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FLORAL ANATOMY OF *ALPINIA SPECIOSA* AND *HEDYCHIUM CORONARIUM* (ZINGIBERACEAE) WITH PARTICULAR REFERENCE TO THE NATURE OF LABELLUM AND EPIGYNOUS GLANDS

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Abstract: The floral anatomy of *Alpinia speciosa* Schum. and *Hedychium coronarium* Koenig. (Zingiberaceae) was investigated for an understanding of the structure and nature of the labellum and epigynous glands. The observation presented in this study supported the classical view of the labellum as a double structure rather than a triple or five-parted structure, as were proposed previously. The dorsal carpellary bundles in the studied species only continue into the style, fade out in the stigmatic tissue without feeding sepals or labellum as suggested in the previous studies. The glands in both studied species are found with very prominent masses of vascular tissue. The presence of vascular tissue seems to be connected with the more organized nature of the gland. Furthermore, the present study appears that the glands are not merely epidermal emergence of the ovary and similar to nectarines which may be vascularized.

Keywords: Zingiberaceae, *Alpini*a, *Hedychium*, floral anatomy, labellum, epigyny glands.

Introduction

Anatomical features are widely used in systematics, identification, placing anomalous groups in a satisfactory position in classification and explanation of the origin, position and nature of every plant organ. The vascular system of the flower has been studied extensively with regard to taxonomic and phyletic relationships among the angiosperms and to the morphological nature of the flower and its parts [MURTY, 1958; SUBRAMANYAM, 1960; EL-SHAFEY & al. 1966; AL-NOWAIHI & KHALIFA, 1973; ESAU, 1976; BARABE, 1981; ALVAREZ, 1988; KUMAR & MANILAL, 1988, 1992; MATTHEWS & ENDRESS, 2002; SAJO & al. 2004; SHARAWY & KARAKISH, 2005]. The use of the vascularization pattern in the interpretations of various phenomena in the flower is more conservative because it is less subjected to alteration by the habitat factor than the organs themselves [PURI, 1951]. Since, the classical research of van TIEGHEM (1871), the floral anatomy has been used as a reliable source of information in floral studies. Subsequent work by the same author and that of his followers, especially HENSLOW (1891), placed this research on a sound footing. He stated that the importance of this line of research lied in the fact that the origin, position and union of every organ were bounded to the particular cord or trace in the axis which subsequently enters it. SAUNDERS (1925) revived more interest in this field of study but her extensive and detailed work was mainly directed towards the application of floral morphology to her

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theories on carpel polymorphism. Further progress in floral anatomical studies took place, particularly in India [PURI, 1961] and the USA (e.g. EAMES, 1961). In addition, the internal anatomical structure of the flower provides many useful characters which can be used in taxonomic and phylogenetic studies. SUBRAMANYAN (1960) mentioned that the internal characters of flowers may be more useful in certain cases than the external because of the frequent persistence of the vascular supply of the lost organs after all the external evidence has disappeared.

Zingiberaceae flowers are bisexual, irregular, commonly in bracted spikes, heads, or panicles, one or more under each bract. Perianth of 6 parts in a calyx series and corolla series. Calyx tubular or somewhat spathe like, generally 3-toothed. Corolla tubular, unequally 3-lobed. Fertile stamen 1, the 2-anther-cells separated by connective. Labellum, one adnate at the base to the corolla tube and showy. Lateral staminodes petaloid free from the labellum. Ovary inferior, 1-celled, or sometimes 2-3 celled many ovules. Epigynous glands called stylodes form 2 erect outgrowths on top of the ovary. Style linear, held between the anther-thecae.

More than any other part of the Zingiberaceae flower, the labellum received a deal of great attention. PANDY (1989) stated that the labellum is two- or three- lobed and is produced by the fusion of the two laterals staminodes from the inner whorl of the stamens. In contrast, HEYWOOD (2001) stated that the labellum is produced from the fusion of two staminodes from the outer whorl of the stamens. Since the nineteenth century two theories about the nature of the labellum have been presented that it either has a double or a triple nature [PAYER, 1857; Van TIEGHEM, 1868, 1871]. GREGORY (1936) and ZHANG & al. (2009) supported that the labellum is triple in constitution from external morphology and flower vasculature. Other evidences are obtained from previous studies may very well be adduced in support of the classical conception, that the labellum is double nature [PAYER 1857; Van TIEGHEM 1868, 1871; PAI, 1963]. On the other hand, LIAO & al. (2008) indicated in *Alpinia hainanensis* that the labellum incorporates elements of five androecial members rather than two or three, as suggested by previous authors for Zingiberaceae flowers.

In addition, the epigynous glands in most Zingiberaceous flower are simply epidermal appendages of the ovary [VALETON, 1918; GREGORY, 1936; RAO, 1963]. According to their opinions the epigynous glands do not contain any vascular tissues. The presence of vascular tissues seems to be connected with a more organized nature of the glands [PURI, 1951; ESAU, 1953]. Furthermore, it appears that the glands are not merely epidermal emergences of the ovary. Comparative observation in the variation of their form, structure, development and vasculature seem to suggest strongly that they are more deeply connected with organs of the ovary [PAI, 1961, 1965; BHAT, 1993; KIRCHOFF, 1997; LIAO & al. 2008; ZHANG & al. 2009].

The flower of Zingiberaceae has a higher degree of organ specialization especially in the nature of labellum and the epigynous glands [BORAH & SHARMA, 2012; NGAMRIABSAKUL & al. 2000]. However, the origin and course of the floral vasculature may provide credible evidence to understand the nature of these floral structures. So, the present work deals with the study of floral anatomy of *Alpinia speciosa* and *Hedychium coronarium* (Zingiberaceae) to interpret the nature of the labellum and the epigynous glands in the studied taxa.

Materials and methods

Flower buds were collected during (2010) from the Botanical Garden of Faculty of Science, Ain Shams University, Cairo, Egypt. Five to eight flower buds of the studied taxa were fixed and preserved in F.A.A. and embedded in paraffin wax, then serially sectioned at 10-15µ according to the conventional method [JOHANSEN, 1940]. Sections stained in crystal violet-erythrosine (saturated in clove oil) combination. Drawings were made at bench level by the aid of a “Ken a vision Microprojector” Model x 1000. The magnification is given by Beck stage micrometer scaled to 0.1 and 0.01 mm.

Descriptive anatomical terms related to the floral vascularization are (i) the vascular supply to the floral organ is termed “trace” when it is still in the receptacular tissue and joined with the central stele and (ii) the same is termed “bundle” when it enters the organ.

Results

In *Alpinia speciosa*, at the pedicel level, the vasculature bundles are arranged in two rings; the outer ring consists of numerous bundles whereas the inner ring is composed of more or less fused ones (Fig. 1a, b). At the floral axis beneath the ovary, the outer ring is further branched forming numerous vascular traces whereas the inner ring contains three large bundles and three small radially bundles alternating with each other (Fig. 1c).

At the base of the ovary, the three large bundles of the inner ring diverge outwards and become oriented higher in the ovary wall as the three carpellary dorsal bundles which eventually enter the style. Meanwhile, the remaining three small bundles are divided radially to form six bundles; three outer and three inner on the same radii (Fig. 1d). The former outer three bundles (septal carpellary bundles) also travel out exhibiting extensive branching during their outward transit. The latter inner three vascular bundles fuse to certain distance and then separate again to form three united masses whereas, at higher level divided into six ventral carpellary bundles two for each carpel and then fade out gradually while progressing upwards till the top of the ovary (Fig. 1e-h).

At the top of the ovary, the three septal carpellary bundles increase in size, divide into two or three bundles and bear outer and lateral branches, adjacent ones of which fuse to form an anastomosing vascular plexus. Some bundles of the outer ring are associated with this plexus but the median and marginal bundles of the sepals remain unaffected (Fig. 1h-k). Many vascular bundles are derived from this anastomosing plexus to supply petals, glands, androecium and style.

A calyx tube starts to separate and is supplied by three large bundles (sepal median bundles) alternating with many small bundles from the outer ring of the vascular bundles. Meanwhile, each of the sepal median bundles divides on each side once and then supply sepal marginal (Fig. 1l).

The glands are initially attached to the inner column of tissue (androcorolla tube) for a short length and become free at little higher within the tube. Two masses of vascular bundles which are derived from the vascular plexus enter the two glands and extend in an antero-posterior direction (Fig. 1m).

At higher level, after the complete separation of the calyx tube, the corolla tube is detected. Higher up, three ridges of the corolla tube are developed on its outer face, one posterior and the other two antero-laterals. Some of the bundles in the corolla tube divide and some of them travel out into the ridges. The latter subsequently separates from an inner cylinder and represents the three petals. The larger, posterior petal embraces the two other smaller, antero-laterals (Fig. 1n).

Thus, the outer three stamens are morphologically absent; the vasculature of the outer three stamens is not detected. The remaining inner cylinder represents the fused bases of the two lateral staminodes (labellum) and the posterior fertile stamen. This cylinder splits into two segments, a flat posterior one and thick crescentric anterior one. The flat part contains five bundles; one median and four laterals traces. In the upper part of the anther, the connective is very much reduced in width and appears as a narrow plate connecting the large anther lobes on either side. The median bundles split into two small bundles and each of the resultant bundles travels towards the laterals. The connective itself, splits in the middle and separates from the anthers a little later. The bundles in the connective run in the split crest of the anther and then disappear (Fig. 1n-p).

The thick crescentric anterior segment is the base of labellum. One of the bundles is on the mid-anterior line and develops a shallow groove on its inner face. This bundle divides higher up to two small bundles. The daughter bundles also shift laterally; these lateral bundles soon undergo further splitting forming the marginal supplies of the labellum (Fig. 1o).

The filament of the fertile stamen is mostly slender and deeply grooved. In such groove, the style extends between the anther cells and the capitate stigma is protruding. This style contains a flattened canal and three bundles which extended to stigma (Fig. 1p).

In *Hedychium coronarium***,** two rings of bundles, each with variable numbers of strands, run in the floral axis beneath the ovary. The outer ring is composed of 16 vascular bundles, while the inner ring consists of six vascular bundles (Fig. 2a).

At the base of the ovary, the vascular bundles of the outer ring travel out exhibiting extensive branching giving rise to three sepal median bundles alternating with the numerous sepal marginal bundles and three large vascular complexes that are alternating with the loculi of the ovary (Fig. 2b, c). At a little higher, the six large bundles of the inner ring separate into three septal carpellary bundles and three ventral carpellary bundles. At the beginning of the ovuliferous zone, the latter bundles split to six small bundles that send traces to the ovules. They bear no branches in the non-ovuliferous zone (Fig. 2d, e). At the upper portion of the ovary, the ventral carpellary bundles fade out gradually till they are lost completely at the base of the stylar tissue (Fig. 2f-h).

On the top of the ovary, at the beginning of separation of calyx tube, the remaining vascular bundles of the outer vascular ring and the three carpellary septal bundles coalesce and form temporary anastomosing vascular plexus (Fig. 2g-j). At a little higher, the anastomosing vascular plexus is differentiated into three large bundles and many small bundles which later supplied petals, glands and androecium.

The former large bundles are recurved downwards from the top of the ovary as dorsal carpellary bundles then bent up again to feed the style. The three dorsal carpellary bundles continue into the style and gradually fade out in the stigmatic tissue (Fig. 2k).

The glands are initially attached to the androcorolla tube for a short length and become free at little higher. Two masses of vascular bundles enter the two glands that extend in an antero-lateral direction (Fig. 2k, l).

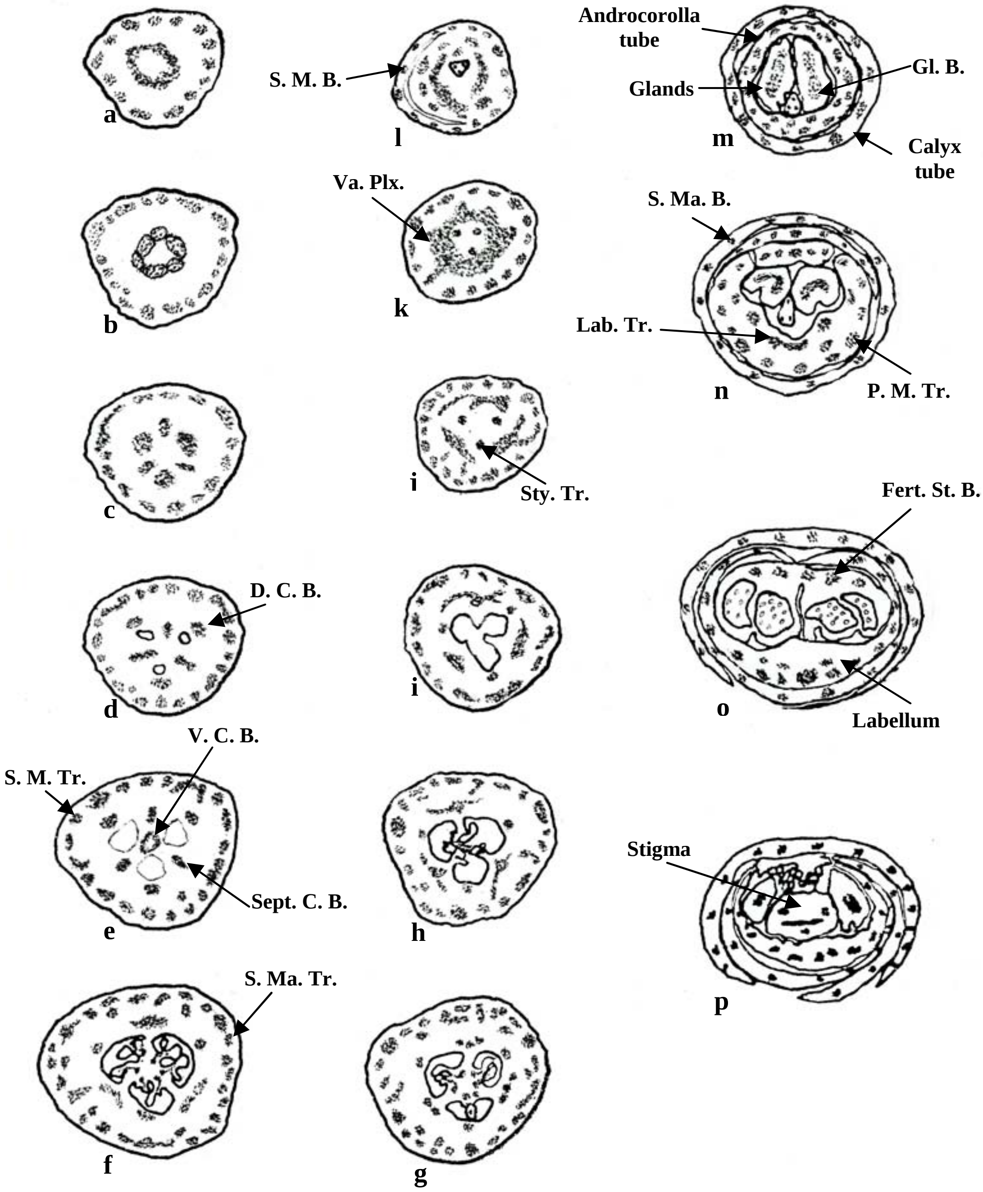

Fig. 1 a-p. Serial transverse sections from the pedicel upwards through the flower of *Alpinia speciosa*. D. C. B. = Dorsal Carpellary Bundles; Gl. B. = Glandular Bundles; Fert. St. B. = Fertile Staminal Bundle; Lab. Tr. = Labellum Trace; P. M. Tr. = Petal Median Trace; S. M. B. = Sepal Median Bundle; S. M. Tr. = Sepal Median Trace; S. Ma. B. = Sepal Marginal Bundle; S. Ma. Tr. = Sepal Marginal Trace; Sept. C. B. = Septal Carpellary Bundle; Sty. Tr. = Stylar Trace; V. C. B. = Ventral Carpellary Bundle; Va. Plx. = Vascular plexus.

At a higher level, the outer whorl of the perianth leaves (calyx) disappears whereas the inner whorl of the perianth leaves (corolla tube) starts to separate from the androcorolla (hypanthium) tube. At a little higher, the corolla tube develops three petals, one posterior and two in antero-lateral position. The three median petal bundles in the corolla tube divide and some of them travel out into the petals as marginal petal bundles. The larger posterior petal embraces the two other smaller, antero-laterals petals (Fig. 2m, n).

After the separation of the inner perianth leaves (corolla), the outer petaloid staminodes separate from the inner three stamens. The former outer stamens are consisting of two staminodes fused together forming a large compound labellum (Fig. 2n). The inner whorl of stamens is represented by one posterior fertile stamen and two lateral staminodes.

The remaining tissue of the vascular plexus is differentiated into the six staminal bundles mass in two rings; three in each ring (Fig. 2m, n). The outer three bundles represented by two large postero-laterals bundles and one anterior small bundle. The former two bundles supply the labellum (posterior-lateral staminodes); each one divides into two and the resultant bundles travel laterally in the labellum. The latter anterior small bundle gradually decreases in size and completely disappears (Fig. 2o).

The inner three staminal bundles divided into numerous small bundles; five of them supply the filament of the fertile stamen, while the remaining supplies the two lateral staminodes. The five vascular bundles of the fertile filament are represented by one median and four laterals. These five bundles exhibiting extensive branched into small traces. In the upper part of the anther the connective tissue appears as a narrow plate connecting the two large anther lobes. At the top of the anther, the connective tissue itself splits in the middle and separates from the anther. The vascular bundles in the connective tissue run in the split part of the anther and fades out (Fig. 2n, o).

The style is filiform enveloped within a channel extending between the anther lobes. The capitate stigma protrudes from the top of the anther. The stigma contains a small triangular canal and three strands flanking the arms of the triangular canal (Fig. 2p).

Discussion

In earlier floral anatomical studies of members of the family Zingiberaceae, GREGORY (1936) and RAO & al. (1954) describe the presence of twelve bundles; nine peripherally disposed in groups of three each and three forming a central group in the pedicel. PAI (1965) interprets the median bundles of the peripheral three sets as derived from the inner rings which initially, contain six bundles. He suggested that the presence of the three median bundles of the peripheral sets in the outer ring occurs only as a secondary feature. Evidently, he said that GREGORY (1936) and RAO & al. (1954) based their description from sections taken a few microns beneath the ovarian loculi and not from lower levels. In the present study, the pedicel vasculature consists of two rings of vascular bundles; the outer ring consists of numerous vascular bundles and the inner ring is composed of six vascular bundles.

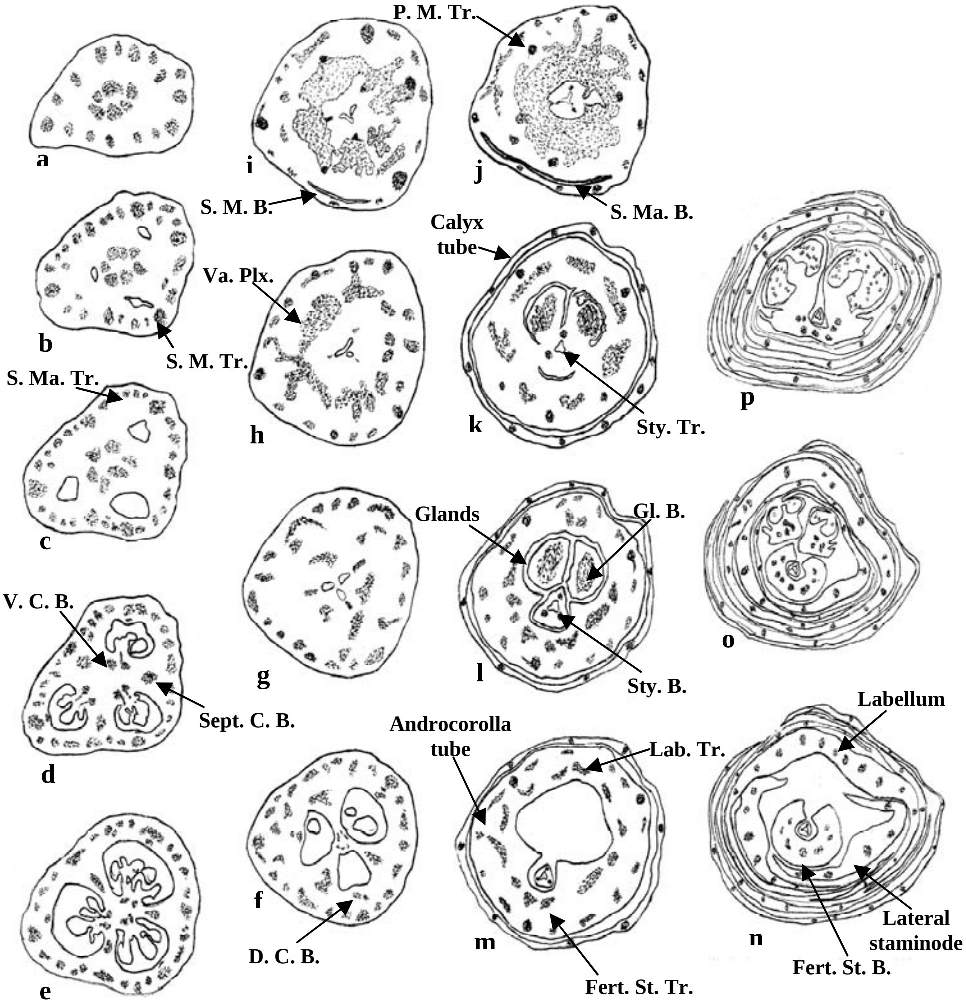

Fig. 2 a-p. Serial transverse sections from the pedicel upwards through the flower of *Hedychium coronarium*. D. C. B. = Dorsal Carpellary Bundles; Gl. B. = Glandular Bundles; Fert. St. B. = Fertile Staminal Bundle; Fert. St. Tr. = Fertile Staminal Trace; Lab. Tr. = Labellum Trace; P. M. Tr. = Petal Median Trace; S. M. B. = Sepal Median Bundle; S. M. Tr. = Sepal Median Trace; S. Ma. B. = Sepal Marginal Bundle; S. Ma. Tr. = Sepal Marginal Trace; Sept. C. B. = Septal Carpellary Bundle; Sty. B. = Stylar Bundle; Sty. Tr. = Stylar Trace; V. C. B. = Ventral Carpellary Bundle; Va. Plx. = Vascular plexus.

According to GREGORY (1936), the three peripheral bundles, after supplying the sepals, precede upwards and are divided laterally; terminated in a number of outer stamens (staminodes) and the original median one is continued into the style. In the present investigation, the pedicel vasculature of *Alpinia speciosa* and *Hedychium coronarium* consists of two rings; outer and inner vascular bundles. In these two species, each sepal is supplied by median sepal bundles and numerous marginal sepal bundles that originate from the outer vascular ring. It is pertinent to note here that the median bundles of the peripheral vascular groups run as the median bundles of sepals, leaving no vascular tissue at all at the top of the ovary. PAI (1965), also in his study on *Elettaria cardamomum*, observed that the median of the peripheral sets which run opposite the loculi represent the median traces of the sepals.

After the separation of the calyx tube, three daughter bundles which were derived from the vascular plexus are in turn derived from the inner ring of the pedicel vasculature, function as median traces of the petals. Later on, these three median petal traces start to ramify into numerous marginal petal traces.

In the earlier studies on Zingiberaceous flowers the presence of the carpellary ventral bundles in the axile region of the ovary is not mentioned. These have been observed in the specimens studied here. Such vascular bundles are also present in some species of the Zingiberaceae but referred to as the placental bundles concerned with the bearing of branches to the ovules. According to GREGORY (1936), PAI (1961) and LIAO & al. (2008), ovular traces are borne by the three parietal bundles (septal bundles) running opposite the septa which constitute the inner of the two rings in the pedicel of the *Cardamom* and *Alpinia* flowers. It may be pointed out here that the three bundles to which GREGORY (1936), PAI (1961) and VAN TIEGHEM (1968) refer are compound cords, being the fusion product of the carpellary septal (parietal) and the carpellary ventral (placental) bundles. In this study, just below the base of the ovarian loculi, these cords split into the constituent strands; the inner carpellary ventral traces and the outer carpellary septal bundles. The former provide traces to the ovules, but this was not mentioned in Gregory's investigation. The later bundles (carpellary septal bundles) are completely absent in *A. speciosa*, while in *H. coronarium* they coalesce at the top of the ovary with other bundles and form anastomosing vascular plexus. In *A. hainanensis* the three parietal bundles divide into five strands; of which the outer strands enter into the petals and the remaining enter into the functional stamen and in the labellum [LIAO & al. 2008].

In the studied species, the dorsal carpellary bundles in *A. speciosa* are derived from the inner vascular ring at the base of the ovary and just below the locules of the ovary. While in *H. coronarium*, at the top of the ovary, three bundles are derived from the vascular plexus. These bundles are recurved downwards as dorsal carpellary bundles and bent up again to the style. In both studied species the three dorsal carpellary bundles continue into the style and fade out in the stigmatic tissue. Unfortunately, few references mentioned the dorsal carpellary bundles in previous studies on Zingiberaceous flower. These carpellary dorsal bundles in *A. hainanensis* divided into five traces, of which the outer strand becomes the median bundle for each sepal and the inner strand runs into the style [LIAO & al. 2008]. While in *H. forrestii* the carpellary dorsal bundles continue to feed the petaloid staminodes [ZHANG & al. 2009].

GREGORY (1936), regards the glands of some members of Zingiberaceae as simply epidermal appendages of the ovary, since according to him, they do not contain any vascular tissue. RAO (1963) argued that the Gregory explanation needs confirmation. In the present study, the glands in both studied species are found with very prominent masses of vascular tissue. The presence of vascular tissue seems to be connected with the more organized nature of the gland and similar to nectarines which may be vascularized [ESAU, 1953]. Furthermore, the present study appears that the glands are not merely epidermal emergences of the ovary. Comparative observations on the variation in their form, structure, development and vasculature, seem to suggest strongly that they are more deeply connected with ovary [PAI, 1961; KIRCHOFF, 1997; ZHANG & al. 2009]. However, it must be mentioned that Gregory (1936) was correct in as much as he associated the glands with the ovary and, at the same time regarded them as of no particular significance in morphological considerations of the Zingiberaceous flower.

In *A. speciosa*, the outer three stamens are morphologically absent and the vasculature of these three outer stamens is not detected. While the inner stamens are represented by one posterior fertile stamen and two staminodes united to form the labellum (lip). The absence of the three outer stamens in this species was also detected in *Curcuma amada* [PAI, 1962], *Kaempferia scaposa* [RAO & PAI, 1959] and in *A. hainanensis* [LIAO & al. 2008]. The mid-anterior bundle in the floral tube of *A. speciosa* is derived from the vascular plexus and showed an earlier splitting into two small traces at the base of the labellum. PAI (1965) explained that the mid-anterior bundle is divided into two strands in the labellum of *Elettaria cardamomum*.

GREGORY (1936) and ZHANG & al. (2009), in the study of the labellum in *E. cardamomum* and *H. forrestii* assumed that the labellum is a triple structure. While LIAO & al. (2008) indicate that the labellum of *A. hainanensis* incorporates elements of five members rather than two or three as suggested by previous authors for Zingiberaceae flowers. Data of the present study may be added in support of the classical conception suggested by PAYER (1957) and VAN TIEGHEM (1868, 1871), that the labellum is a double structure rather than triple or five. This mid-anterior bundle may conveniently be interpreted as a composite bundle being the fusion product of the marginal bundles of the two component member of the inner staminal whorl. The variation seen in the course of this bundle in some of the plants studied bears testimony to this contention. In *Zingiber macrostachyum*, there are no mid-anterior bundles, but laterally on either side of the mid-anterior line, the marginal bundles of the two constituents are present and they continue upwards into the two segments of the labellum [RAO & PAI, 1959]. The same condition occurs in some other species e.g. *Curcuma decipiens* [PAI, 1962], *Kaempferia scaposa* [RAO & PAI, 1959] and *Curcuma amada* [PAI, 1962]. The resultant traces also continue for the rest of the length of the labellum and into the two segments.

Gregory supports from the external morphology his contention that the labellum is triple in constitution. He found it more or less three-lobed which he considers significant from the point of view of its morphological nature. In this study, the flower of *A. speciosa* shows a median apical split in the labellum. PAI (1965) suggested that when the apical notch of the labellum is absent, the median trace in the labellum continue for some length and then clearly exhibits a bifurcation. This might once again be used in support of the contention that the development of the mid-anterior traces in the labellum is related to the degree of connation of its two components. Although, the connation has progressed in some flowers removing all external indications of the constitution of the labellum, floral anatomy helps us to understand its morphological nature. In this connection, the remarks of WILLIS (1948) are significant. According to him, the labellum in Zingiberaceae might be two- or three lobed but it still comprises only the two antero-lateral members of the inner androecial whorl. The evidence provided by THOMPSON (1933, 1936), KIRCHOFF (1997) and BARAH & SHARMA (2012) on the morphological, anatomical and organogenesis studies of family Zingiberaceae may well be agreed with the present study and the classical conception that the labellum is of a double nature rather than triple or five-parted.

In *Hedychium coronarium* the flower has a single pollen-bearing stamen and four petaloid staminodes that are united in various ways. In tribe *Hedychieae* the most common interpretation of these fusions is that of EICHLER (1884). According to this interpretation, two staminodes are fused to form a large compound labellum (or lip), two other stamens form the lateral staminodes [KIRCHOFF, 1977]. In the present study, at the top of the ovary, the outer stamens are represented by three vascular bundles; two bundles supply the two postero-lateral staminodes that unite to form the posterior labellum, the third bundle (anterior bundle) fades out and disappear. Like the vasculature of labellum of *A. speciosa*, the labellum of *H. coronarium* well is adduced in support the classical theory, that the labellum is double in its nature.

The anterior outer staminal bundle is fading out. Thus, this stamen (anterior outer stamen) is morphologically absent. The evidence obtained from the floral ontogeny of *H. coronarium* carried by THOMPSON (1933, 1936) and KIRCHOFF (1977) supported the presence of three outer stamens but the primordium of the anterior one ceases growth soon after its initiation.

Conclusions

The present study supports the old classical concept, that the labellum is a double structure rather than triple or five-parted that was suggested by previous authors. Also, confirmed the ontogenetically evidence for the origin of the outer stamens in *A. speciosa* and *H. coronarium*. The fertile stamen of the inner whorl in the both studied species (*A. speciosa* and *H. coronarium)* is supplied by five vascular traces. These five bundles exhibiting extensive branched into small traces to feed the two large anther lobes. In addition, the investigation indicates that the ovary glands of the two studied species are vascularized.

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