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Proteins present in tannin cenocyte mother cells in *Sambucus racemosa* L.

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Abstract

It has been demonstrated that protein content and concentration are higher in mononucleate tannin-cells than in the parenchyma cells of *Sambucus racemosa*. Cytoplasmic and nuclear free acid proteins markedly prevail here. It is believed that they may be enzymatic proteins. Increase of acid proteins content within the nucleus of tannin cells causes an increase of the nucleus size. The content of nuclear bound basic proteins in tannin cells, may be lower than in the neighbouring parenchymal cells.

INTRODUCTION

Karyological investigations of the tannin elements in *S. racemosa* L. (Z o b e l, 1975) demonstrated that the mononucleate tannin cell giving rise to mixoploid tannin cenocytes differ from the surrounding cells of the parenchyma in size, shape, stronger staining cytoplasm, larger dimensions of the nucleus and a large nucleolus. Single tannin drops were found in the vacuoles of these cells. If the DNA content in mononucleate tannin cells is compared with that in the neighbouring parenchymal cells, it is seen that it is similar or lower than in the parenchyma. The observed increase in volume of the tannin cell nuclei was perhaps due to a higher protein content. The correctness of this supposition is supported by the fact of a higher affinity of the tannin cells to iron haematoxylin, probably due to the quantitative differences and different character of the proteins present in the tannin cells.

The investigations were undertaken in order to define the character of the cytoplasmic and nuclear proteins and evaluate them quantitatively in the mononucleate tannin cells and the neighbouring parenchymal cells.

MATERIAL AND METHODS

The experimental material consisted of the growth apexes of lateral *Sambucus racemose* L. shoots. The observations were made on material fixed in acetoalcohol (1 : 3 vol/vol) for 3 h. The fixative removes some histons, but also tannins, the presence of which would obscure the picture. Since the observations concerning protein content in the tannin cells and the neighbouring parenchymal ones were made for comparison on the same preparations, therefore the error resulting from the method of fixation may be considered the same in both kinds of cells. After dehydration and clearance of the fixed material in a series of alcohols and xylene, it was embedded in a paraffin block and oblong 15 μm thick microtome sections were prepared. Longitudinal sections through the shoot growth apexes were stained with 0.25 per cent Coomassie blue solution. Coomassie Brillant Blue G — 250 (Serva CI 42655) stains a special type of proteins at suitable pH. After staining the proteins with Coomassie blue, qualitative and quantitative measurements can be made by means of a cytophotometer. This staining was used for the first time for quantitative biochemical investigations by Fazekas et al. (1963) and later by Meyer and Lamberts (1965), Meizel (1966) and Chrambach et al. (1967). Bramhall et al. (1969), on the other hand, describe staining with Coomassie blue as a method of quantitative protein determination five times more sensitive than the Naphthalene Blue Black of Folin-Lowry methods. The sensitivity of this method is compared with the UV method at wavelength 2150—2200 Å applied by Waddal and Hill (1956).

Sections were prepared from 5 apexes and placed on object glasses, two from each apex next to each other. Quantitative and qualitative measurements were made separately for each object glass. The preparations were separated into 4 groups:

— hydrolyzed 15 min in 5 per cent TCA at 95°C and stained 15 min in 0.25 per cent Coomassie blue, pH 2.66 for determination of bound and free proteins;
— not hydrolyzed and stained 15 min in 0.25 per cent Coomassie blue, pH 2.66 for determining the free proteins content;
— hydrolyzed 15 min in 5 per cent TCA at 95°C and stained 15 min in 0.25 per cent Coomassie blue, pH 8.3 for determination of bound and free alkaline proteins;
— not hydrolyzed and stained 15 min in 0.25 per cent Coomassie blue pH 8.3 for determination of free alkaline proteins.

The preparations were then washed several times in fresh solutions with appropriate pH withouth dye until their colour ceased to change.

The stain with pH 2.66 was prepared by dissolving 250 mg Coomassie Brillant Blue G-250 (Serva CI 42655) in 100 ml of solution

composed of: 5 parts CH_3OH, 1 part CH_3COOH, 5 parts H_2O. pH of this solution is about 2.60.

To obtain pH 8.3, 250 mg of the stain was dissolved in 100 ml of solution comprising 5 parts CH_3OH, 1 part NAOH, 5 parts H_2O. The stain prepared in this way is pH 8.2—8.3. pH was checked each time before staining.

Protein content was determined by the cytophotometric method at $\lambda = 6100$ Å with a cytophotometer connected with a writing arm (photometer devised in the Department of Pathological Anatomy of the Pomeranian Medical Academy in Szczecin). From each of the above mentioned 4 groups of preparations 4 tannin and 4 parenchyma cells were examined. For each cell 5 parallel cytophotometric records were taken serving for mean extinction calculation. This method allowed quantitative estimation of proteins (in working units — AU) occurring separatly in the cell nucleus, the cytoplasm and the cell as a whole with an accuracy up to 5 per cent (Wied, 1966; Wied and Bahr, 1970). In view of the method of fixation and the fact that hydrolysis with 5 per cent TCA removes proteins only in small amounts, mainly acid ones and F_1 fractions of histones (Kłyszejko, 1967). The sections were investigated comparatively on the same preparations, and the tannin and neighbouring nontannin cells on the same sections.

RESULTS

Preliminary analysis of the preparations stained with Coomassie blue showed a stronger staining both of the cytoplasm and the nuclei in the mononucleate tannin cells as compared with that of the neighbouring parenchyma cells (Plate I, fig. 1).

Cytophotometric studies confirmed these observations. The results of measurements listed in Table 1 concern:

— total protein content in tannin cells and neighbouring parenchyma cells,

— content and concentration of free proteins,

— total protein content in the cytoplasm and nucleus of tannin cells and neighbouring parenchyma cells,

— content and concentration of free proteins within the cytoplasm and nucleus.

The protein content is expressed in working units (AU) (extinction × surface area computed with the planimeter). Protein concentration is expressed by extinction values. The precise content of bound nuclear and cytoplasmic proteins was not determined because part of them was removed during hydrolysis in 5 per cent TCA.

Measurement concerning the amount of nuclear and cytoplasmic proteins and total protein content in tannin cells and the parenchyma showed their increased content in mononucleate tannin cells (Table 1). To characterize the investigated proteins the values of total protein concentration in the cell (staining with Coomassie blue at pH 2.66 after previous hydrolysis Plate I, Figs 2 and 3) were compared with those of basic protein (staining at pH 8.3 after previous hydrolysis of the preparations, Plate II, Figs 6 and 7). Acid protein concentration resulted from the difference between the above named two values (Table 1). Basic protein concentration in these two types of cells is more or less the same (differencea within 10%). The significant difference concerns acid proteins the content of which is 6 times higher in tannin cells then in the parenchymal ones.

Measurement results obtained for preparations stained with Coomasie blue at pH 2.66 (Plate II, Figs 4 and 5) when compared with the cytophotometer readings for preparations stained with Coomassie blue at pH 8.3 (Plate III, Figs 8, 9, 10 and 11) both without hydrolysis give information on the concentration of free acid proteins and free basic proteins.

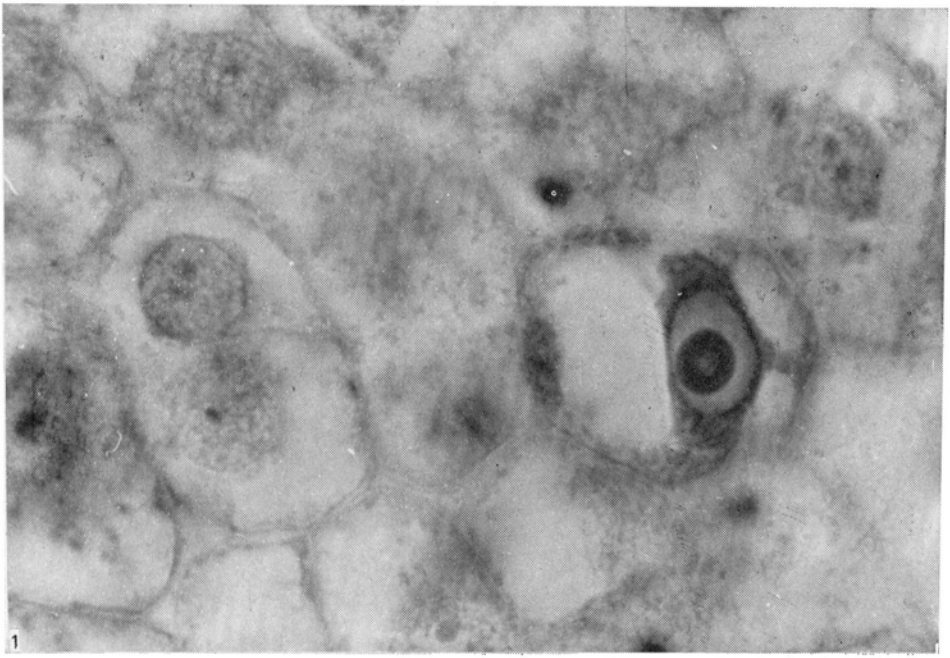
The concentration of all free proteins in the tannin cells and those of the neighbouring parenchyma does not differ significantly, the concentration of free basic proteins, however, is 3 times higher in the tannin cells, whereas a higher concentration of free acid proteins is characteristic for the neighbouring parenchyma cells.

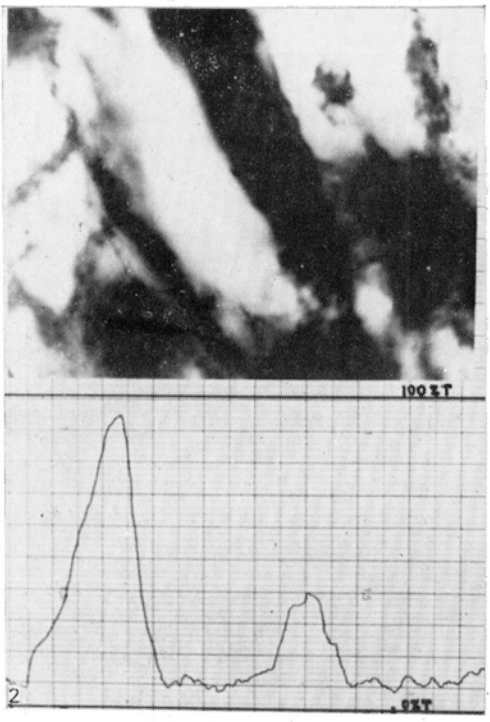
The comparison of hydrolyzed and nonhydrolyzed preparations shows that protein extraction by acid hydrolysis involved to a greater extent the parenchymal cells than the tannin ones. This was particularly true of acid proteins: in the parenchymal cells acid protein concentration diminished by 0.55 and in the tannin cells by 0.17 (Table 1). This may indicate a different character of the acid proteins in both types of cells (different isoelectric points).

Then the character of proteins was analyzed separately for cytoplasm and nucleus. Values of total protein concentration (staining at pH 2.66 in hydrolyzed preparations), free and bound basic protein concentration (staining at pH 8.3 after previous hydrolysis) and free and bound acid protein concentration (difference of the preceding two values) were compared (Table 1).

Both in the nucleus and in the cytoplasm of tannin cells total protein concentration (free and bound acid and basic) is higher as well as free with bound basic ones. The difference in acid protein content is significant.

The data concerning free acid with basic protein concentration (staining at pH 2.66) substracted of the only free basic (staining at pH 8.3) allowed to determine the concentration of free acid proteins (Table 1).





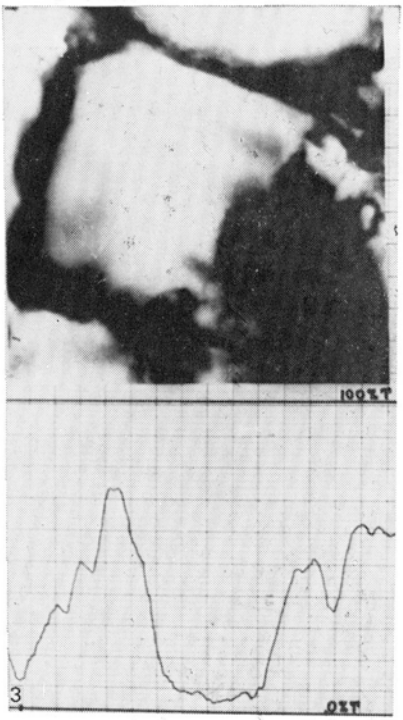


Fig. 1. Segment of longitudinal section through first internode of *Sambucus racemosa* L. Apical shoot. Mononucleate tannin cell and the neighbouring perenchymal cells are visible. Acetoalcohol, Coomassie blue, pH 8.3, × ca. 200

Fig. 2. Diagram showing protein concentration in Mononucleate tannin cell. Acetoalcohol, Coomassie blue, pH 2.66 after hydrolysis, × ca. 1500

Fig. 3. Diagram showing protein concentration in cell of parenchyma in the neighbourhood of tannin cells. Acetoalcohol, Coomassie blue, pH 2,66 after hydrolysis, × ca. 1500

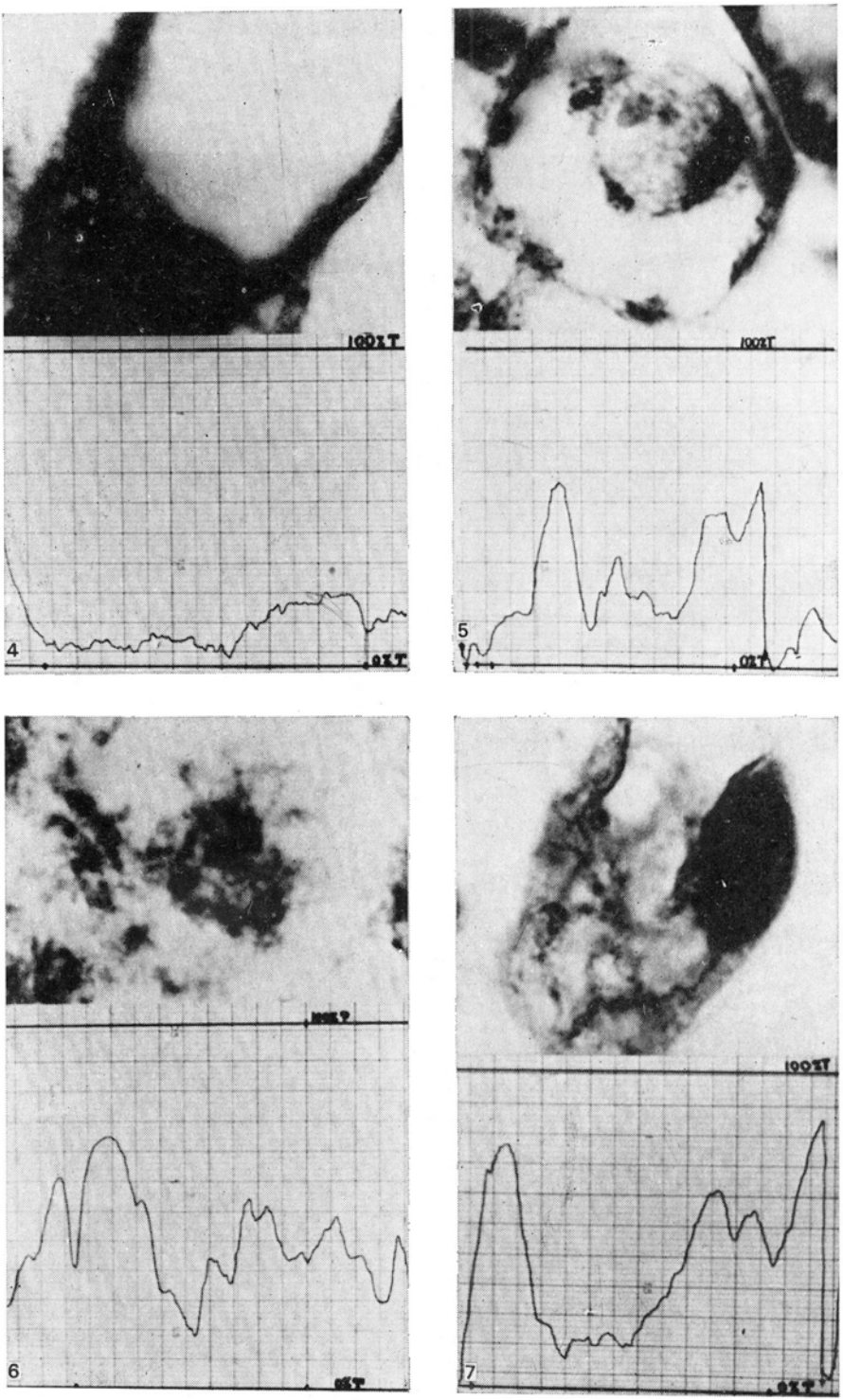
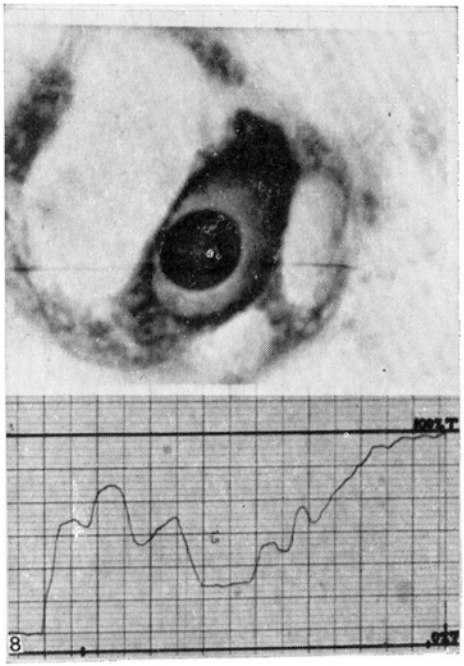


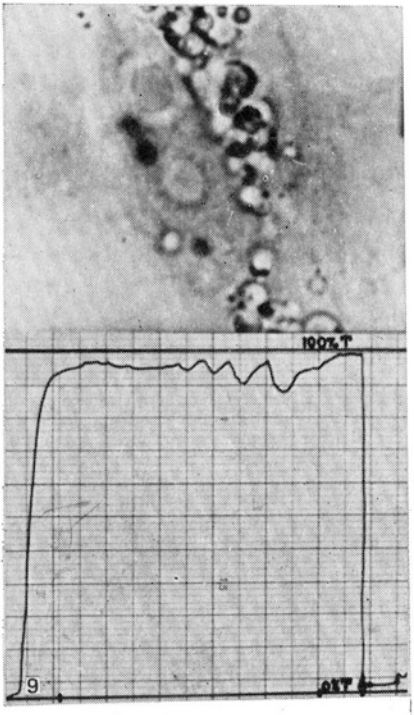
Fig. 4. Diagram showing protein concentration in mononucleate tannin cell. × ca. 1500

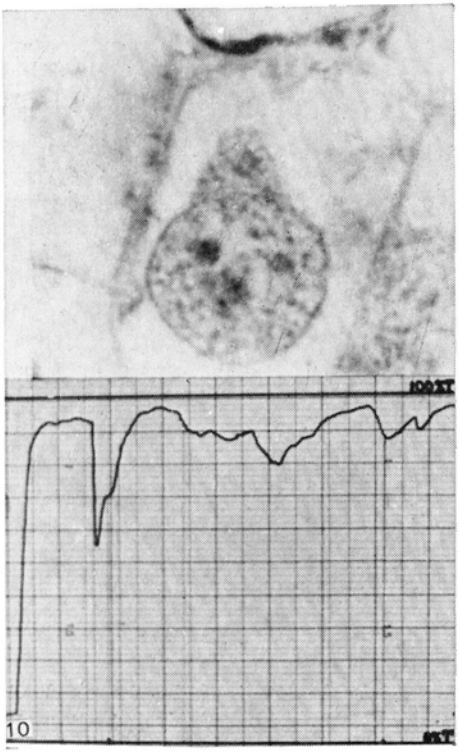
Fig. 5. Diagram showing protein concentration in parenchymal cell. × ca. 1500

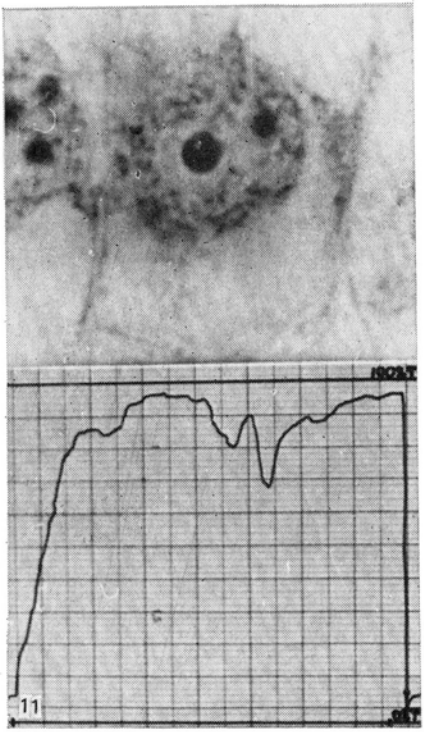
Fig. 6. Diagram showing protein concentration in parenchymal cell. × ca. 1500

Fig. 7. Diagram showing protein concentration in mononucleate tannin cell. Acetoalcohol, Coomassie blue. Fig 4 and 5 — pH 2.66 without hydrolysis, × ca. 1500; Figs. 6 and 7 — pH 8.3 after hydrolysis, × ca. 1500









Figs 8, 9. Diagrams showing protein concentration in mononucleate tannin cells × ca. 1500

Figs 10, 11. Diagrams showing protein concentration in parenchymal cells. All acetoalcohol, Coomassie blue, pH 8.3 wihout hydrolysis, × co. 1500

Table 1

Protein content and concentration in mononucleate tannin cells and neighbouring parenchymal cells

Staining conditions	Proteins	Kind of cells	Total protein content in cell (AU)	Cell sufrace area (planimetric unites)	Protein content		Sufrace		Protein concentration on planimetric unit			Photo. no.
					cytoplasm	nucleus	cytoplasm	nucleus	whole cell	cytoplasm	nucleus	
pH 2.66 after hydrolysis	free and bound acid and basic	tannin	177	312,2	122	55	164,1	53,2	0,56	0,46	1,27	2
		parenchymal	109,3	352,8	77,6	41,7	309,5	43,3	0,31	0,25	0,96	3
pH 8.3 after hydrolysis	free and bound basic	tannin	116,8	370,6	96,4	20,4	336,6	34	0,31	0,28	0,60	7
		parenchymal	103,3	377,4	72,2	31,1	316,2	61,2	0,27	0,22	0,51	6
	free and bound acid (by subtraction)	tannin							0,25	0,18	0,67	
		parenchymal							0,04	0,03	0,45	
pH 2.66 without hydrolysis	free acid and basic	tannin	269,4	496,4	163,6	105,8	401,2	95,2	0,55	0,41	1,11	4
		parenchymal	153,6	267,3	116,1	37,5	211,2	56,1	0,56	0,55	0,69	5
pH 8.3 without hydrolysis	free basic	tannin	42,0	317,6	26,3	15,7	262,2	55,7	0,13	0,10	0,29	8 and 9
		parenchymal					191,3	76,5	0,048	0,034	0,054	10 and 11
	free acid (by subtraction)	tannin	9,0	267,7	4,85	4,13			0,42	0,31	0,82	
		parenchymal							0,512	0,516	0,636	

In general in both kinds of cells free basic protein concentration is lower then that of free acid proteins, but in the parenchymal cells the concentration of free basic proteins is very low. The concentration of free basic with acid proteins and free acid ones is higher in the nucleus of tannin cells, whereas in cytoplasm it is lower. If to compare the amount of free acid proteins and that of basic free proteins in the tannin cells both in the nucleus and the cytoplasm, the content of the former is three times higher whereas in the neighbouring parenchymal cells it is more than ten times higher than of the latter.

Partial removal of protein by hydrólysis in 5 per cent TCA is specific for the two types of cells analyzed. It was noted that mainly the cytoplasmic proteins of the parenchyma, particularly the acid ones are hydrolytically partially removed. The loss in nuclear acid proteins after hydrolysis is markedly smaller. In the tannin cells acid proteins are also partly removed by hydrolysis, this, however, concerns both the nuclear and cytoplasmic proteins in the same range. Since the loss of cytoplasmic proteins is particularly high in the parenchyma cells, it would seem that the cytoplasmic proteins in tannin cells are of more neutral character.

DISCUSSION AND CONCLUSIONS

The process of cell differentiation is closely connected wtih changes in their metabolism. These changes concern particularly protein metabolism, the changes being of both quantitative and qualitative character.

This fact was the outset point for the suggestion advanced by the author of a difference in the character of the proteins in the cells initiating tannin cenocyte development, in relation to those contained in the neighbouring parenchyma.

The cytophotometric method applied here allows quantitative estimation of proteins in these different types of cells. The use of Coomassie blue which specifically stains, in dependence on the experimental conditions, various protein groups made possible identification of their character.

On the basis of analysis of preparations stained with Coomassie blue at various pH values, directly or after previous hydrolysis in 5 per cent TCA, data were obtained concerning free and bound acid and basic protein contents.

These values are burdened with a certain inaccuracy resulting from the fact that the method of fixation applied and hydrolysis in 5 per cent TCA partly remove histons and to a smaller extent other proteins (Daly and Mirsky, 1954; Hnilica and Busch, 1963; Bennett, 1967, Wren and Nutt, 1967).

It was found that total protein content, both acid and alkaline, is always higher in tannin cells as compared with that in the neighbouring parenchyma. This is particularly true of cytoplasmic proteins, in spite (or perhaps because) of the former cells having a larger vacuole.

This higher protein content, especially of acid proteins, within the cytoplasm may be due to the fact of their complex binding with tannin. Formation of tannin-protein complexes has been described among other authors by Gustavson (1949), Mejbaum-Katzenellenbogen (1959a, b), Mejbaum-Katzenellenbogen and Kudrewicz-Hubicka (1966).

Winthin the tannin cell nuclei a much higher content of free acid and alkaline proteins was noted. Their amount is on the average three times that in the nuclei of the neighbouring parenchymal cells. It proved, on the other hand, that the total pool of alkaline proteins, bound and free, within the tannin cell nuclei may be lower with marked prevalence of free alkaline proteins (5 × more than in the nuclei of the parenchymal cells). It may, thus be concluded that the amount of bound alkaline proteins in the tannin cell nuclei is significantly smaller as compared with that in parenchymal cells. These differences may result from a different life cycle of these two cell types. In karyolological investigations on tannin cenocytes (Zobel, 1975) it was demonstrated that the mononucleate tannin cells contain as much or less DNA than do the neighbouring parenchymal cells. The lowered DNA level results in a lower histon content in the tannin cells. The here presented results seem to indicate that the tannin cells produce proteins of different character, which may result in a specific composition of the karyolymph since these proteins are free.

The observed higher protein content in mononucleate tannin cells points to a more intensive protein metabolism.

The results obtained lead to the following conlusions:

1. Mononucleate tannin cells differ from the surrounding parenchymal ones by their protein content:

(a) the content and concentration of proteins is higher in the tannin cells;

(b) protein content in these cells is higher both in the nucleus and in the cytoplasm.

2. In the nuclei of tannin cells the content of free proteins composing the karyolymph is higher, the amount of bound basic proteins remaining similar.

3. The cytoplasm of tannin cells is rich in proteins both bound and free.

4. The widest quantative difference in relation to the neighbouring parenchyma cells concerns the higher amount of bound proteins in the cytoplasm of tannin cells.

5. The bound proteins in the tannin cell cytoplasm consist mainly of acid proteins, but with a much higher pH than the cytoplasmic acid proteins in the neighbouring parenchymal cells.

6. The higher content of proteins of various types in tannin cells is evidence of their different metabolism.

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Białka występujące w komórkach macierzystych cenocytów garbnikowych u Sambucus racemosa L.

Streszczenie

Stosując metodę cytofotometryczną odczytywano ilość cytoplazmatycznych i jądrowych białek w preparatach barwionych błękitem Coomassie przy różnych wartościach pH. Przedmiotem obserwacji były jednojądrowe komórki garbnikowe, rozwijające się w młodych pędach *Sambusus racemosa* L. oraz sąsiadujący z nimi miękisz.

Preparaty podzielono na cztery grupy:

— hydrolizowane 15 min. 5% TCA w temperaturze 95° C i barwione błękitem Coomassie przy pH. 2,66
— nie hydrolizowane i barwione przy pH 2,66
— hydrolizowane 15 min. 5% TCA w temperaturze 95° C i barwione błękitem Coomassie przy pH 8,3
— nie hydrolizowane i barwione przy pH 8,3

Pomiarów ilości białek dokonano stosując cytofotometr przy długości fali 6100 Å.

Na podstawie otrzymanych wyników można wnioskować, że komórki macierzyste cenocytów garbnikowych charakteryzują się wysoką zawartością białek kwaśnych i zasadowych zarówno związanych, jak i nie związanych. W jądrze komórki garbnikowej zawartość białek kwaśnych i zasadowych nie związanych jest większa, natomiast zawartość białek zasadowych związanych jest taka sama jak w komórce sąsiadującego miękiszu. Na terenie cytoplazmy komórki garbnikowej białek zasadowych i kwaśnych zarówno związanych, jak i nie związanych jest więcej niż w otaczającym ją miękiszu.

Przypuszcza się, że zaobserwowane różnice w zawartości białek cytoplazmatycznych i jądrowych w komórce inicjującej rozwój cenocytu garbnikowego wynikają z jej specyficznego metabolizmu związanego z procesem różnicowania. Zwiększona pula cytoplazmatycznych białek kwaśnych może być źródłem dla tworzących się kompleksów taninowo-białkowych, których obecność jest charakterystyczna dla komórek i cenocytów garbnikowych.