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CYTOPHOTOMETRIC ANALYSIS OF ASCORBIC ACID (AA), RIBONUCLEIC ACIDS AND SULFHYDRAL PROTEINS DURING EMBRYO GENESIS IN *COIX LACRYMA JOBI* L.

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ABSTRACT

Histochemical studies reveal that all the organogenetic centres of the proembryo are uniformly stained. At first indentation, a difference in localisation pattern arises. The globular embryo is physiologically the most active stage in development. Positive correlation is shown to exist by the regressions.

FRESH fertilised carpels of *Coix* were fied in the fluids of Maheshwari[1], Carnoy[2], and neutral formalin. The sections were stained with pyronin[2] G for RNA and DDD test of diazo red[3] for –SH. Alcoholic silver nitrate at 0–3° C[4] and pH 2–2·5 was used as a fixative for the localisation[4] of ascorbic acid due to its quick penetration and the specificity[5] of the reaction. Control slides were prepared for all the metabolites. The absorbance of the exposed chromophore was measured by a simple cytophotometer[6] devised in our laboratory. The assembly follows Beer's law of linear relationship between the absorbance and section thickness. The green filter (500–570 μm) served for red chromophore of RNA and –SH while the measurement of black silver grains was performed under white light. E. values were multiplied by cell area to obtain the total content of the metabolite per cell. E. values are divided by cell area to obtain the concentration per unit area of the cell. Correlations between AA and RNA, AA and –SH and RNA and –SH were established by the regression method. E. values were used to calculate the regression equations and to trace their trend lines.

The zygote is a strongly polarised cell. Its proximal pole shows vacuolated cytoplasm containing less amount of AA, RNA and –SH proteins while the distal pole shows a strong staining reaction. As the development proceeds, the stain intensity (e. values) for AA, RNA and –SH in the derivatives of *ca* rises and reaches its peak when the embryo is globular. Another small peak is visible when the second indentation takes place. At maturity the stain intensity considerably declines. The amount of these metabolites (Table I) (expressed in terms of content per unit area) shows similar changes observed for the stain intensity except one fall at the 16-celled pro-embryo. This

TABLE I

The quantitative data of ascorbic acid, RNA and –SH proteins during embryogenesis

Stage of embryogenesis	Extinction value (e. value)			Cell area (in μ^2)	Content per unit area of the tissue		
	Ascorbic acid	RNA	–SH Protein		Ascorbic acid	RNA	–SH Protein
Zygote ..	0·13	0·06	0·04	5396·6	701·6	323·8	215·9
Pro-embryo—							
(*a*) 2-celled ..	0·21	0·13	0·08	2,860·0	600·6	114·4*	288·8
(*b*) 4-celled ..	0·22	0·22	0·17*	2,376·3	522·8	522·8	403·98*
(*c*) 16-celled ..	0·33	0·23	0·19	1,577·9	520·4	351·9	300·7
Globular embryo ..	0·63	0·47	0·28	1,731·5	1,107·2	815·5	326·7
Indented embryo—							
(*a*) First indentation ..	0·44	0·24	0·1	1,251·1	564·5	310·3	129·5
(*b*) Second indentation ..	0·37	0·3	0·27	1,464·3	669·2	468·0	467·7
Mature embryo ..	0·32	0 05	0·13	1,633·5	492·0	90·7	188·0

* Values derived from the regression equation.

TABLE II

The regression values of ascorbic acid, RNA and –SH proteins in different combinations

Stage	Regression values of ascorbic acid (X) *versus* RNA (Y) X = 0·71 Y + 0·1838 Y = 0·8 X – 0·0042				Regression value of ascorbic acid (X) *versus* –SH protein (Y) X = 1·4 Y + 0·12 Y = 0·51 X – 0·05976				Regression value of RNA (X) *versus* –SH protein (Y) X = 0·02 + 1·25 Y Y = 0·07 + 0·47 X			
	X	Xl	Y	Yl	X	Xl	Y	Yl	X	Xl	Y	Yl
Zygote	0·13	0·22	0·06	0·06	0·13	0·176	0·04	0·06	0·06	0·07	0·04	0·0982
Pro-embryo—												
(*a*) 2-celled	0·21	..	..	0·126	0·21	0·332	0·08	0·04	0·13	0·04	0·08	..
(*b*) 4-celled	0·22	0·34	0·22	0·13	0·22	..	0·17	0·052	0·22	..	0·17	0·1734
(*c*) 16-celled	0 33	0·35	0·23	0·22	0·33	0·38	0·19	0·108	0·23	0·2575	0·19	0 1838
Globular embryo	0·63	0·51	0·47	0·46	0 63	0·51	0·28	0·26	0·46	0·37	0·28	0·2905
Indented embryo—												
(*a*) First indentation	0 44	0·36	0·24	0·31	0·44	0·26	0·1	0·164	0·24	0·145	0·1	0·1838
(*b*) Second indentation	0·37	0·39	0·3	0·25	0·37	0·498	0·27	0·128	0·3	0·3575	0·27	0·211
Mature embryo	0·32	0·22	0·05	0·21	0·32	0·302	0·13	0·103	0·05	0·1825	0·13	0·0932

is related to the small size of the embryonal cells at this stage. The globular embryo has the highest rate of synthesis of AA, RNA and –SH proteins (Fig. 1). Its physiological state is most active. The tiered arrangement of four, eight and sixteen cells does not show any difference in the staining reaction. The organogenetic centres of the pro-embryo are all physiologically equally active. Schultz and Jensen[7] could not detect any apparent differences until the formation of a heart-shaped embryo in *Capsella*. The first difference in the localisation pattern in *Coix* is observed during the first notch in the elongated embryo. At this stage the germinal face which is the future plumule-radical axis reveals a higher staining reaction than the abgerminal face which forms the scutellum. At maturity the staining intensity decreases indicating the slowing down of the synthetic processes.

In the regression of these metabolites (Table II), the points representing the e. values of AA, RNA

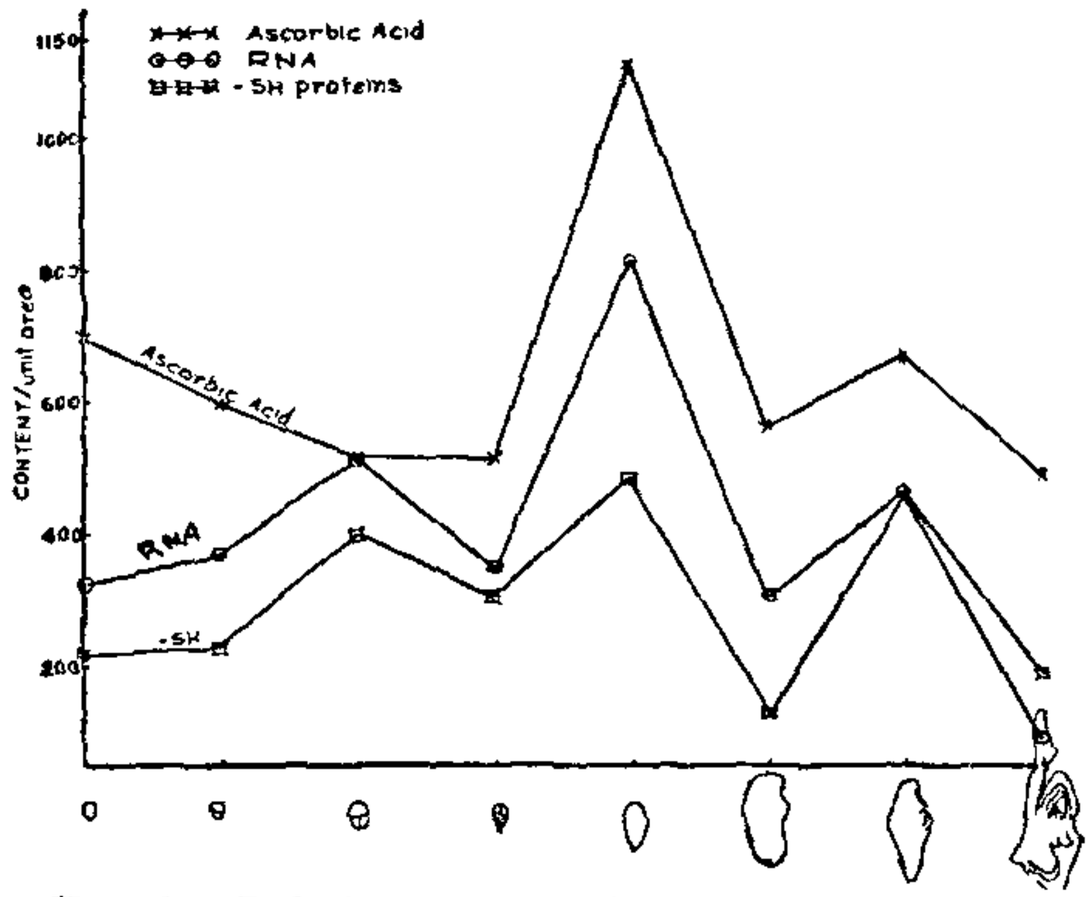

FIG. 1. Relative changes in the content of AA, RNA and sulfhydral proteins per unit area of the tissue during embryogenesis.

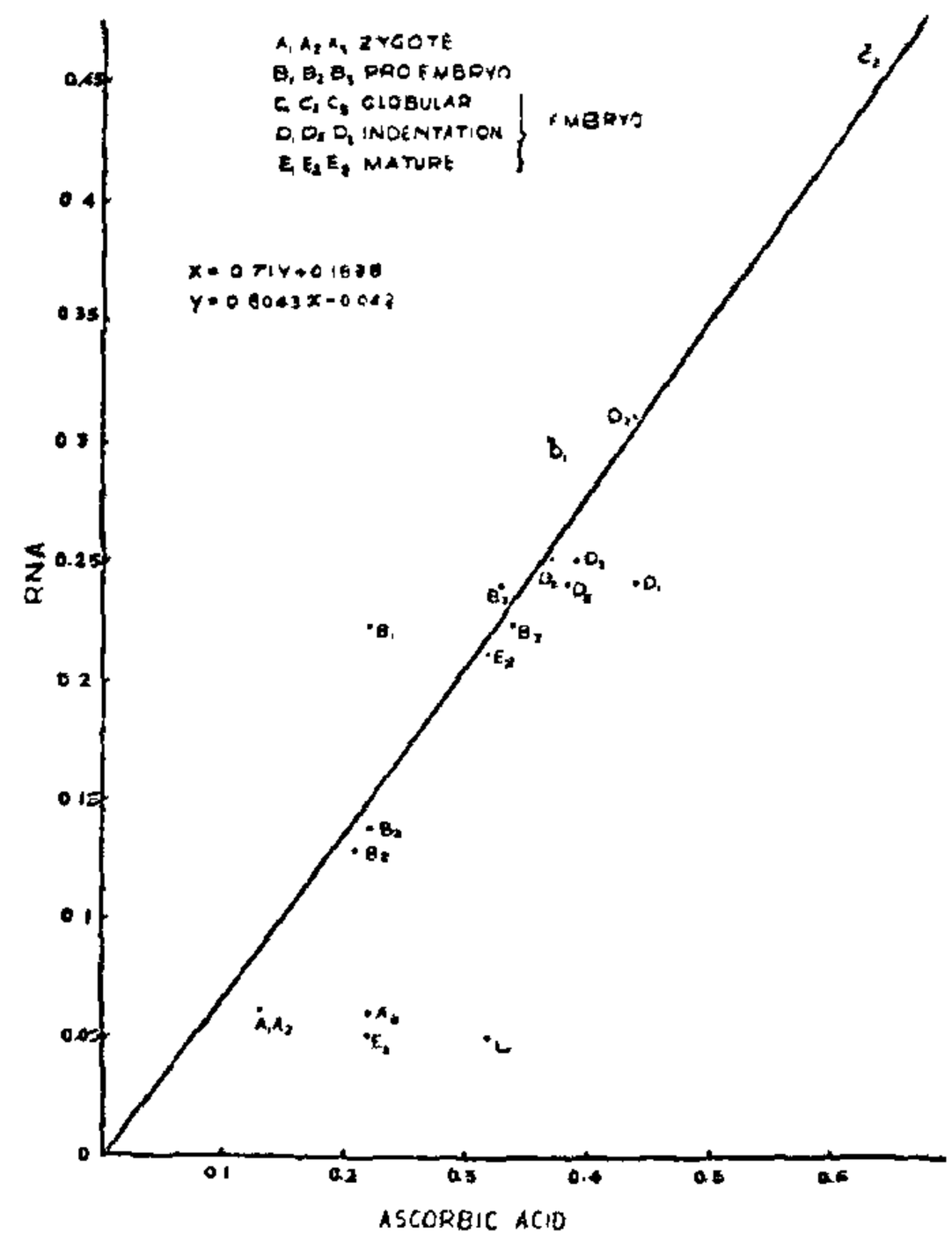

FIG. 2. The regression trend line derived for AA (*x*-axis) and RNA (*y*-axis). The slope of the line shows a positive correlation.

and –SH proteins at the zygote ($A_1 - A_3$), globular ($C_1 - C_3$) and indented stage ($D_1 - D_3$) lie (Fig. 2) close to the central trend line while those at the pro-embryo ($B_1 - B_3$) and mature ($E_1 - E_3$) embryos are far from it. Thus a positive correlation amongst the above three metabolites is very sharp at the zygote, globular and indented stages (Fig. I) while in the rest of the stages, it is comparatively weak.

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1. Shah, C. K., *Le Nature. Canadien*, 1962, 89, 330.
2. Alfert, M. and Geshwind, I. I., *Proc. nat. Acad. Sci. (U.S.)*, 1953, 39, 991.
3. Barnett, R. J. and Seligman, A. M., *Science*, 1952, 116, 323.
4. Dave, I. C., Saxena, O. P., Abraham, P. G. and Pandya, R. B., *Vidya J. Gujarat Univ.*, 1969, 12, 199.
5. Chinoy, N. J., *Histochemie*, 1969, 20, 105.
6. Chinoy, J. J., Shah, C. K., Madhavan Unni, N. V. and Neelakandan, N., *Interntl. Seminar on Physiology of Differentiation in Plants, Simla*, 1971, p. 23.
7. Sister, R. Schultz and Jensen, W. A., *J. Ultrastru. Res.*, 1968, 22, 376.