, but on the relative arrangement of the particular characters it has not been necessary to determine exactly the intensity and duration of the exposition to light. After 6 weeks the culture was stopped and the chlorophyll content in the callus tissue determined by means of Comar, Tscheile method (1942, quoted after Modern Methods of Plant Analysis, Berlin 1955).

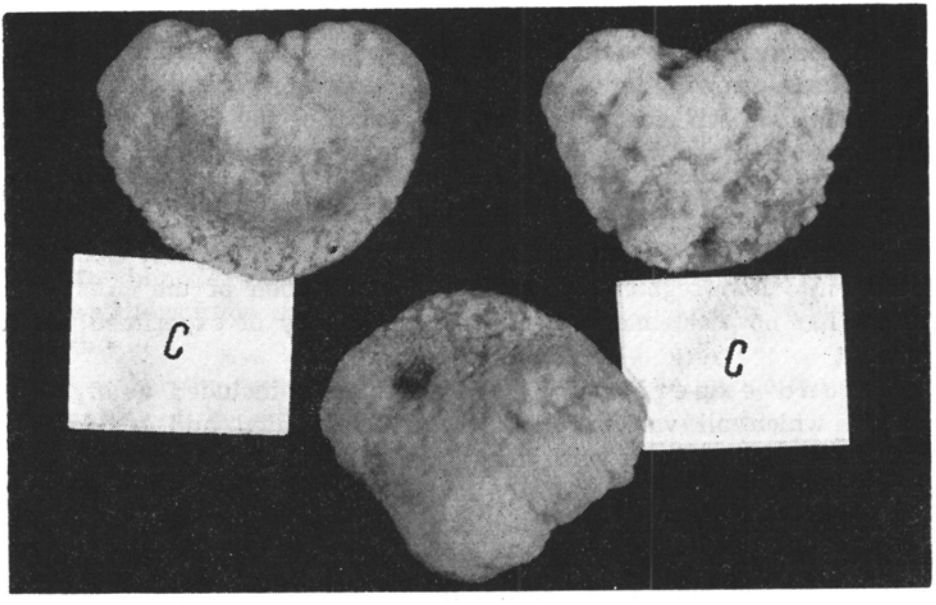
RESULTS

First experiment: From the variety Lenka 6 large coniform roots have been chosen, their average dimensions being as follows: lenght — 27 cm., diameter in the upper part of root — 5 cm., average weight — 24 dkg. These roots have been cut into 6 segments and from each of them three explants have been taken. After 6 weeks of culture the calluses, growing on the explants deriving from the same root segment, have been compared. On the strength of this comparison it has been stated that the pigmentation of calluses has been similar with the exception of two roots where, beginning from the middle segment the negligible differences of pigmentation have been observed. Similar results have been obtained with three roots of the variety Nantejska divided into four segments. Chemical determination of the chlorophyll content has not been made, owing to the scarcity of experimental material.

Second experiment: This experiment included a larger series, in which all varieties have been investigated and besides, the experimental treatments with the varieties Amsterdamska and Lenka have been replicated. Owing to the fact that the variety Lenka has roots of both shapes (cylindric and coniform), each type of them has been estimated separately. Results are summarized in the table 1. As follows from the data of table 1, calluses of the variety Amsterdamska, growing on explants taken from the particular root segments have shown very similar or nearly identical chlorophyll content. In an experiment with



Phot. 1. Carrot Lenka, callus grown from explants isolated from the same root section
root section



Phot. 2. Carrot Nantejska, callus grown from explants isolated from same root section
root section

Table 1

Chlorophyll content in calluses of 4 carrot varieties, according to the segment from which explant has been taken
(in mg. per 100 g. of dry matter)

Varieties	Experiment	Root segment	Number of calluses	Chlorophyll content a	Chlorophyll content b	Chlorophyll content a+b	Chlorophyll ratio a : b
Amsterdamska	Experiment A 12 roots	A	11	19.5	10.8	30.5	1.81
		B	12	22.9	12.7	35.6	1.79
		C	11	23.9	10.1	33.3	2.29
		D	12	18.8	10.4	29.2	1.81
		E	11	19.3	7.1	26.5	2.71
		F	7	25.0	13.7	38.7	1.83
		G	9	16.9	13.5	30.4	1.25
	Experiment B 16 roots	A	15	21.8	16.4	38.2	1.34
		B	16	23.2	15.0	38.2	1.53
		C	15	23.0	15.3	38.3	1.50
		D	12	23.1	14.8	38.0	1.56
		E	12	23.1	15.0	38.1	1.54
Lenka	Experiment A 6 coniform roots *)	A	13	30.5	15.5	46.0	1.97
		B	16	27.0	13.0	40.0	2.07
		C	16	20.8	10.6	31.9	1.96
		D	11	22.8	11.5	34.3	1.98
		E	10	19.9	10.5	30.4	1.90
	Experiment B 20 coniform roots	A	20	22.4	11.4	33.8	1.96
		B	20	23.7	12.3	36.1	1.92
		C	20	23.1	12.8	35.9	1.80
		D	20	17.8	9.8	27.6	1.82
	Experiment C 21 cylindric roots	A	20	20.7	12.7	33.4	1.62
		B	21	19.9	10.4	30.2	1.91
		C	20	16.7	9.5	26.2	1.75
		D	21	12.8	6.8	19.6	1.88
Nantejska	27 roots	A	27	26.6	15.8	42.4	1.69
		B	27	32.8	20.4	53.1	1.60
		C	27	30.1	17.1	47.1	1.76
		D	27	29.7	18.0	47.6	1.65
Londyńska	Experiment A 14 shorter roots (5 segments)	A	13	38.0	19.4	57.5	1.80
		B	13	38.5	19.6	58.0	1.96
		C	14	36.6	19.1	55.7	1.91
		D	12	28.7	15.8	44.5	1.81
	Experiment B 13 longer roots (6 segments)	A	12	37.6	20.3	58.2	1.85
		B	12	41.6	20.2	61.7	2.06
		C	13	39.7	20.1	59.8	1.97
		D	12	35.4	19.7	55.1	1.80

*) In this experiment 3 explants have been taken from each root segment

Table 2

Chlorophyll content in explants and calluses of carrot roots, which have been separated into the orange and green groups
(in mg per 100 g of dry metter)

	Experiment Root number	Kind of chlorophyll	Explants		Calluses	
			Group of roots		Group of roots	
			orange	green	orange	green
Nantejska	I	a	traces	7.3	33.8	43.5
	roots:	b	„	5.0	19.0	22.6
	orange 21	a+b	„	**12.3**	**52.8**	**66.5**
	green 22	a : b		1.48	1.78	1.92
	II	a	traces	7.8	42.6	41.4
	roots:	b	„	4.6	22.8	22.0
	orange 23	a+b	„	**12.4**	**65.4**	**63.3**
	green 20	a : b		1.70	1.86	1.88
	III	a	traces	2.9	24.0	21.9
	roots:	b	„	2.2	14.4	10.3
	orange 23	a+b	„	**5.1**	**35.5**	**32.2**
	green 20	a : b		1.32	1.66	2.11
Lenka	I	a	traces	2.4	43.9	35.9
	roots:	b	„	1.3	24.2	19.9
	orange 19	a+b	„	**3.7**	**68.1**	**55.7**
	green 21	a : b		1.74	1.81	1.80
	II	a	traces	2.0	31.7	35.6
	roots:	b	„	1.1	16.3	19.8
	orange 20	a+b	„	**3.1**	**47.4**	**55.4**
	green 11	a : b		1.82	1.95	1.79
	III	a	traces	4.9	46.1	36.9
	roots:	b	„	2.7	23.4	19.9
	orange 22	a+b	„	**7.6**	**69.4**	**56.8**
	green 24	a : b		1.80	1.97	1.85

12 roots the quantity of chlorophyll in calluses fluctuated within the limits from 26.5 to 38.1 mg%, but this variance has a random and undirected character. In an experiment with 16 roots the variance of respective data is negligible and affords only decimal fractions. Results with the variety Lenka have shown that there the chlorophyll content in calluses, growing on explants, decreased in the direction to the root apex i.e. in the experiment A — from 46.0 mg% to 30.30 mg%, in the experiment B — from 33.8 to 27.6 mg%, in the experiment C — from 33.4 to 19.6 mg%. Apart from the above mentioned data, this phenomenon is confirmed by additional observations of 8 roots divided into 5 segments for which chemical determination of the chlorophyll content have not been made. Results, concerning the roots, separated with respect to their shape (coniform or cylindric) are similarly arranged, hence the root shape within this variety does not influence the chlorophyll

formation. With the variety Nantejska a decrease of the chlorophyll content in calluses, growing on explants deriving from inferior root parts, have not been observed. Differences of the respective data fluctuated within the limits from 42.4 to 53.1 mg% and are of a random character. Instead, with the variety Londyńska such tendency is perceptible i.e. in the experiment A a distinct decrease has been observed for the segments C and D, while in the experiment B for the segment D. Negligible yields of calluses obtained on explants deriving from the last root segments (E, F) do not allow to make chemical analysis of these calluses. None the less, observations of the pigmentation of these calluses allow to state that their chlorophyll content is distinctly lower than that in the segments D.

Third experiment: Roots, taken from the three different field experiments with the varieties Lenka and Nantejska have been separated, with respect to their pigmentation visible on their longitudinal sections, into two groups i.e. orange and greening in the upper part of the root corresponding to the segment A. Explants from the segment A have been taken and the chlorophyll content simultaneously determined in their homologues. As follows from these data (table 2) explants from orange roots contained trace quantities of chlorophyll, whereas explants of greening ones contained from 3 to 12 mg% of this pigment. Chemical analyses of calluses showed that independently from the chlorophyll content in explants, calluses of both groups synthesized a similar quantity of this pigment, and the differences occurring have no connection with the chlorophyll content in explants. Since all determinations have been made separately for each field experiment, they may be considered as three replications of the same investigation.

DISCUSSION

Investigations on the ability for chlorophyll synthesis in calluses grown on explants, deriving from the various parts of carrot roots, showed that this process is connected to a great extent with the root shape. The varieties with narrow cylindric roots as Nantejska and Amsterdamska showed similar results of analyses for all segments. In the coniform roots of the varieties Lenka and Londyńska the quantity of chlorophyll in calluses decreased towards the root apex. This connection is not a merely simple relation, since deviations may be caused either by the different number of roots (various number of contaminated roots) or by the fact that calluses, grown on explants isolated from the particular roots, have been of unequal size. The lack of differences in results, concerning the roots of the variety Lenka, separated with respect to their shape, calls in question whether the genetic factor, within the same variety, has a greater importance than the root shape.

It is necessary to quote that the root shape depends also to a considerable extent upon growth conditions (Banga 1954; De Bruyn, Smeets 1959), therefore, in the variety differentiated genetically with respect to its root shape, it is difficult to eliminate the influence of environment.

To manage with an homogenous material in the investigations on calluses is of great importance. The statement that several explants taken at the same level of the root, exhibit nearly identical ability for the chlorophyll synthesis may be useful for this type of investigations. After possible verification of obtained results on the more larger assortment of varieties as well as that of their relation to other characters it may be possible to use the explants, taken from the same root level, as the parallel samples for physiological or biochemical investigations. It may be stressed also that in cutting the explants we took special care to take a similar quantity of the xylem tissue as that of the phloem one. In the case of changing this ratio different results might be obtained (Caplin 1962, 1963; Danilina, Łuss, Butienko 1965). All experimental results have been obtained on the mineral nutrient medium without growth substances. It is known, however, that IAA and 2,4-D affected the chlorophyll synthesis (Hildebrandt et al. 1963; Venketeswaran 1966) and a series of other substances of this type have not been hitherto investigated in this respect. It is also known that an interaction exists between the kind of growth substances as well as their concentration and the response of particular plant species (Mayer 1956), therefore, it may be possible that such an interaction occurs also with the varieties. With the aim to avoid the influence of supplementary factors on the chlorophyll synthesis the experimental tissues have been cultured on poor media. Therefore, as a result smaller yields of calluses have been got than those obtainable on more complicated media and not in all experiments chemical determination of the chlorophyll content have been possible owing to the scarcity of the experimental material. The data, obtained from taking of orange and greening explants, are very interesting. The greening grade of the explant does not affect the chlorophyll content in the callus. It may be supposed, therefore, that the quantity of produced chlorophyll in the callus is determined either by conditions under which it has been growing or by the genetic factor working independently from environmental conditions, which caused the greening of some roots within the variety.

CONCLUSIONS

1. Calluses, deriving from explants isolated from the same segment of the root, are greening in similar way.

2. The chlorophyll content in calluses, deriving from the different root segments, is either similar or decreases towards the direction of the

root apex. This is connected probably with the root shape of the particular variety.

3. No dependence have been ascertained between the chlorophyll content of calluses and the greening grade of the root tissues, from which explants have been isolated.

SUMMARY

The ability for chlorophyll synthesis in calluses, grown in vitro, on root explants, deriving either from the same root segment or from various ones as well as from orange and greening roots, have been investigated in four Polish carrot varieties. It has been found that calluses, deriving from the same root segment are greening similarly. The chlorophyll content in calluses of explants from various root segments is either similar or decreases towards the root apex, according to the root shape (cylindric or coniform) of the given variety. The ability for chlorophyll synthesis in calluses does not depend on the greening grade of the root part, from which the explant originates.

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(Entered: September 24, 1967.)

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Tworzenie się chlorofilu w hodowanych in vitro tkankach kalusa czterech odmian marchwi u eksplantatów izolowanych z różnych partii korzenia

Streszczenie

U czterech polskich odmian marchwi badano zdolność syntetyzowania chlorofilu w kalusach wyrosłych na eksplantatach korzeniowych w hodowli in vitro, pochodzących z tego samego odcinka lub z różnych odcinków korzenia, oraz z korzeni pomarańczowych i zazielenionych. Zaobserwowano, że kalusy pochodzące z tego samego odcinka korzenia zazieleniają się podobnie. Zawartość chlorofilu w kalusach eksplantatów różnych odcinków jest albo podobna, albo zmniejsza się ku wierzchołkowi korzenia, w zależności od kształtu korzenia danej odmiany (walcowaty czy stożkowy). Zdolność do syntezy chlorofilu w kalusach jest niezależna od stopnia zazielenienia tej części korzenia, z której eksplantat pochodzi.