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Nucleic Acids and Nitrogen Contents of Cells in the Dividing and Elongating Zones of *Vicia faba*

Masahiro R. ISHIDA, Namio SHINKE and Sango BABA

Botanical Institute, College of Science, University of Kyoto

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It seems to be there no longer any reasonable objection to the view that RNA is concerned in protein synthesis in the cells. It is not improbable to assume, therefore, that the amount of nucleic acids and nitrogen in the cells of permanent tissues may be less than that in the cells of meristematic tissues, because the cells in the meristematic tissues contain dense protoplasm and divide repeatedly while those in the well differentiated cells carry large vacuoles and do not divide. The results of the biochemical study carried out by several investigators, however, seem to be not always harmonious with the assumption stated above (*cf.* HOLMES, MEE, HORNSEY and GRAY, 1955; JENSEN, 1958; and others).

It is intended in the present study to compare the amounts of nucleic acids and nitrogen in the dividing zone to those in the elongating zone of the young roots of *Vicia faba* in order to see whether the assumption mentioned above is correct or not.

Material and Method

Seeds of *Vicia faba* were soaked in tap water for 18 hours at 30°C and then they were germinated and grown for 4 days at the same temperature. Seedlings from 7 to 10 cm in length were selected. Young roots of these seedlings were cut transversally and following two segments were used in this study.

Segment A (0–5 mm from the root apices). This part of roots is called the 'dividing zone' in this paper, for the sake of convenience because many mitotic figures are found in the tissues of this segment. Most of the cells in this segment appear meristematic, that is, they contain dense protoplasm and large nuclei.

Segment B (15–25 mm from root apices). The cells in these segments are large and markedly elongated having large vacuoles. As the mitotic figures

are hardly found in this part of the roots, this part is called "elongating zone" in this paper.

About five hundred root tips were used in each biochemical estimation of nucleic acids and nitrogen.

a) *Separation and estimation of nucleic acids.* DNA, RNA, phospholipids, phosphoproteins and acid soluble P-containing substances were isolated from root tip cells following the SCHMIDT and THANNHAUSER method with a slight modification (SCHMIDT and THANNHAUSER, 1945; SUGIYAMA, SHINKE and ISHIDA, 1954).

Phosphorus contained in each fraction stated above was estimated by phospho-vanado-molybdate method (SUGIYAMA, SHINKE and ISHIDA, 1954). Essential point of this method is as follows: Organic substances in each fraction were decomposed with perchloric acid (PCA) over a micro-burner. To 5 ml of decomposed sample soln, 0.6 ml of 50% HNO_3 and 1.0 ml of 10% ammonium molybdate soln were then added followed by an addition of 3.4 ml of ammonium vanadate soln[1]. Orange color developed after 10 min. Then the transmittance of the reaction color was measured by a BECKMAN's spectrophotometer at the wavelength of 405 mμ.

b) *Estimation of total nitrogen.* The amount of total nitrogen contained in the cells of the dividing and the elongating zone was determined by micro-Kjeldahl method modified by PARNAS (1938) as follows: Materials were homogenized in ice-cold distilled water and then 1 ml of the homogenate was placed in a Kjeldahl flask, followed by an addition of 2 ml of concentrated sulfuric acid. The mixture was boiled until it became colorless. One ml of H_2O_2 (30%) was added to the cooled mixture and was boiled again for 10 minutes and then cooled. The oxidation by H_2O_2 was repeated once more. Then 20 ml of 30% NaOH were added. A receiving flask containing 1 ml of a mixture of methyl red and methylene blue[2] and 25 ml of N/25 sulfuric acid was placed under the dilivery tube and then distilled for 5 minutes. The sulfuric acid soln was titrated by N/25 NaOH.

Result

The results obtained in the present study are summarized in the following table, showing the amounts of phosphorus contained in DNA, RNA, phospholipids, phospho-proteins and acid soluble substances as well as the amount of total nitrogen in the cells of the dividing and the elongating zone of the roots.

In this table, it is seen that amount of DNA-P and RNA-P contained in one gm of the fresh and dry tissues of the dividing zone is markedly larger

1) Dissolve 1.175 gm of ammonium metavanadate in 10 ml conc. HNO_3 and add 460 ml distilled water.

2) Fifteen ml of 0.1% methylene blue aqueous soln were added to 100 ml of 0.03% methyl red aqueous soln.

Table 1. Amount of phosphorus and total nitrogen in the roots of *Vicia faba*.*

Amount	mg/gm of F. W.		mg/gm of D. W.		10^{-9} mg/cell	
Tissues / Fractions	Divid. zone	Elongat. zone	Divid. zone	Elongat. zone	Divid. zone	Elongat. zone
P in acid soluble fraction	0.635	0.301	6.546	5.794	18.5	93.5
P in lipid fraction	0.235	0.031	2.417	0.590	6.8	10.3
P in DNA fraction	0.183	0.008	1.883	0.061	5.5	2.8
P in RNA fraction	0.544	0.015	5.610	0.419	12.7	6.8
P in phospho-protein fraction	Negligible	Negligible	Negligible	Negligible	Negligible	Negligible
P in residual fraction	Negligible	Negligible	Negligible	Negligible	Negligible	Negligible
Total N	8.496	2.304	87.591	44.314	235.0	707.0

* The values shown in this table are the average of three estimations.

than that found in the elongating zone, but amounts of both DNA-P and RNA-P contained in a single cell of dividing zone are only twice as large as those found in a single cell of the elongating zone. P content between per one gram and per single cell is due to a fact that the number of cells contained in one gram of the dividing zone tissue is far larger than that contained in those of the elongating zone tissue. Among amounts of P per 1 gm of fresh tissue, per 1 gm of dry tissue and per one cell shown in the table, the last one is most important from a physiological view point, because a single cell may be regarded as a physiological unit of a tissue or an organ.

The fact stated above that the amounts of DNA-P and RNA-P contained in a single cell of the dividing zone are larger than those of the elongating zone, is quite in accord with the result of the cytophotometric study carried out by Mr. E. YOKOMURA[3] in our laboratory that the relative amount of DNA per cell of the dividing zone is larger than that of the elongating zone. Moreover the result obtained by him shows that the relative amount of DNA per cell of the dividing zone differs markedly in different nuclei while that in the elongating zone differs only a little.

It is concluded, therefore, that the amount of DNA-P per cell in the dividing zone (5.5×10^{-9} mg/cell), obtained in the present biochemical study, is nothing but an average of different DNA-P amount of many cells. Contrary to the above case, amount of DNA-P per cell in the elongating zone (2.8×10^{-9} mg/cell) shows a stable amount contained in a single cell of the permanent tissue of this plant.

3) To be published later.

The ratios RNA-P/DNA-P in a single cell of the dividing and the elongating zones are 2.31 and 2.43 respectively. These values are nearly equal to the values which have frequently been reported in the cells of some animal organs which show active protein or nucleoprotein synthesis.

Contrary to the DNA-P and RNA-P content in a single cell, amounts of P in phospholipids, phosphoproteins and acid soluble substances per single cell are larger in the elongating zone than those in the dividing zone.

It must be noted that the total nitrogen content in a single cell is larger in the elongating zone than that of the dividing zone. This fact is probably due to the largeness in volume of the cells of the elongating zone. A discussion on the relation among the protein synthesis, RNA and DNA contents of the cells in the dividing and elongating zones will be made in a subsequent paper.

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