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RESEARCH ARTICLE

# Study of Floral Biology of Insulin Plant *Costus pictus* D. Don

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## Abstract

This study deals with the reproductive biology of plant *Costus pictus* D. Don, commonly known as "Insulin plant" that have potential medicinal properties. It provides information regarding unique whorls of flower. Pollen and stigma morphology is studied through SEM analysis. The present studies were carried out on 50 inflorescence spike. Flowers of *C.pictus* D. Don have a labellum of five fused sterile stamens. Single fertile petaloid stamen is present.. Bilobed stigma appendage is attached to the wet bilamellate stigma. Flower shows co-occurrence of stamen and stigma. Pollen grains are somewhat asymmetrical in shape due to large size. Pollen are pantoporate as examined in SEM analysis. Pollen surface is found to be psilate during SEM examination but pores are ornamented. Floral nectary is present inside, above the ovary in prolongation region. Stigma receptivity was high in mature flowers while bud stage showed very few bubbles. The present study will help to scientists, taxonomists, and students to study floral biology of flower and inflorescence for identification, conservation and breeding purposes.

**Keywords:** *Costus pictus*, Pollen, Insulin, labellum, pantoporate, nectary.

## Introduction

Since ancient times, plants have been harnessed for their therapeutic properties, earning the distinction of "medicinal plants." The Rig Veda, an ancient Indian text, contains the earliest documented evidence of medicinal plant use. Today, plants remain a vital source of natural products and pharmaceuticals, driven by their remarkable pharmacological properties. *Costus pictus* D. Don , commonly referred to as the American insulin plant or spiral flag Sharma A et al. (2021). *C. pictus* D. Don belongs to the Costaceae family within the order Zingiberales. This family comprises four genera and approximately 200 species. Notably, Costus is the largest genus, boasting over 148 recognized species. Originating from Mexico and tropical regions, *C. pictus* D. Don has been introduced to India in recent years. The Costaceae family is distinguished by its unique floral structure, characterized by a solitary fertile stamen and five infertile stamens fused into a prominent, petaloid labellum. Traditionally, *C. pictus* D. Don has been employed in Ayurveda medicine due to its anti-diabetic properties. The plant's primary phyto-constituent, diosgenin, exhibits significant pharmacological activities, such as anti-diabetic, antioxidant, anti-inflammatory, and hypoglycemic effects. The study of floral biology will enlighten upon the pattern of structure of flower including reproductive parts of insulin plant *C. pictus* D. Don.

## Materials and Methods

This study has been carried out on *Costus pictus* D. Don species of Costaceae which is medicinally important. This work was carried out from October 2022 until October 2024 in plants growing in experimental garden, size 10 feet x 4 feet. The mature flowers were collected and preserved in F.A.A. Pollen grains were isolated with the help of acetolysis method. The taxonomic macro and micro characters were analyzed at the place of occurrence in the field as well as in the laboratory.

In floral biology taxonomic characters were studied in the field as well in the laboratory after fresh flower harvesting. Various characters related to stamen and pistil

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were studied in terms of stamens per flower, length of stamen, length of style and stigma were measured with the help of scale in millimeters.

### *Pollen morphology*

Following Erdtman's (1969) acetolysis method, pollen structure was studies under light microscope (LM) and Phase contrast Microscope (PCM). Mature flowers were plucked and anthers taken out and were crushed in distilled water. The crushed anther material with pollen grains was subjected to centrifugation for about 2-3 minutes at 2000 rpm. After centrifugation was completed, discard the solvent and add to the pellet, fresh acetolysis solution prepared in 1:9 (conc. $H_2SO_4$:$C_4H_6O_3$) ratio. Water bath was used to bring the temperature of solution to 80°C-100°C. Indicator of rise in temperature was change of solution to brown colour. Then it was centrifuged. Then the pellet was washed two-three times by $CH_3COOH$ solution. In the end, 50% glycerine was poured out to the pellet. Tip of the needle was immersed in the jelly and bring it in contact to the pellet so that pollen get stick to it. Now on the slide, mount the pollen which are stick to the jelly and seal with paraffin wax after covering the cover slip. Ten pollen of each species were taken for average measurement.

### *Scanning Electron Microscope (SEM) study*

Most of the specimens used during investigations were obtained from plants growing in natural conditions. Pollen and stigma were obtained from the fresh flowers. While during the scanning, the anther was placed on the slide and teased thoroughly in alcohol .The fresh pollen grain and stigma were mounted on metal stub with the help of double sides carbon tape. Uniform dusting of gold was done with the help of Quorum tech Q 150T S plus thin film gold coating machine for 10 minutes. The pollen grains and stigma were scanned in Thermoscientific Apreo S SEM at Institute Instrumentation Centre (IIC), IIT main campus, Roorkee, UK, India.

Pollen production (total number of pollen per anther) in each species was counted with the help of cell or spore counting method i.e., by the use of haemocytometer. The preserved anthers were crushed in distilled water in plastic tubes and then sieved into a centrifuge tube. The pollen grains were centrifuged at 2000-3000 rpm to form a pellet. This pellet was taken in 10 ml of water. Then one drop water containing pollen was placed on the haemocytometer and covered with coverslip. Pollen grains of 8 outer squares were counted. The 10 readings were taken of each sample and average was calculated

Total number of pollen per anther = Average number of pollen grains x 10000/25

Receptivity of stigma was tested by using hydrogen peroxide catalase activity method (Dafni ,1992). Hydrogen peroxide solution and distilled water and pistil of flowers were taken. Stigma were immersed into hydrogen peroxide (80%) solution and observed the bubbles through naked eyes.

## Result And Discussions

Taxonomic Characters: *Costus pictus* D. Don is a perennial plant with distinctive features of vegetative and reproductive shoot (Fig.1a). The plant has an erect or spreading stem, dark red or maroon in colour (Fig.1b). Plants attain height of 58-88 cm. The rhizome and shoot exhibit nodes and inter-nodes. Leaves arise from nodes with sheathing bases, and leaf lamina emerges from the sheath's apex. Abaxial surface of leaf is waxy and adaxial surface is shiny and dark green in colour (Fig.1c). Leaves and bracts are arranged in a right-handed spiral pattern (Halle, 1967). The plant produces a single shoot type, known as homoplyadic, combining vegetative and reproductive portion. The vegetative shoot transforms into a reproductive shoot or inflorescence, marking a transition from vegetative growth to flowering. Rhizomes remain dormant in the soil for 3-4 months during winter season. Shoot sprouting commences in the month of March from the underlying rhizomes.

Flowering season was reported from May to November (2022-24). Inflorescence arises from a leafy shoot emerging from the rhizome(Fig.1a). Floral shoot is in form of a cone or spike which bear bracts and flowers in a spiral manner. Floral shoot or cone grows upward with older flower at the base of

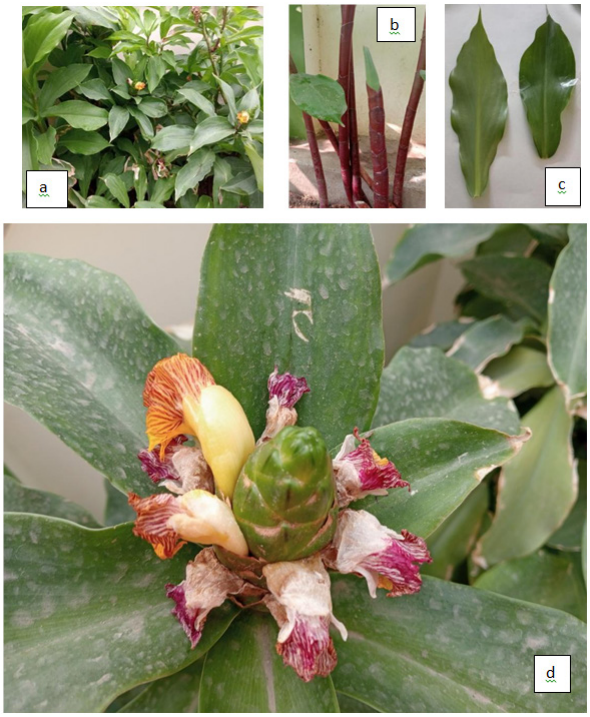


**Figure 1 (a-d):** a: *C. pictus* D.Don crop, b: Stems dark red colored, c: Abaxial and Adaxial surface of leaves, d: Inflorescence showing green bracts with dark green nectar guides, open flower and wilted flowers in acropetal manner.

the cone and younger flowers at the top showing racemose fashion. Inflorescence is racemose type. Flowers arise in an acropetal manner (Fig.1d). According to WesternKamp and Claben-Bockhofi (2007) this type of flower structure is present in ten other families of monocotyledonous plants including family Costaceae. Bud stage and mature flower was dissected to understand the development of floral parts (Fig.2a, b, c, d). Individual flower is globose, length 7.5-8.5 cm, and width 5.8-6.9 cm. Ovate, large green bracts with hidden part red in colour are arranged in spiral manner on the cone, measuring in length 2.4-3.2cm and width 2.5.- 3.0 cm. Dark green colour line is present on the bracts which serves as an extra floral nectary (Fig. 1d). Flowers are arranged in a spiral manner in the axil of the bract. Single red coloured folded bracteole is present that surrounds the flower. Length of bracteole is 1.7-2.3 cm (Fig.2d,e). Each flower is membraneous or yellow, complete, zygomorphic, and hemaphrodite . Reddish stripes are present on apex of yellow flower. Perianth consists of calyx and corolla. Tubular 3-lobed red spathe like calyx is 1-1.3cm in length. Median member of calyx is anterior, and aestivation is imbricate. Corolla is 3-lobed and yellow in colour, strong, oblong. Corolla lobes are unequal, in which median lobe is larger and upcurved.

Structure of androecium is median on an oblong petaloid process. Single fertile petaloid stamen is present. Structure of labellum is cup or funnel-shaped. Labellum is a unique structure of the Costaceae family. Labellum is made up of five fused sterile staminodes. The lower part of the labellum is united with the stamen forming a second tube (Maas 1972). Callens *et al*. (2018) suggested that labellum is extremely fascinating as it represents a special floral structure crucial for interacting with pollinators from evolutionary point of view. The fertile stamen consists of two anthers measuring about 9.0mm and .07mm in length & width respectively (Fig.2 f,g). Anthers dehisce through longitudinal slits. Anthers are adnate, and introse. Nectar guide is present in the reflexed inner side of the petaloid stamen. Nectar guide helps in pollination as it guides pollinator towards the nectary.

Gynoecium is three carpellary. Pistil consists of stigma, long and thin style and ovary (Fig. 2h,i). Wet, papillate, Group III type stigma bears dorsal two-lobed appendage, which is about 1.50-2.512 mm long and 2.0-3.308 mm wide. Bilobed appendage on dorsal side is 2.012-2.512 mm in length ( Fig. 2j). Style 4.8- 10.367 mm long, is thin in structure and positioned between the two narrowly elliplictic thecae forming anther of the fertile stamen ( Fig. 2f). Stigma and style are hairy. SEM analysis of stigma shows 2-lamellate structure with bilobed appendage (Fig. 2 m,n). Beak

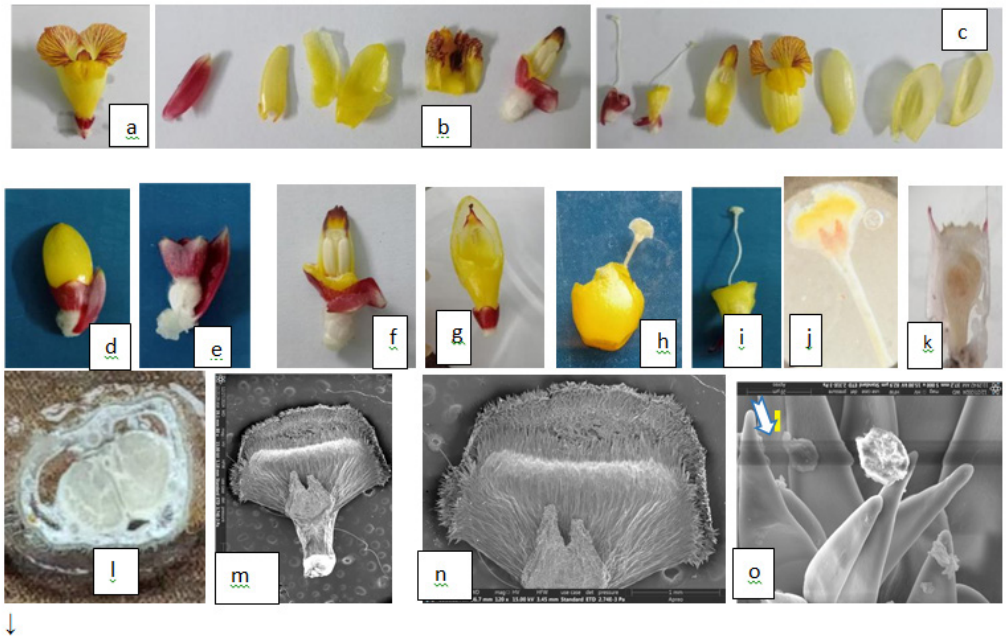


**Figure 2 (a-l):** a: Flower of *C. pictus* D.Don, b: Floral whorls of bud of *C. pictus* viz: bracteole, petals,under developed folded labellun, single fertile stamen on petalloid process with dark red nectar guide, pistil with style positioned in between two anther lobes, c: Floral whorls of mature flower of *C. pictus,*viz: bracteole, petals, cup shaped labellum, single fertile stamen on petalloid process, pistil with style positioned in between two anther lobes , d*:* bud of *C. pictus* with bracts and bracteole e: flower after fertilization with only persistent calyx and folded bract, f: Stamen on petalloid process, style and stigma in bud stage of *C. pictus,* g: discolored petaloid stamen process,stamen, style and stigma of mature flower of *C. pictus* flower after anther dehiscence, h: Stigma of bud stage, i: Stigma and long style of mature flower, j: enlarged stigma with bilobed appendage, k: L.S of ovary of *C. pictus* showing locules, ovules and prolongation region of internal nectary, l: T.S. of ovary of *C. pictus* showing locules and prolongation region of nectary. SEM microphotographs of stigma of *C. pictus* D.Don Fig 2(m-o)-m: microphotograph of stigma, n: magnified 2-lamellate stigma with bifid appendage, o: a portion of receptive stigma showing papillae with emerging beak of pollen grain

emergence starts when the pollen reaches the receptive papillae of bi-lamellate stigma (Fig. 2o).

The co-occurrence of anthers and stigma and their placement on the petaloid structure at the entrance of the flower opening helps the pollen deposition on the pollinators's dorsal surface and subsequent transfer to the receptive stigma of another flower is the feature of costaceae family which is also reported in *C.pictus* D. Don.

Ovary is tricarpellary, syncarpous, inferior, bilocular with axile placentation (fig. 2l). Ovary is about 9mm in length and 1.0 cm in breath, with 7-8 ovules arranged in axile placentation. Ovary show three partitions in longitutinal view. The topmost part above the ovary in the central axis of the flower doesnot contain any locular region but interfloral nectary is situated in this region. This part is distinguished as prolongation region (fig.2k,l). Below prolongation region, locular region is situated which contains ovules. It is the main part of the ovary. Below the locular region is sublocular region. Our studies are similar to the work on *Monocostus uniflorus, Tapeinocheilas ananassae, Dimerocostus strobilaceus and Costus dubius as* reported by Newman et *al.* (1992). A floral nectary is positioned above the locules and extends to the top into the prolongation region of the ovary in the centre of the floral axis. Abnormal feature of the ovary examined is the third locule which is reduced. Further studies are to be carried out so that it can be find out whether two or four rows of ovules per locule is advanced or primitive condition of costaceae. Floral nectary cannot be identified externally but can only be seen in the transvers or longitudinal section (Fig: 2a-o). Although literature on nectary of *C.pictus* D.Don is not reported but ovary of other members of family Costaceae shows floral nectaries which are the prominent feature of ovaries of the family.The family shows autapomorphy in relation to the position and structure of the nectaries. Charestiristic feature of the family Costaceae is the structure of ovary, divided into three parts with prolongation part on the top of the ovary. According to Newman et.al (1992), work on ovary of Costaceae, nectaries of the family are gynopleural or at least derieved from gynopleural nectaries.

Our observations are similar to the work of Samanta. A. *et al.* (2023) on plant *Hellenia speciosa* (J. Koening) as most of the member of Costaceae show similar structures, though *C.pictus* D.Don is not studied too much.

*Pollen morphology*

After acetolysis of mature pollen grain, they were mounted

**Figure 3:** *C. pictus* D. Don pollen in phase contrast microscope

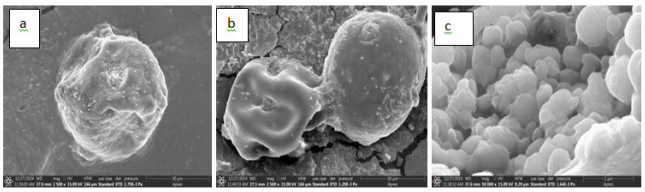


**Figure 4:** SEM images of stigma and pollen of *Costus pictus* D.Don (a-c)- a: single pantoporate pollen grain, b: dehydrated pollen grain showing infoldings and pores, c: sunken pore membrane ornamented

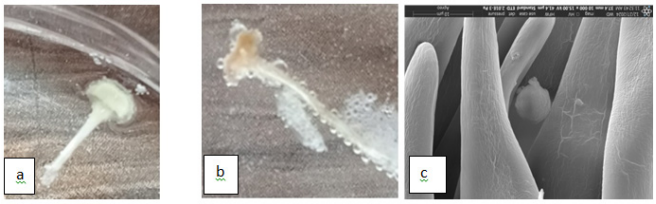


**Figure 5:** (a-c) Stigma of *C. pictus* D. Don a: Stigma receptivity in young stigma, b: presence of numerous bubbles showing stigma receptivity in mature stigma, c: SEM microphotograph of a part of mature stigma showing papillae with pollen grain

with glycerine and observed in the phase contrast microscope (Fig. 3) and SEM (Fig. 4a-c). Pollen grains were larger in size and after mounting appeared deformed. Shape of the pollen was found to be sub-spherical or slightly asymmetrical. Diameter of pollen grain was 66- 92µm. Polar axis of pollen grain ranges from 72.6 µm to 88.3 µm and equatorial axis ranges from 66.2µm to 98.6µm. Pores were present all over the surface. Pollen were 7-9 pantoporate and sunken (Fig4a). Length of the pores ranges from 9.415µm to 61.5µm and width ranges from 2.4 to 4.6µm. Pollen were yellow to pale yellow in colour. Exine is smooth and appears to be psilate (fig 4a). Usually psilate pollen is indicative for anemophily ( Foegri and Iversen 1989). Exine thickness ranges from 2.5 to 4.0µm. Exine is thickened at pores. Pore membrane is ornamented as appeared in SEM analysis(Fig 4c). In SEM analysis pores are found to be covered with knob like papillae (Fig. Intine is thin and smooth with 1.3 to 3.4µm thickness. Infolding of pollen grains are formed due to dehydration (Fig4b). In SEM image fig 2(o) pollen beak developd on the receptive stigma. The plant produces numerous pollen grains. The total count of pollen (average of ten readings) was 12200+/-1514.21.

***Receptivity of Stigma***

Pollen receptivity of stigma was observed by 80% Hydrogen peroxide st in *C.pictus* D.Don. Fresh young and mature flowers were collected and stigma was incised. Incised stigmas of young and mature flowers were immersed in the $H_2O_2$. Bubbles were formed. Number of bubble formation was much higher in mature stigma in comparison to young stigma where bubbles were not observed. In SEM analysis pollen with emergence of beak were reported in receptive stigma (Fig. 5a-d)

## Conclusion

Study of taxonomic characters and flower characters as well as SEM analysis of pollen and stigma of *C. pictus* will help taxonomists, scientists, researchers, and students in identification, phylogenetic and evolutionary studies. Further investigations of *C.pictus* D. Don in biochemical and biotechnological studies will be helpful in pharmaceutical industry.

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