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CROTALARIA LABURNIFOLIA POLLEN – BIOCHEMICAL ANALYSIS

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ABSTRACT

A pollen grain is a microscopic body that contains the male reproductive cell of plant. It is crucial in plant's fertilization process. Good fruit set and high crop yield depend to a great extent on healthy and viable pollen grains. The present paper deals with analysis of carbohydrates, DNA, RNA, free amino acids and total protein content in pollen of the *Crotalaria laburnifolia.* The biochemical evaluation of these metabolites was performed by standard methods. Estimation of carbohydrates was done by Anthrone method. The estimation of DNA and RNA were done by using UV Spectrophotometer. Amino acids estimation was done by Ninhydrin method and separation and identification by thin layer chromatographic method and estimation of protein was done by Lowry's method. Amino acids like Leucine, Phenylalanine, Methionine, Aspartic acid, Histidine, Tryptophan were found to be present.

KEYWORDS: Biochemical evaluation, amino acids, protein, Carbohydrates, DNA, RNA, *Crotalaria laburnifolia.*

INTRODUCTION

Palynology is the study of pollen grains produced by seed plants (angiosperms and gymnosperms) and spores (pteridophytes, bryophytes, algae and fungi). Pollen and spores differ in their function, but both result from cell division involving a reduction by half of the chromosome content (meiosis) (Moore *et al.* 1991). Pollen grains are the house of male gametes, but spores are usually the resting or dispersal phase of the fern, algae, etc. Pollen grains represent the male portion of the reproductive process in plants and trees. These tiny bodies are swirling in the air (anemophily) and on the legs of insects (zoophile) so that they can join the female part of the plant to create a new seed. This important process is known as fertilization. Flowers have a simple structure and can be pollinated by many different types of animals. Some flowers have a more specialized structure and depend on one type of insect, or another type of animal, to pollinate them. Being the carrier of male genetic material, pollen is also essential for the life cycle of other living organisms, e.g. it forms the principal source of normal non-liquid food for pollinators. Pollen grains consist of nutrient-rich inner protoplasm ("intine"), surrounded by an exceptionably durable coat ("exine") and the grains are often covered by oil-rich and fragrance-rich "pollenkit" (a substance derived from tapetal tissue in the anther). Pollen contains abundant protein and free amino acids, although the proportion of these nutrients can vary widely among plant taxa. In addition to lipids and starch, the protein is intended for the growth of the pollen tube and this nutrient in particular, that makes pollen an appropriate food for the developmental stages of insects. There is abundant evidence for pollen and spore eating by insects in the fossil record. Pollen evolved from the microspores of seedless vascular plants, appearing in the fossil record during the Carboniferous. Spores and pollen ingested and excreted by insects during the Late Carboniferous to Miocene eras are sometimes even identifiable to source plant taxa (Labandeira 1997). Fossil insects associated with pollen feeding include thysanurans, diaphanopterodean nymphs, adult hemipteroids, protorthopterans and grylloblattids (Labandeira 1997). Modern pollen-eating insects occur in at least 14 orders. A report has been published of a pollen-collecting sphecid wasp in Sri Lanka (Krombein & Norden 1997; Hymenoptera: Sphecidae: Crabroninae), Because of the larger size and the importance of pollen in pollination and to insects, pollen will be emphasized.

Crotalaria laburnifolia (Fabaceae) is a shrub and the flowers are bright yellow and often marked with red with no odour. It blossom during July- May initiation of flowering is in July – August and peak flowering is in September - February and termination of flowering is from March – May. Inflorescence length 22.4±5cm and number of flowers 17.2±3.5. The flower opens during 12.00 to 18.00 and flower falloff from the inflorescence within 36hrs. Anther dehiscence is longitudinal. Pollen grains are semi wet and ovoid with 1mm size and golden yellow in colour. Stigmas are receptive from the time that the flowers start to open (assessed using the hydrogen peroxide test) but the receptivity of the stigmas does not reach its peak until 24 hours after anthesis.

Number of stamens 10 and number of ovules 20. Thus, the present study would give an inner view of the chemical nature of the *Crotalaria laburnifolia.*

MATERIAL AND METHODS

Carbohydrates

Pollen sample (100mg) was ground in a motor at -20^{o}C for 10 min. then 5ml of 2.5N HCl was added to the frozen powder and hydrolyzed by keeping it in a boiling water bath (Sadasivam and Manickam, 1992), cooled, neutralized with sodium carbonate and then centrifuged. The quality of carbohydrates was determined from the standard graph according to the method described by Anthrone method.

Proteins

Pollen sample (500mg) was crushed and homogenized with 5-10 ml of 0.5M Tris buffer (P^{H} 7.6) and then centrifuged for 15min. The supernatant was used for protein estimation following the Lowry method.

Total free amino acids

Pollen sample (500mg) was crushed and homogenized with 5-10ml of 80% ethanol and then centrifuged. The supernatant was collected and used for estimation of total free amino acids by following the Ninhydrin method.

DNA & RNA

Pollen sample (500mg) was crushed and homogenized with 5-10ml of 80% ethanol and then centrifuged. The supernatant was collected and used for estimation of DNA and RNA by using UV Spectrophotometer.

RESULTS AND DISCUSSION

Although man often experience discomfort on contact with pollen but in other living organisms pollen is essential for their life cycle, man has long has been a consumer of pollen and pollen containing foods. Pollen products are now being marketed as human food supplements, with various nutritional and health benefits often claimed for the user. Since about 1960, research has shown that pollen is beneficial when incorporated in feed rations of certain farm animals. These newer use of pollen are distinct from the interactions of insects with pollen. The adaptive and parallel evolution of insects with flowers is the key to survival and reproduction of many plant species. Pollen quality must be assessed to find plant fertility, to monitor pollen state during storage, in ecological or taxonomic studies and in research on pollen biochemistry, genetics and stigma interactions, incompatibility systems and fertilization. Total carbohydrate, protein, free amino acids, DNA and RNA were estimated to know the chemical nature of the pollen grains of *Crotalaria laburnifolia*.

The carbohydrate levels in pollen grains were found to be species specific. For 1g of pollen grain extract the carbohydrate content was found to be 320µg/ml (Table 1). Quadrio (1928) and Mameli (1952) reported that the pollen grains of anemophilous plants are generally starchy, whereas pollen of entomophilous plants are rich in fat and sugar which are known to serve nutrients to the insects. They claimed that the carbohydrates are the principal metabolic substrates used by the germinating pollen which occurs in the form of cytoplasmic polysaccharide in the cell walls.

For 1g of pollen grain extract the protein content was found to be 2000µg/ml (Table 1). For 1g of pollen grain extract the Total free amino acids content was found to be 79950µg/ml (Table 1). Free amino acids like Leucine, Phenylalanine, Methionine, Aspartic acid, Histidine, Tryptophan were found to be present. Pollen is the food of the young bee and it is approximately 40% protein. It is considered one of nature's most completely nourishing foods. It contains nearly all nutrients required by humans. About half of its protein is in the form of free amino acids that are ready to be used directly by the body. Such highly assimilable protein can contribute significantly to one's protein needs. Pollen is frequently called as "the perfect complete food". Just as the busy bee represents a role model for an active and productive member of the society the high performance athletes also quoted that their performance is due to eating this miracle food pollen.

In most pollen, the nucleic acids constitute about 2% of the total dry weight (Amal Kumar Mondal et al 1997). For 1g of pollen grain extract the DNA content was found to be 2595 µg/ml at 260nm and at 280nm it is 45µg/ml (Table 1). For 1g of pollen grain extract the RNA content was found to be 2076 µg/ml at 260nm and at 280nm it is 36µg/ml (Table 1). DNA content at levels of about 0.5% of dry weight and RNA content varying from 0.6-1% have been reported by Stanley and Linskens, 1974.

Table 1: Chemical constituents of pollen grain of Crotalaria laburnifolia.

Chemical constituent for 1g of pollen grain	Method/ Instrument used	Result obtained
Carbohydrates	Anthrone method	320±0.2µg/ml
Proteins	Lowry's method	2000±0.3µg/ml
DNA	UV Spectrophotometer	2595 ±0.3µg/ml at 260nm 45±0.1µg/ml at 280nm
RNA	UV Spectrophotometer	2076±0.2 µg/ml at 260nm 36±0.1µg/ml at 280nm
Total free amino acids	Ninhydrin method	79950±0.5µg/ml

CONCLUSION

Good fruit set and high crop yield depend to a great extent on healthy and viable pollen grains. And for germination of pollen grain a good food store is very important. During germination those stored foods are utilized for growth and development and for effective pollination in one way and for protecting the ecofriendly insects on the other. The above finding conforms to the results of other studies on the composition of amino acids in pollen.

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