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Floral Ontogeny in *Alpinia oxyphylla* Miq. (Zingiberaceae) and Its Systematic Significance

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Abstract

Floral organ development of *Alpinia oxyphylla* Miq. begins with the initiation of sepal primordia, and then the three common primordia comprising petal and inner whorl androecial members. Each common primordium separates into a dorsal petal and a ventral androecial member. The adaxial common primordium begins to separate first to produce the functional stamen and the adaxial petal. Subsequently the two abaxial common primordia separate to form two abaxial inner androecial members and two abaxial petals. After the three common primordia completed their differentiation, three outer androecial members are formed, of which the two adaxial primordia have a slow growth and finally become two lateral staminodes, while the abaxial primordium ceases growth and disappears gradually. The gynoecium is the last floral structure to initiate. Soon after the initiation of gynoecial primordium, the two abaxial inner androecial members form two secondary primordia. Compared to the development of the anther on the fertile stamen, the secondary primordia may be homologous with the primordia of the pollen sacs. This provides new evidence supporting the view that the labellum was derived from the two inner whorled androecial members.

Introduction

The Zingiberales is a natural order of monocotyledons consisting of eight families, namely, Heliconiaceae, Strelitziaceae, Lowiaceae, Musaceae, Zingiberaceae, Costaceae, Cannaceae and Marantaceae (Tomlinson, 1982; Dahlgren and Rasmussen, 1983). Among the eight families, Heliconiaceae, Strelitziaceae, Lowiaceae and Musaceae are often informally referred to as the banana group, and flowers of those four families possess either five or six fertile stamens. The remaining four families form the monophyletic ginger group. Flowers in the ginger families possess either one fertile stamen with two anther sacs (Zingiberaceae and Costaceae) or one stamen with only one anther sac (Marantaceae and Cannaceae) (Kress *et al.*, 2001; Rudall and Bateman, 2004). A typical flower of angiosperm consists of four whorls of different floral organs, they are from outside to inside: sepals in whorl 1, petals in whorl 2; stamens in whorl 3 and carpels in whorl 4. Nevertheless, there are labellum and lateral staminodes in the whorl 3 besides the stamen in ginger group. Fig. 1 shows a flower diagram of Zingiberaceae and a picture of mature flower of *Alpinia oxyphylla* Miq. to indicate the positions of lateral staminodes and labellum. Inspite of several opinions about the nature of the labellum and the lateral staminodes in the ginger family based primarily on morphological and anatomical evidences expressed in the past (e.g. Burrt, 1972; Gregory, 1936; Rao, 1963; Raghavan and Venkatasubban, 1941; Willis, 1948), the recent evidences support labellum being derived by the congenial fusion of two inner staminodes (e.g. Kirchoff, 1997, 1998).

In angiosperms, many structures that are absent in mature flower have vestigial remains that can be observed during the development of floral primordium (Endress, 1994). Accordingly, floral organogenesis and development can reveal not only the early state of the floral structure in the flower, but also the homeosis of floral structure. Kirchoff has described the aspect of floral development of several species in the Zingiberaceae (Kirchoff, 1988a, 1997, 1998) and other Zingiberales (Kirchoff, 1983, 1986, 1988b), and Box and Rudall (2006) have investigated the floral ontogeny in *Globba*.

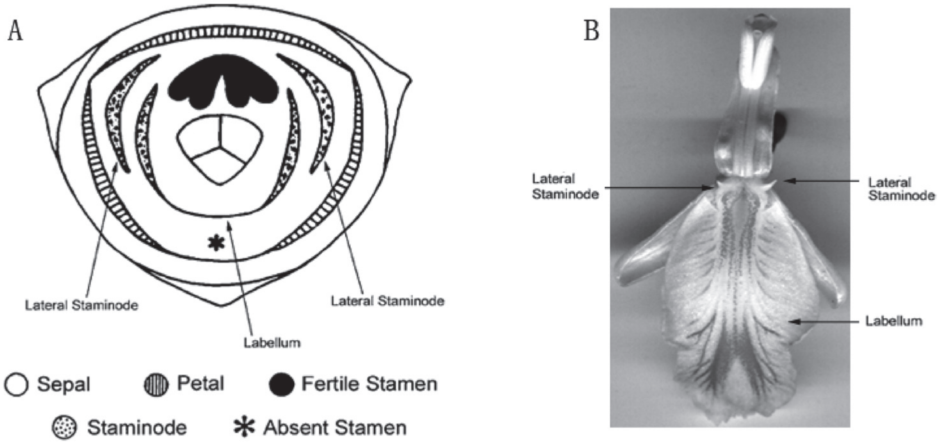


Figure 1. Floral diagram of Zingiberaceae and a mature flower of *Alpinia oxyphylla*. A. Floral diagram of Zingiberaceae (cited from Kress *et al*, 2002); B. mature flower of *Alpinia oxyphylla.*

In the genus *Alpinia* only one species, *Alpinia calcarata* (Haw.) Roscoe, was studied (Kirchoff, 1988a). Here we describe the floral development of *Alpinia oxyphylla* to provide additional information for the species within the genus *Alpinia* and present additional evidence to a better understanding of the origin of the labellum and the lateral staminodes in the family Zingiberaceae.

Materials and Methods

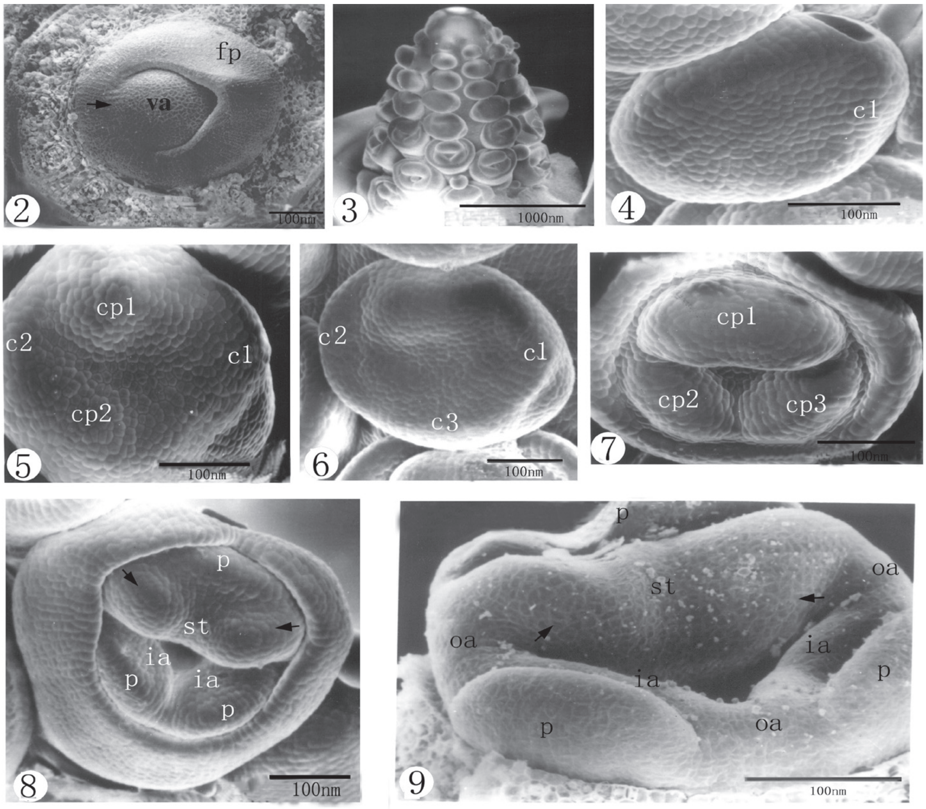
Inflorescences of *Alpinia oxyphylla* were collected from the ginger garden of South China Botanical Garden, Chinese Academy of Sciences. Fresh materials were fixed using forlmalin-acetic acid-alcohol (FAA) (Berlyn and Miksche, 1976) for two days, and then stored in 70% alcohol. The vouchers (collection number: *J. J. Song 2004-03*) were deposited at SCBG. Specimens used for scanning electron microscope (SEM) were dissected in 70% alcohol under Wild Stereo Microscope, dehydrated in a series of alcohol running up to 100%, treated with isoamyl acetate, and then critical-point dried using CO_2. Gold-sputtered specimens were observed and photographed under the SEM.

Results

The shoot apex of *Alpinia oxyphylla* is a domed shape and more or less symmetrical structure and produces leaves in an alternate arrangement (Fig. 2). A crescent-shaped primordium initiates on one side of the apex, which indicates the initiation of a leaf. This leaf primordium has a rapid growth and forms a cap-like structure; gradually encloses the apex from above. When this cap-like structure encloses half of the shoot apex, another foliar primordium begins to initiate on the other side of the apex and repeats the same growth process (Fig. 2). After about the differentiation of 17-20 leaves, the shoot apical meristem converts itself into an inflorescence meristem.

The inflorescence of *A. oxyphylla* is a raceme (Wu and Larsen, 2000) and there are about 50 to 70 flowers that are initiated in acropetal order in one inflorescence (Fig. 3). Floral organ development begins with the initiation of sepal primordium. The three sepal primordia are initiated sequentially at the three angles of the floral shoot apex (Figs. 4, 5). These three primordia continue to enlarge and gradually separate from the central part of the floral primordia to form a ring of calyx primordium (Figs. 6, 7, 8).

Soon a bulge initiates at the adaxial side of the midsection of the floral primordium after the initiation of the sepal primordium (Fig. 5).



Figures 2-9. Floral organogeny of *Alpinia oxyphylla:* 2. The vegetative apex (va) and the foliar primordium (fp). The black arrow indicates the initiation of a new foliar primordium. 3. The inflorescence shoot. 4. The first sepal (c1) is initiated on a flower bud. 5. Sepals (c1, c2) labeled in order of initiation; cp1, cp2: the two primordia of petal and inner whorl androecium. 6. Three sepals (c1,c2, c3) begin to form a ring calyx primordium. 7, The complete formation of three primordia (cp) labeled in order of initiation. 8, The three primordia separate into petal and inner whorled androecium (ia) [p, petal; st, stamen; ia, abaxial inner whorled androecial member]. Black arrows indicate pollen sac of the anther. 9. Formation of three outer androecial members (oa) [p, petal; st, stamen; ia, abaxial inner whorled androecial member]. Black arrows indicate pollen sacs.

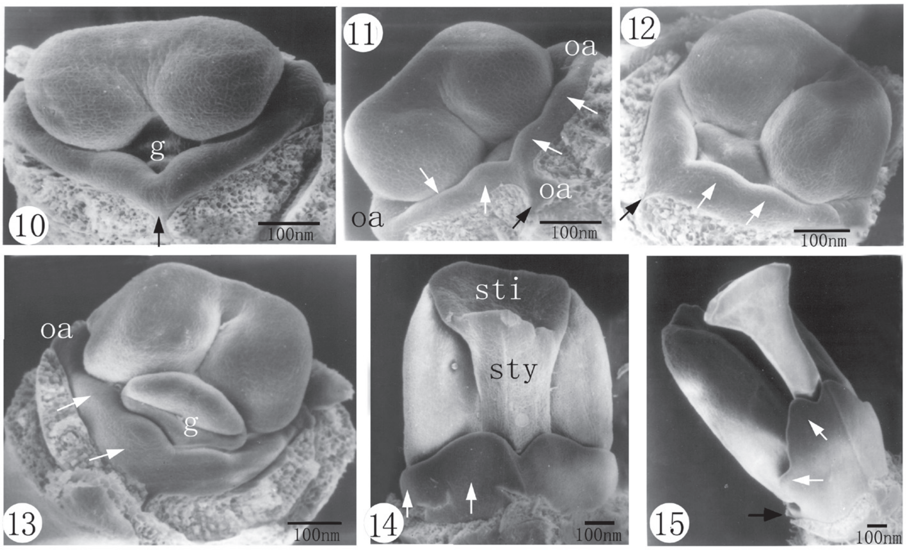
Thereafter, two other bulges form in a counter-clockwise order at the abaxial side of the floral primordium (Figs. 5, 6). These are the common primordia of three petals and the three inner whorl androecial members. The three common primordia arrange triangularly and fuse basally to form the floral cup. However, there is an unequal development of these three primordial: the adaxial one becomes obviously larger than the abaxial two (Fig. 7). The center of the floral cup is depressed, and the depression becomes deeper with the growth of the three common primordia (Figs. 6, 7). The three petals and their associated androecial members are formed from the separation of these three common primordia. Each primordium separates into a dorsal petal and a ventral inner whorled androecial member. The adaxial primordiun begins to separate first and produces the functional stamen and the adaxial petal. Subsequently the two abaxial primordia separate more or less simultaneously to form the two abaxial inner androecium and two abaxial petals (Fig. 8).

Additionally, the primordium of the fertile stamen differentiates into two bulges soon after its formation and these two bulges grow rapidly to give rise to the locules (pollen sac) of the anther (Figs. 8, 9, see arrows). The two abaxial inner androecial primordia then fuse with each other, and ultimately form the labellum. After the three common primordia complete their differentiation, three outer androecial members begin to form (Fig. 9): two of them appear beside the stamen primordium, one presents at the position outside the fused part of the two abaxial inner androecial primordia. The two adaxial primordia of the outer androecial members form the lateral staminodes when the flower finishes differentiation (Fig. 15, see black arrow). The abaxial one ceases growth soon after its initiation and disappears gradually (Figs.10-12, see black arrows).

The three petals undergo rapid growth after their initiation and enclose the inner floral organs gradually. The floral primordium has differentiated calyx, petal, inner and outer whorl androecial members at this stage. The gynoecial primordium is the last one to initiate; it appears in the depression formed by the development of outer whorls (Fig. 10). The gynoecial primordium grows rapidly following initiation and soon forms the stigma and style (Figs. 13-15). Soon after the initiation of gynoecial primordium, the two abaxial inner androecial primordia, which ultimately form the labellum, each begin to produce two secondary primordia respectively (Fig.11, see white arrows). The size of the four secondary primordia is similar when they are just formed (Fig.11), but there is an unequal development among them. The abaxial two grow faster than the adaxial two, which results in the difference of their sizes (Fig.12). Furthermore, the difference between their sizes becomes greater with growth and it is these four secondary primordia eventually form the semi-oval labellum when all floral organs finish the ontogenetic differentiation (Figs. 12-15).

Discussion

Kirchoff (1983, 1986, 1988a, b, 1997, 1998) studied the floral organogenesis in Zingiberales and established the pattern of floral ontogeny in the order, which in general, is highly conserved at the family level. The floral organ development of *A. oxyphylla* fits well into this general pattern.



Figures 10-15. Floral organogeny of *Alpinia oxyphylla*: 10. The initiation of the gynoecium (g). Black Arrow indicates the abaxial outer whorled androecial member. 11. Two abaxial inner androecial members begin to form secondary primordia (white arrows) [oa, outer whorl androecium]. 12-13. Size differentiation (see white arrows) between the secondary primordia of inner androecium. 14. The differentiation of stigma (sti) and style (sty). 15, The adaxial outer whorl androecium with subulate appendage (see arrow).

The initiation sequence of the floral organ is sepal, petal and inner androecium, outer androecium, gynoecial primordium, which resembles the developmental pattern reported for *A. calcarata* (Kirchoff, 1988a). In our study of *A. oxyphylla*, some of the developmental characters observed are like *A. calcarata*: (1) there is a lag between the formation of the inner and outer androecial whorls, that is, the outer androecial members do not begin to initiate until after the petals and the inner androecium are distinctly formed; (2) the three common primordia initially are asymmetric, and originated on the adaxial side of the floral shoot apex; and (3) the shape of the floral cup. However, these characters observed in the floral ontogeny of two species of *Alpinia* are different or partly different from what was described by Kirchoff (1988a) for other members of the ginger group. Kirchoff (1988a) had used the characters of floral ontogeny in his phylogenetic analysis of the family. Our data on the floral ontogeny may add information to the understanding of the relationship between the floral development and evolution of the individual groups in the family.

The formation of the labellum and the lateral staminodes of species in Zingiberaceae has received much attention. Various interpretations have been advanced. Brown (1830) regarded the labellum and the two subulate appendages as the outer whorled stamens, and the two epigynous glands and the functional stamen as the inner whorled stamens. Raghavan and Venkatasubban (1941) had the same point of view on the basis of work on *A. calcarata.* But Rao (1963) proved that the two epigynous glands of Zingiberaceae are merely an outgrowth from the upper surface of the ovary. Gregory (1936) gave an interpretation that the stamen and the lateral portions of the labellum belong to the inner whorl, while the median part of the labellum and the two subulate appendages belong to the outer whorl on the basis of his work on *Elettaria cardamomum* (L.) Maton. Others, like Willis (1948), believed that the functional stamen and the labellum represented the inner whorled stamens. Liao *et al.* (2006) studied the floral vasculature of *Alpinia hainanensis* and showed that the labellum is supposed to represent five members of the androecium: its two marginal and the median portions are derived from three members of the outer androecial whorl and its two lateral parts represent the two members of the inner whorl. The recent evidences supporting the origin of labellum is derived from the congenial fusion of two staminodes, and the two lateral staminodes represent the outer androecial whorl, the anterior member of this whorl being absent.

The floral development study reported by Kirchoff (1997, 1998) supported the interpretation that the primordia of the two inner staminodes are joined by the intercalary growth to produce the labellum, while the abaxial outer androecial member ceases growth soon after initiation and contributes only initially to the formation of the labellum. The other two outer androecial members form the two lateral staminodes. In our study, the floral development of *A. oxyphylla* also supports this interpretation. Moreover, the two abaxial inner androecial primordial differentiated into two secondary primordia respectively. Compared with the development of the anther on the fertile stamen, the two secondary primordia maybe homologous to the primordia of two locules (pollen sac) of the anther. From this point of view, the four secondary primordia observed by us in *A. oxyphylla* that eventually form the labellum, may represent the fusion of four pollen sacs of two stamens. This brings forth new evidence for the view that the labellum was derived from the two inner whorled androecial members.

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References

Berlyn G.P. and J.P. Miksche. 1976. *Botanical Microtechnique and Cytochemistry*. Iowa State University Press, Ames.

Box M.S. and P.J. Rudall. 2006. Floral structure and ontogeny in *Globba* (Zingiberaceae). *Plant Systematics and Evolution* **258**: 107–122.

Burrt, B.L. 1972. General introduction to papers on Zingiberaceae. *Notes from the Royal Botanic Garden Edinburgh* **31**:155-165.

Dahlgren R. and F.N. Rasmussen. 1983. Monocotyledon evolution: characters and phylogenetic estimation. *Evolutionary Biology* **16**: 255-395.

Endress, P.K. 1994. *Diversity and Evolutionary Biology of Tropical Flowers*. Cambridge University Press, Cambridge.

Gregory, P.J. 1936. The floral morphology and cytology of *Elettaria cardamomum* Maton. *Botanical Journal of the Linnean Society* **50**: 363-391.

Kirchoff B.K. 1983. Floral organogenesis in five genera of the Marantaceae and in Canna (Cannaceae). *American Journal of Botany* **70**: 508–523.

Kirchoff B.K. 1986. Inflorescence and development in the Zingiberales: *Thalia geniculata* (Marantaceae). *Canadian Journal of Botany* **64**: 859–864.

Kirchoff, B.K. 1988a. Floral ontogeny and evolution in the ginger group of the Zingiberales, pp. 45-56. In: P. Liens, S.C. Tucker and P.K. Endress (eds.). *Aspects of Floral Development*. Berlin Stuttgart.

Kirchoff B.K. 1988b. Inflorescence and flower development in *Costus scaber* (Costaceae). *Canadian Journal of Botany* **66**: 339–345.

Kirchoff, B.K. 1997. Inflorescence and flower development in the Hedychieae (Zingiberaceae): *Hedychium*. *Canadian Journal of Botany* **75**: 518-594.

Kirchoff, B.K. 1998. Inflorescence and flower development in the Hedychieae (Zingiberaceae): *Scaphochlamys kunstleri* (Barker) Holttum. *International Journal of Plant Sciences* **159**: 261-273.

Kress, W.J., L.M. Prince, W.J. Hahn and E.A. Zimmer. 2001. Unraveling the evolutionary radiation of the families of the Zingiberales using morphological and molecular evidence. *Systematic Biology* **50**:926-944.

Kress, W.J., L.M. Prince and K.J. Williams. 2002. The phylogeny and a new classification of the gingers (Zingiberaceae): evidence from molecular data. *American Journal of Botany* **89**:1682-1696.

Liao, J.-P., P. Zou, Y.-J. Tang, J.-J. Song, Z.-Y. Xie, Q.-G.. Wu and T.-L. Wu. 2006. Floral vasculature in *Alpinia hainanensis* in relation to the nature of the labellum in gingers. *Nordic Journal of Botany* **23**: 545-553.

Rao, P.J.M. 1963. The epigynous glands of Zingiberaceae. *New Phytologist* **62**: 342-349.

Raghavan, T.S. and K.R. Venkatasubban. 1941. A contribution to the morphology and cytology of *Alpinia calcarata* Rosc. with special reference to the theory on Zingiberous flowering. *Proceedings of the Indian Academy of Sciences* **B13**: 300-325.

Rudall, P.J. and R.M. Bateman. 2004. Evolution of zygomorphy in monocot flowers: iterative patterns and developmental constraints. *New Phytologist* **162**: 25–44.

Tomlinson, P.B. 1982. Phylogeny of the Scitamineae - morphological and anatomical considerations. *Evolution* **16**: 192- 213.

Willis, J.C. 1948. *A Dictionary of the Flowering Plants and Ferns.* Cambridge.

Wu, T.-L. and K. Larsen. 2000. *Zingiberaceae*, pp. 322-377. In: Wu, Z. and P.H.Raven (ed.). *Flora of China*, vol. **24**. Science Press and Missouri Botanical Garden Press, St. Louis.