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DEVELOPMENTAL AND MORPHOLOGICAL STUDY OF THE COLEORHIZAE IN *HEMEROCALLIS* (LILIACEAE)

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Abstract

The developmental morphology of the coleorhizas of *Hemerocallis* was studied by paraffin method in this paper. Our results show when their seeds germinate the coleorhiza stretches out the testa first, cracks radiately and reflexes with the radical elongation, and these splits atrophie and leave a ring of projections around the between the primary root and hypocotyl in their seeding development. The study of the embryonic development of *Hemerocallis* shows the coleorhiza is formed by the residual suspensor, the proper next to the suspensor, and cells from the meristematic cellular band occurred in the radical. It is different from the coleorhizas presented in the cycads and grasses, in which the coleorthizsa are composed of the residual suspensors. The coleorhiza occurred in the genus may be partially structurally homologous with those of the cycads and grasses.

Introduction

Sutton and Tinus (1983) described that the coleorhiza is a sheath surrounding a grass embryo. The root and its root cap are enclosed in a coleorhiza which, in the young embryo, is continuous with the suspensor. Little and Jones (1980) also indicted the coleorhiza is a protective sheath-like structure enclosing the radicle in grass embryos. The coleorhiza is widely present in the grasses and cycad seedlings (e.g. Chamberlain, 1919, 1935; Avery, 1930; Merry, 1941; Wardlaw, 1955; Hooft, 1970; Bierhorst, 1971; Rao, 1971; Foster & Gifford, 1974; Fahn, 1982; Walsh, 1990; Pant & Sing, 1991; Liu, 2010). However, Stevenson (1990) supplied that a true coleorhiza is unique to the grasses and that the cycads do not have a true coleorhiza. Comparing the embryonic development of the cycad and grasses, Robbertse & Grobbelaar (2011) observed that the coleorhiza existing in cycad embryos derives from the distal part of the suspensor and postulated that the coleorhiza in grasses also derives from the distal part of the suspensor and that the two structures are therefore structurally homologous.

Many researches about the physiological, biochemical, germination conditions, and anatomy of seeds and seedlings were done (e.g. Walsh, 1990; Fisher, 2008; Secorun & Souza, 2011; Ahmad *et al*., 2012; Escapa *et al*., 2012; Jamil *et al*., 2012; Murtaza & Asghar, 2012; Nadeem *et al.*, 2012; Liu *et al*., 2013; Wu *et al*., 2013; Shaukat *et al*., 2013), but the coleorhiza structure was not found except cycads and grasses. A sheath-like structure occurred on the primary root of *Hemerocallis citrina* Bar. was observed in our early study. Our aims are to study carefully the sheath-like structure and embryonic development of *Hemerocallis* L. including *H. citrina* and other species, to know if the sheath-like structure is a coleorhiza, to compare the structure with the coleorhizae presented in the cycads and grasses, and to understand the similarities which may present in them.

Materials and Methods

The plants of *Hemerocallis citrina* were collected from Qianshan in Liaoning, and other materials used were collected in Heilongjiang, *H. lilio-asphodelus* L., *H. middendorfii* Trautv. & Mey. from Jingpohu, *H. fulva* L. from *Forest Botanical Garden of* Harbin. The taxa collected were identified by Prof. M.Y. Liu, and cultured in the Botanic Garden of Harbin Normal University. The embryonic development of *H. citrina* and the seed germination of all species used were studied in detail. The ovaries and seedlings of different developmental periods were placed in FAA for a minimum of 24 h, stained by hematoxylin. The fixed materials were dehydrated through an ethanol series and embedded in paraplast (Li, 1987). Serial sections were made at 6 µm, and photographed using an Olympus BX51 microscope Leica DH205 anatomical lens and digital camera DP70.

Results

The development from zygote to seedling of *Hemerocallis citrina* and the seeding structure of *H. lilio-asphodelus*, *H. middendorfii* and *H. fulva* were studied for showing the sheath-like structure occurred in their primary roots.

The embryonic development of *Hemerocallis citrine*: The four-embryo of *H. citrina* is developed by longitudinal division of the apical cell at chalazal pole and transversal division of the basal cell at micropyle pole (Fig. 1A). Two cells of four-embryo at chalazal pole develop into a globular embryo and the other two cells at micropyle pole develop into a polystichous and multi-cellular suspensor (Fig. 1B). The divisions of suspensor cells are slower than those of the embryo. The suspensor cells next to the proper divide faster than those of the suspensor at micropyle pole, therefore the suspensor appears as an inverted trapezium in shape. The globular embryo develops further into an ellipsoidal embryo (Fig. 1B, C). The suspensor cells at the micropyle pole begin to wither gradually when the cotyledon primordium appears. Some suspensor cells next to the proper, which join the proper development, were observed (Fig. 1C), and they remain on the tip of the coleorhiza (see below) (Fig. 1G). When the bud primordium appears, the original cells of the radical begin to divide and form a division center. The meristematic cells of the division center close to the micropyle pole further increase and form an arc-shaped band composed of meristematic cells (Fig. 1D, E).

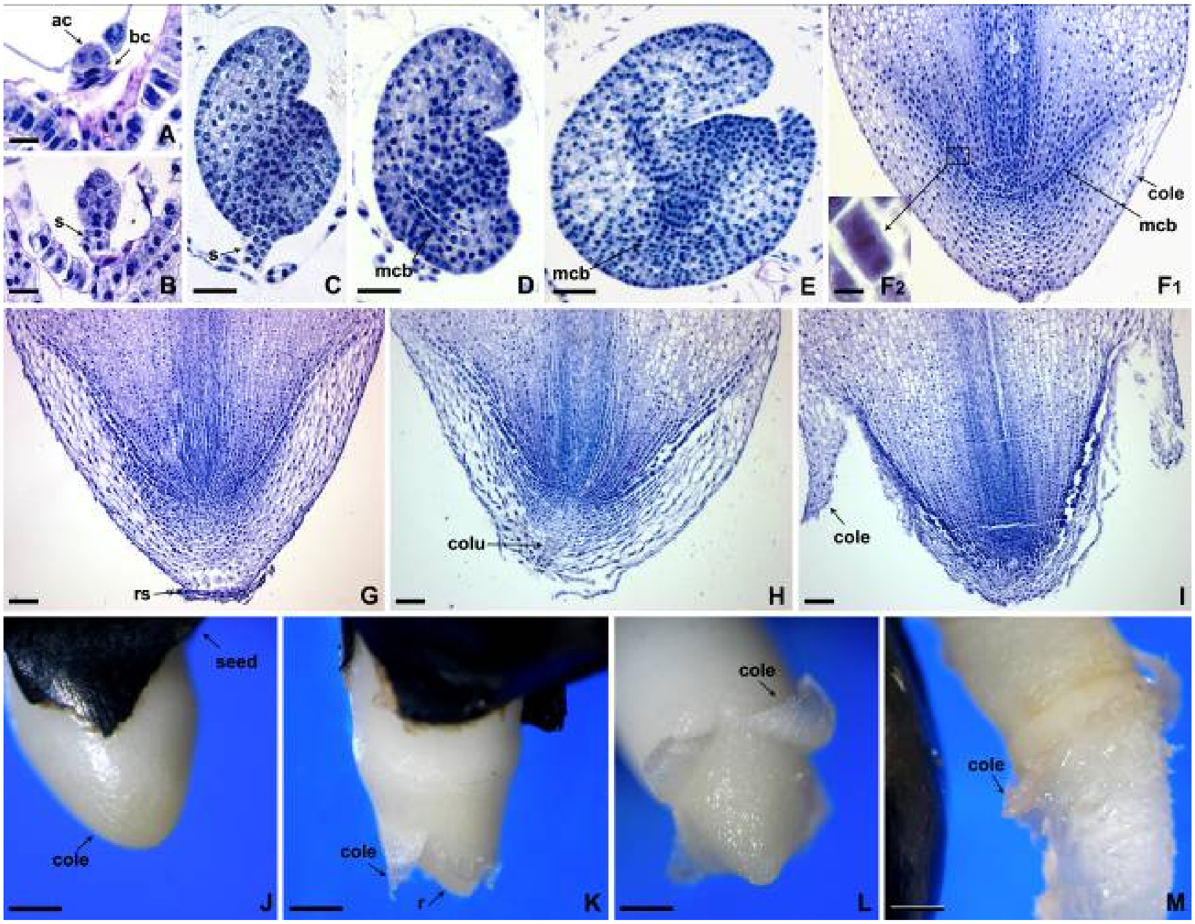

Fig. 1. Morphology and anatomy of the coleorhiza development in *Hemerocallis citrina*. A. Four-cellular proembryo. B. Globular embryo with polystichous and multi-cellular suspensor. C. Ellipsoidal embryo with withered suspensor cells. D-F_1. D. Meristematic cellular band; E. A cap-like meristematic cellular band; F_1. A sheath-like structure (coleorhiza). F_2. The cell in division in meristematic cellular band; G. Some rudimental suspensor cells remained on the tip of coleorhiza. H. Columella. H-M. Broken coleorhiza. M. A ring projection between primary root and hypocotyl; ac = apical cell, bc = basal cell, cole = coleorhiza, colu = columella, mcb = meristematic cell band, r = radical, rs = residual suspensor, s = suspensor, scale bar = 0.02 mm in A-B, 0.05 mm in C-E, 0.1 mm in F_1-I, 0.01mm in F_2, 1 mm in J-M.

The development of the sheath-like structure: When the seed embryo of *Hemerocallis citrina* is formed, the cells of the meristematic cellular band divide further and some cells in division could be observed (Fig. 1F_2). These cells join the proper next to the division center of the radical and form a sheath-like structure with the residual suspensor together, which cover the radical. (Fig. 1F_1, G, J). At the stage, the columella developed from the meristematic cellular bands and the meristematic center of the root are formed (Fig. 1H).

When the seed of *H citrina* germinates, the testa is broken and the radicle covered by the sheath-like structure stretches out (Fig. 1G, J) and the sheath-like structure is broken with the radical elongation (Fig. 1H, K), and the broken sheath-like structure reverses and withers gradually (Fig. 1I, L, M), and leaves a ring of projection between the primary root and the hypocotyl (Fig. 1M). Other taxa of *Hemerocallis* (*H. lilio-asphodelus, H. middendorfii and H. fulva*) studied also have the sheath-like structures as in *H. citrina* in shapes and structures and they are not shown in the paper.

Discussion

Hemerocallis is growing in cold, drought, and barren environment (e.g. Qiu, 2008; Shao, 2009), the sheath-like structure could protect the radicle as in the coleorhizaes of the cycads and grasses. Brown (1960) described the origin of the coleorhiza was stated differently, which is as part of the base of the embryo remaining after the true primary root differentiates. However, Guignard and Mestre (1970) states that the coleorhiza is a degenerated primary root, whereas the normal root arising above the coleorhiza is adventitious. In the cycads and grasses the coleorhiza develops from the distal part of the suspensor (Robbertse & Grobbelaar, 2011). Feng *et al*., (2003) described that the upper portion of the hypoblast contributes to the formation of the coleorhiza of *Zea mays* L. However our study showed that the sheath-like structure of *Hemerocallis* differs from all above. It is very similar with those of the cycads and grasses in shape, but it is connected with the radicle in the seed embryo. The structure was also found in the cycads (Robbertse &

Grobbelaar, 2011), but differs from the grasses which have a gap between the coleorhiza and radicle (e.g. Esau, 1977; Xu & Zhu, 1982). The sheath-like structure occurred in the genus may be partially structurally homologous with those of the cycads and grasses, and should be a coleorhiza.

The polystichous and multi-cellular suspensor is also found in the grasses and the cycads as in *Hemerocallis* (e.g. Esau, 1977; Fahn, 1982; Xu & Zhu, 1982; Dogra, 1992; Raven *et al*., 1992; Black *et al*., 2006; Lin, 2009). In dicotyledonous plant embryos where there is no coleorhiza, the suspensor is either absent as in the Euphorbiaceae or mostly consists of a single strand of cells attached to the initials of the radicle of the embryo proper and it is impossible for the radicle to grow into the suspensor as in the case of the cycads and the grasses (Raven *et al*., 1992; Robbertse & Grobbelaar, 2011). However, Tian & Shen (1991) indicated that *Astragaius mongholicus* (Bge.) Hsiao in Fabaceae has a four-strand cellular suspensor, which is a sheath-like structure in the species (pers. obs.). It will be interesting to know if its sheath is also a coleorhiza, if all plants with the polystichous multi-cellular suspensors have the coleorhiza and the function of the polystichous multi-cellular suspensors in the coleorhiza formation.

Conclusion

According to the features of zygote and seeding development, the sheath-like structure occurred in *Hemerocallis* is a coleorhiza which could protect the radical in the seeding development. The polystichous and multi-cellular suspensors may be related to the formation of the coleorhiza. The coleorhiza may present in other seed plants with the polystichous and multi-cellular suspensors and it is necessary to study the coleorhiza structures, their variation, origins and homology which may present in the seed plants.

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