CYTOCHEMICAL ANALYSIS OF POLLEN AND POLLEN TUBES OF *CROTALARIA JUNCEA* (SUNN HEMP.)

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Abstract

The cytochemical analysis of pollen and pollen tube of *Crotalaria juncea* showed a rich deposit of all reserved matabolites in the pollen at the time of shedding. The pollen tubes after germination were rich in cyto- plasmic polysaccharides, proteins, RNA, lipids and cellulose. Longer tubes revealed irregular distribution of cytochemicals and rich matabolites, whereas in some ascorbic acid and pectic substances were confined only to the tip region before bursting. Pollen and pollen tube wall also showed positive staining for wall materials such as DNA, cellulose and pectic substances.

Introduction

Extensive studies have been made by Heslop-Harrison (1970), Southworth (1973), Malik and Mehan (1975), Panchaksharappa and Rudramaniyappa (1972, 1976) and other workers on the cytochemical aspects of pollen and pollen tube growth in different plants. They also have studied the physiology of pollen and pollen tubes. Present study has been undertaken to know the cytochemical localization of polysaccharides, proteins, RNA, ascorbic acid, cellulose, starch and pectins in germinated and ungerminated pollen of *Crotalaria juncea* L.

Material and Method

The mature buds were collected for germination purpose in the early morning hours, 6.00 a.m. to 8.00 a.m. Fresh pollen grains prior to anthesis were then sown in the srandardized culture solutions of 5%, 10%, 15%. .....to see the effect on the pollen germination and length of the pollen tubes by adopting hanging drop techniques. Pollen and pollen tubes of various lengths in relation to time were then processed for cytochemical analysis.

Pollen grains from freshly dehisced anthers of *C. juncea* were best germinated in 20 per cent sucrose solution. Pollen tubes started growing within 10 minute in a culture solution. Pollen germination was observed after 2 hrs, 4 hrs, 6 hrs, 8hrs and 24 hrs from the time of sowing of pollen. Ungerminated and germinated pollen were transferred to slides on 1 per cent gelatine with the help of a fine needle and it was immediately killed by 4 per cent formaline and then air dried. Like this as many as slides needed were prepared and they were processed for the cytochemical analysis.

For localization of biochemical constituents like polysaccharides, proteins, RNA, DNA, lipids, ascorbic acid, cellulose, starch and pectins, the following methods were followed:

1. *Polysaccharides:*
   Periodic acid schiffs method. PAS+ve test (Jensen, 1960)
2. *Proteins:*
   Ninhydrin-Schiffs reaction (Yasuma & Ichikawa, 1953)

3. *RNA:*
   Azure B method (Flax & Himes, 1952)
4. *DNA:*
   Fuelgens test (Gomori, 1952)
5. *Lipids:*
   Sudan black B method (Baker, 1947)
6. *Ascorbic acid:*
   Silver nitrate method (Daye *et al.*, 1968)
7. *Cellulose:*
   IKI-H_2SO_4 method (Johansen, 1940)
8. *Starch:*
   IKI test (Johansen, 1940)
9. *Pectins:*
   Hydroxylamine-Ferric chloride method (Reeve, 1959)

Observations

1. *Polysaccharides*

In the ungerminated pollen a rich cytoplasmic polysachharide deposit was observed. The staining reaction for polysaccharides showed that the pollen cytoplasm contains a large amount of polysaccharides. It was seen that pollen tubes after 2 hours and 4 hours of germination showed intense colouration at the extreme tip of the pollen tube. Wall layers were PAS+ve (Pl. 1, fig. 2). Both exine and intine, however, give a +ve staining reaction. In case of 8 hours and 24 hours of germination polysaccharides more or less uniformly distributed and appeared as purely densely packed granules in the tip of pollen tubes.

2. *Proteins*

Mature pollen were rich in protein contents. Rich protein contents were noticed in the area near the germ pore. Pollen tube was also rich in protein contents. In the larger tubes the rich protein was localized in the tip region while the remaining tube showed the irregular distribution of proteins (Pl. 1, fig. 2). High amount of proteins were detected in the growing part of pollen tubes.

3. *RNA*

Azure B staining method showed RNA as purple. Pollen revealed a rich cytoplasmic RNA contents (Pl. 1, fig. 3). Young pollen tubes after 4 hours of germination showed a rich RNA localization. The observation of pollen grains indicated considerable amount of RNA and uniform distribution of RNA throughout the pollen tube.

4. *DNA*

For DNA stainning Fuelgen's test was adopted, DNA appeared purple in colour (Pl. 1, fig. 4). Pollen wall showed a intense colouration of DNA. In case of 8 hours of germination, it was observed that pollen tubes showed rich quantity of DNA throughout the pollen tubes. But in case of 24 hours of germination tube showed irregular distribution of DNA content near the germ pore.

5. *Lipids*

The test with Sudan black B method for lipids showed the presence of large quantity of lipids in the mature pollen grain and also in the pollen tubes. It showed the uniform distribution of lipids in pollen tubes in all the cases of germination. Exine and intine showed the large amount of lipids showing positive test (Pl. 1, fig. 5).

6. *Ascorbic acid*

Silver nitrate method showed the golden brown colouration for the ascorbic acid. The test showed weak staining of germinated pollen grains. Pollen tubes were observed at 2 hours of germination, showed more amount of ascorbic acid; but in case of 8 hours and 24 hours period of germination they showed gradually less amount of ascorbic acid. The various parts of the pollen tube showed irregular distribution of ascorbic acid only and intine showed variable positive staining reaction (Pl. 1, fig. 6).

7. *Cellulose*

The staining reaction with IKI-H_2SO_4 method showed a very high amount of cellulose in the pollen cytoplasm as well as in germinated pollen grains. High amount of cellulose was detected in the growing part of the pollen tube and also showed a considerable amount of cellulose throughout the pollen tube (Pl. 1, fig. 7).

8. *Starch*

The test with IRI method for the starch revealed the presence of large quantities of starch in pollen grains whereas, a decreased level of starch was observed in the germinated pollen grains. Both the exine and intine gave a positive test whereas, pollen tube showed negative test (Pl. 1, fig. 8).

9. *Pectins*

It revealed presence of pectins only in pollen cytoplasm. Pollen tube wall showed weak staining test. Intine and exine showed presence of pectic substances (Pl. 1, fig. 9).

Discussion

Pollen and pollen tube cytoplasm revealed a rich polysaccharide contents. Pollen wall layers were also PAS positive and showed uniform distribution of polysachharides. Intensity of the PAS positive colouration was uniform throughout the surface of the pollen and pollen tubes. Starch was gradually disappeared from the pollen tubes in *C. juncea*.

During the pollen tube formation sugars were in active state and ulimately wall materials mainly polysaccharides were laid down throughout the pollen tubes. The exogenous sucrose is observed by the pollen tubes and utilized during their growth. This results in an increase in the amount of PAS positive substances in cytoplasm and also PSA positive tinge in wall. This suggests the important role of sugar in metabolism and tube growth.

Southworth (1973) in *Gerbera jamesonii*, Panchaksharappa and Rudramaniyappa (1972, 1976) in *Millets*, Sharma (1977) in *Amaryllis vittata*, Vijayraghavan and Shukla (1976) in *Pergularia daemia*, Nagraj and Rao (1978) in *Asclepia curassavia*, Sathe and Patil (1982) in *Phaseolus trilobus* reported rich PAS positive material in pollen cytoplasm and wall layers.

Ungerminated and germinated pollen also revealed a rich localization of proteins. In pollen, protein was richly localized in the area near the germ pores. However, in larger tube the distribution was not uniform. The site of protein synthesis was the tip region of the tubes as suggested by rich localization of protein in the tip area in the present work. Similarly, protein synthesis at various stages of pollen tubes growth have been reported in plants like pine (Stanley *et al.*, 1958), Tupy (1966) in *Nicotiana*, Linskens (1969) in *Lilly*, Linskens *et al.* (1968) in *Perunia*, and Mascarenhas (1970) in *Tradeschantia*, Rahman and Patil (1980) in *Phaseolus aconitifolius*. In the present study pollen wall layers were protein positive.

In *C. juncea* pollen and pollen tube cytoplasm showed rich RNA content. Jawade, Rahman and Patil (1982) in *Melingtonia hortensis* also reported rich RNA content. The activity of RNA synthesis was confined throughout the region of the tube. A similarity in the localization of RNA and proteins in the present work suggests that RNA and protein synthesis during the tube growth goes hand in hand. Rosen (1971) also suggested synthesis of RNA in the pollen tube while studying the fine structure of pollen tube *in vitro*.

Pollen tube wall showed irregular distribution of DNA and only in the area near the germ pore. Pollen wall layers showed lipid droplets throughout the surface of the exine and intine and intense cytoplasmic lipids in the pollen and pollen tubes at various stages of their germination. Thus it seems that lipid was the storage material in this plant to be used during germination. Sassen (1964) found the germination both in pollen and pollen tubes of *Petunia*.

According to Malik and Mehan (1975) presence of hydrolysing enzyme lipase presumably converts lipids into simple acids which are then used in reproduction during the expansion or elongation phase. He suggested the role of lipid in matobolism and also in building the membrane of the rapidly growing cells.

Ascorbic acid in *C. juncea* showed irregular distribution. Exine and intine showed positive test for the ascorbic acid. Pollen and pollen tubes of *C. juncea* were cellulose positive and the intensity of the colouration was uniform throughout the wall layers of pollen and pollen tube whereas the pectic substances were found only in pollen grains.

Thus it can be concluded from the above study that the pollen of *C. juncea* is self equipped with cytoplasmic matabolic reserves and self sufficient to carry out the physiological activities during pollen tube growth.

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Explanation of Plate

Plate 1

1. Pollen and pollen tube with rich polysaccharide content, pollen and pollen wall is PAS positive.
2. Young pollen and pollen tube with uniform and rich proteins.
3. Pollen grain and the pollen tube tip showing rich cytoplasmic RNA content.
4. Male cells and vegetative cell are equally rich in DNA.
5. Very rich cytoplasmic lipids in pollen as well as in pollen tubes.
6. Weak ascorbic acid contents in pollen tube.
7. Pollen tubes show uniform distribution of cellulose throughout the entire length.
8. Pollen grains are very rich in starch content.
9. Pollen grains reveal very rich pectic substances.


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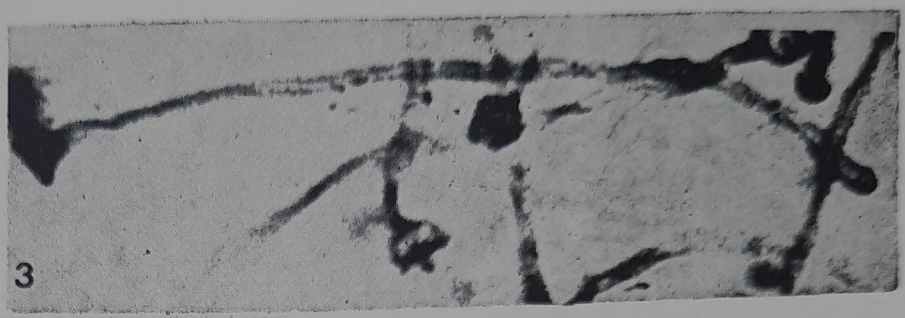
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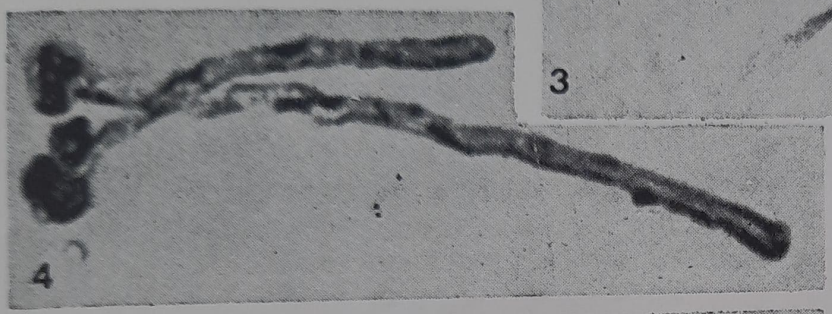
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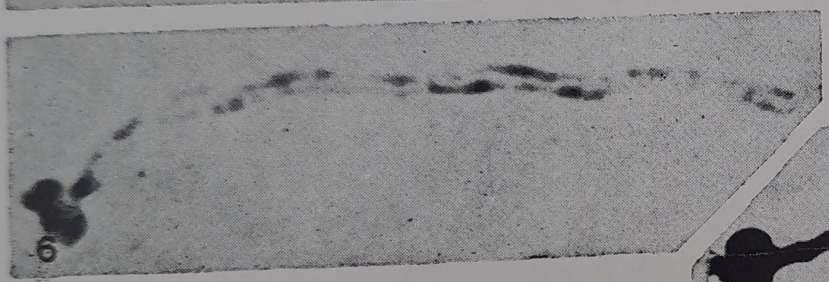
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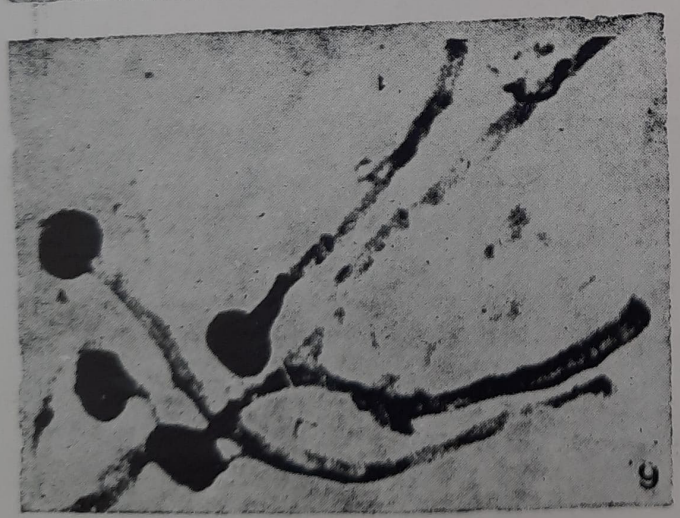
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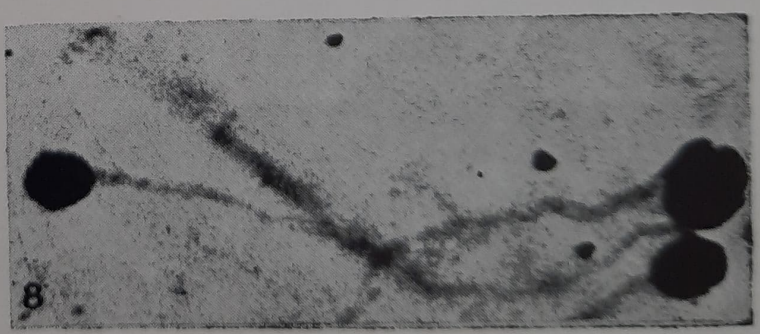
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