Cytophotometric quantification of metabolites in anther of *Cassia auriculata* Linn.

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The paper deals with cytophotometric quantification of DNA, RNA and proteins during various developmental stages of anther in *Cassia auriculata* Linn. The changes in chemical constitution of anther tissues during it's development are verified by calculating relative contents of metabolites per cell. Tetrads show maximum concentration of metabolites per unit area. An attempt is also made to give contributions of metabolites during development of anther.

Key-words - *Cassia*, anther, cytophotometric quantification, metabolites.

INTRODUCTION

PHYSIOLOGICAL and biochemical changes during anther development have been studied by numerous workers (Moss & Heslop - Harrison 1967; Heslop-Harrison 1972; Sauter 1971; Shah & Maruf 1976; Jain 1989). Cell and tissue differentiation in anther depends on the synthesis and concentration of various biochemical substances. Many studies have also indicated that major chemical substances viz. nucleic acids, proteins, enzymes, polysaccharides play a vital role in differentiation of reproductive structures (Ptitchard 1964; Raghavan 1976; Malik *et al.* 1976).

Distribution of DNA indicates high mitotic activity. RNA and proteins play conspicuous role in growth and differentiation of anther tissues. Hence, quantification of DNA, RNA and proteins have been studied in developing anther tissues of *Cassia auriculata* L. An attempt is made to find out relative amount of metabolites synthesised. Their contributions during anther development are also discussed.

MATERIAL AND METHOD

Anthers of *Cassia auriculata* L. at successive developmental stages were collected and fixed in Acetic-alcohol. They were dehydrated, infilterated and embedded in paraffin wax. Serial microtome sections at 10 µm thickness were cut and mounted with gelatine adhesive (Jensen 1962). For histochemical localisation, standard staining procedures were followed.

Staining intensities were measured with the help of cytophotometer. From the extinction values, content of metabolite per cell and concentration per unit area were calculated (Pollister *et al.* 1969). All the values were expressed in arbitrary units (Au).

Metabolite	Method	Colour produced	Wavelength in nm & filter
DNA	Feulgen method (Gahan 1984)	Violet	500-570 Green
RNA	Azur B (Flax & Himes 1952)	Blue	500-570 Green
Proteins	Mercuric Bromophenol Blue (Mazia *et al.* 1953)	Blue	610 Orange

RESULTS

DNA - DNA content in wall layers do not show much variation throughout development of anther. DNA content of sporogenous tissue is 16.14 Au. Reduction in DNA content is observed in pollen mother cells.

Tetrads show maximum DNA concentration (Text-fig.2). Due to increase in cell area and extinction value, pollen grains show maximum 74.21 Au DNA content (Table 1).

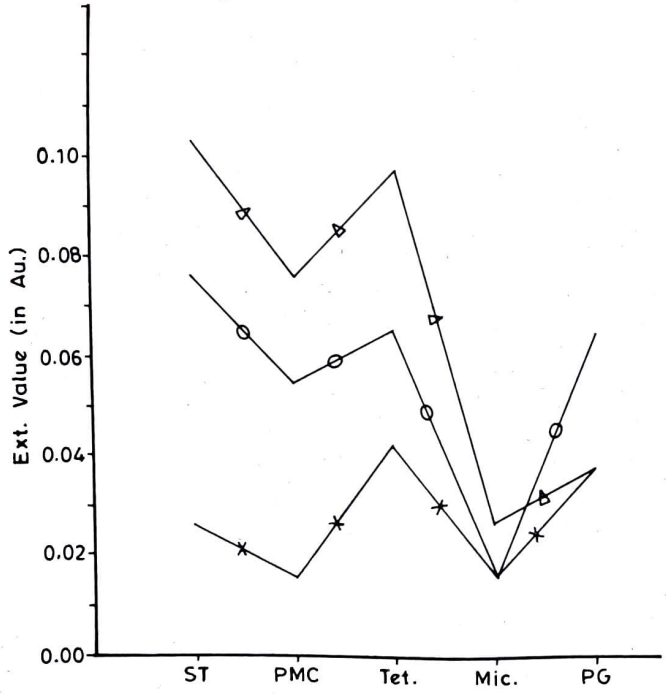
Table 1. DNA quantification in ST and it's derivatives

Name of the species	log Is	E= log Io-log Is	Cell Area (CA) in μm^2	Content/cell = E X CA	Concentration/unit area = E / CA (in the order of 10^{-5})
Cassia auriculata L.					
(log I_0 = 1. 6990)					
Stage ST	1.6721	0.0269	600	16.14	4.48
PMC	1.6812	0.0178	800	14.24	2.22
Tetrad	1.6580	0.0410	452	18.53	9.07
Microspores	1.6812	0.0178	1017	18.10	1.75
Pollen grain	1.6628	0.0362	2050	74.21	1.76

RNA - In contrast to DNA, extinction value for RNA declines from sporogenous tissue to pollen grain stage in wall layers. However, at maturity, due to increase in cell area RNA content remains almost same throughout the anther development. RNA concentration per unit area shows decrease at maturity.

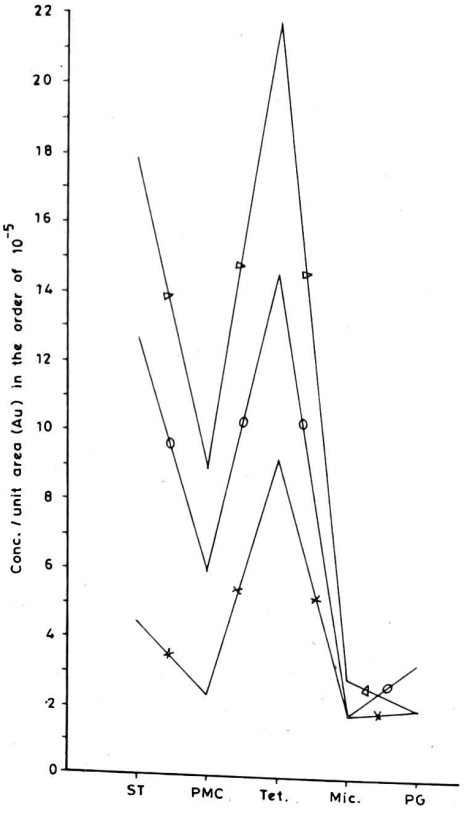
Extinction value of sporogenous tissue is 0.0758. As pollen mother cell differentiates, extinction value declines (Text-fig. 1). Tetrads show maximum RNA concentration per unit area (Table 2). Separated microspores exhibit low RNA content. In mature pollen grains, with increase in extinction value and cell area, RNA content per cell increases considerably.

Proteins - Quantification of proteins in anther tissues was observed to be parallel to that of RNA. Extinction value for proteins declines from sporogenous tissue to pollen grain stage in wall layers. Protein concentration per unit area is very less at anther maturity.



Text-fig. 1. Relative changes in extinction values of metabolites during anther development in *C. auriculata* L. X - DNA, O - RNA, - Proteins, ST - Sporogenous tissue, PMC - Pollen mother cell, Tet - Tetrad, Mic - Microspores, PG - Pollen grain, Au - Abitrary units, Ext - Extinction value.)

Extinction value declines from sporogenous tissue to pollen mother cell stage (Text-fig. 1). Tetrads show maximum protein concentration per unit area (Text-fig. 2). Separated microspores show low protein content. Mature pollen grains exhibit 74.21 Au protein content (Table 3).



Text-fig 2. Relative changes in concentration of metabolites per unit area during anther development in *C. auriculata* L. (X - DNA, O - RNA, - Proteins, ST - Sporogenous tissue, PMC - Pollen mother cell, Tet - Tetrad, Mic - Microspores, PG - Pollen grain, Au - Arbitrary units, Conc - Concentration per unit area.)

DISCUSSION

The extinction value and concentration per unit area of all metabolites are analogous while content per cell is analogous to cell area. Similar results were observed in rice (Jain 1981).

Table 2: RNA quantification in ST and it's derivatives

Name of the species	log Is log	E= log I-Io -log Is	Cell Area (CA) in μm^2	Content/cell = E X CA	Concentration/unit area = E / CA (in the order of 10^{-5})
Cassia auriculata L.					
(log I_0= 1.6990)					
Stage - ST	1.6232	0.0758	600	45.58	12.63
PMC	1.6435	0.0555	800	44.40	6.00
Tetrad	1.6335	0.0655	452	29.60	14.49
Microspores	1.6812	0.0178	1017	18.10	1.75
Pollen grain	1.6335	0.0655	2050	134.27	3.19

Table 3: Protein quantification in ST and it's derivatives

Name of the species	log Is	E= log Io-log Is	Cell Area (CA) in μm^2	Content/cell = E X CA	Concentration /unit area = E / CA (in the order of 10^{-5})
Cassia auriculata L.					
(log I_0= 1.6990)					
Stage - ST	1.5911	0.1079	600	64.74	17.90
PMC	1.6232	0.0758	800	60.64	9.00
Tetrad	1.6021	0.0969	452	43.80	21.40
Microspores	1.6721	0.0269	1017	27.35	2.64
Pollen grain	1.6628	0.0362	2050	74.21	1.76

In contrast to RNA and proteins, DNA content in wall layers do not show much variation throughout anther development. This may be due to diploid nature of nuclei in them.

Rich RNA and protein content is generally associated with growing and differentiating cells (Panchaksharappa *et al.* 1985). This is evident in *Cassia auriculata*, where rich content of these metabolites were observed in wall layers during early development of anther and sporogenous tissue.

Rich DNA, RNA and protein content in sporogenous tissue provides substantial biochemical base essential for division. Reduction in DNA content of late sporogenous tissue and pollen mother cell is obscure. According to Pritchard (1964), reduction in DNA content is due to increase in volume. Present results in *C. auriculata* also support above view.

Extinction values of RNA and total proteins decline from sporogenous tissue to pollen mother cell stage. This decline may be due to dilution effect resulted from growth, since cell area increases from 600 μm^2 (at sporogenous tissue stage) to 800 μm^2 (at pollen mother cell stage). This decrease is due to reduction in ribosome population of meiocytes (Heslop-Harrison 1972).

Tetrads show maximum concentration of all the metabolites per unit area. However, metabolic content per cell in tetrads is less. As per our observations this is due to decrease in cell area and low metabolic synthesis due to deposition of PAS + ve substance.

Although there is increase in cell area, separated microspores show low metabolic contents. It is possible that they may be constantly utilising these substances for their differentiation. It indicates low metabolic activity of microspores.

Mature pollen grains contain rich metabolites indicating high metabolic activity. These are utilised for development of male gametophyte.

Extinction values of RNA and proteins are analogous. Both the metabolites show two peaks, at sporogenous tissue and tetrad stage. This indicates that there is more protein synthesis in presence of more RNA.

In all the anther tissues, quantification of DNA was observed to be less than quantification of RNA and proteins. As anther tissues differentiates, RNA and proteins are required in more amount.

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