Pak. J. Bot., 47(4): 1467-1472, 2015.

THE MORPHOLOGICAL STUDY OF THE FRUIT, SEED AND SEEDLING OF *HYDROCHARIS DUBIA* (HYDROCHARITACEAE)

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Abstract

The structure of the fruit, seed and seedling of *Hydrocharis dubia* in Hydrocharitaceae were studied with light microscope. The results show that the fruit has incompletely six locules with superficial placentation. The seed has anchor-like trichomes occur on the surface and with no endosperm. The seed embryo develops on the water. The hypocotyl hairs appear earlier than the primary root which develops weakly. The adventitious roots are formed with the leaf development. The paracytic stomata are present in the upper epidermis of the young leaf and the air canals are present in the mesophyll. The petiole has a sheath-like base. The seedlings usually die and only a few could develop into mature plants and the development of the species is similar to those of the other taxa in Alismatales. The study provides the morphological evidence for the placement of the Hydrocharitaceae in the order Alismatales.

Key words: *Hydrocharis dubia*, Hydrocharitaceae, Morphological, Seed, Fruit

Introduction

Hydrocharitaceae includes about 17 genera and 80 species, which are aquatic herbaceous plants (Sun *et al*., 1992). The family was placed in Butomales based on the numerous ovules dispersed on the inner surface of the ovary wall and seeds have no endosperm (Hutchinson, 1934). Cronquist (1981) due to its syncarpous and inferior ovary which are more evolutionary characters, placed Hydrocharitaceae in the order Hydrocharitales. In APG (2009) the family was moved to Alismatales which included Alismataceae, Butomaceae, Potamogetonaceae and some other related families.

Hydrocharis includes 3 viz., species *H. dubia* (Bl.) Backer *H. morsus-ranae* L. and *H. chevalieri* (De Wideman) Dandy (Cook & Lüönd, 1982). The morphological features of the genus are such as leaf and bud formation, floral development, the structures of vegetative organs and cortical ontogeny in roots described by Cutter, (1964); Cook & Lüönd, (1982); Tomlinson, (1982); Scribailo & Posluszny, (1984, 1985a, 1985b); Sun *et al*. (1997); Sun & Chen, (1998); Seago *et al*. (1999) and Ru, (2013), but the anatomy of the reproductive organs and seedling of *H. dubia*, the is only one species found in China, remains unknown. The aims of the study are to study the structure of fruit, seed and seedling of *H. dubia*, and provide the morphological evidence for the placement of Hydrocharitaceae indicated by APG (2009).

Materials and methods

The plants of *Hydrocharis dubia* were collected in Hulin of Heilongjiang province and the voucher specimen are kept in the Herbarium of Harbin Normal University (HANU).

Morphological study: The pericarps of the *Hydrocharis dubia* fruits (brown in color) opened irregularly when the living fruits (green in color) were stored in room temperature (20-25^{o}C) about 2-3 months. The mature seeds were collected and placed in the petri dish for germination and the seedling development was observed using Olympus SZX16 stereomicroscope and photographed with Olympus DP26 digital camera and Sony DSC-F717 camera. The testa and the leaf epidermis were peeled off and placed on the slides with 1-2 drops of 50% glycerin. The trichome and stoma were observed with Olympus BX51 light microscope and photographed with Olympus DP70 digital camera.

Aantomical study: The fruit, seed and seedling in different developmental stages were fixed in FAA (70% ethanol) for a minimum of 24 h, dehydrated through a graduated ethanol series and then treated according to Feder & O'Brien (1968) for embedding in glycol methacrylate (GMA). However, a minimum of 24 h was used for the first two infiltrations in GMA and a minimum of 5 days for the third infiltrations. The capsules containing the materials and GMA were placed in an electro thermostat at 60^{o}C for 24 h. Sections of the fruit, seed and seedling, about 2 µm thick, were cut using Leica Ultralcut R. In accordance with the Feder & O'Brien (1968) method, sections were stained with the Schiff-toluidine blue and mounted with Neutral Balsam. Permanent sections were examined with an Olympus BX51 light microscope and photographed (Olympus DP70).

Results

The morphological features of *Hydrocharis dubia* fruits, seeds and seedlings were studied. Their external characteristics are shown in Fig. 1 and anatomical structures could be observed in Figs. 2, 3.

Fruit and seed structures: The fruit of *Hydrocharis dubia* is nearly globose (Fig. 1A), which has six carpels with joined ventral sutures extending almost to ovary cavity subdivided into six lobes, which are filled with massive gelatinous substance (Fig. 2A1). The pericarp is mainly composed of the parenchyma cells. In the young

(green) fruit the exocarp is one layer of rectangular cells (Fig. 2A1). The mesocarp is composed of 11-13 layers of circular cells and some of them are with tannins (Fig. 2A1). Many small vascular bundles (about 110) are scattered in the mesocarp (Fig. 2A2). The endocarp cells are nearly rectangular (Fig. 2A1). Many bitegmic ovules occur on the inner wall of the ovary which has the superficial placentation (Fig. 2A1). Each integument is composed of two layers of cells (Fig. 2B). The exocarp cells of mature fruit are flattened, the well-developed aerenchyma tissue is present in the mesocarp, and the endocarp cells are nearly circular (Fig. 2A3). The seeds enclosed by the gelatinous mass are elliptic, about 1.5-2 mm in length and 0.9-1.1 mm in width (Fig. 1B1).

The testa consists of 3 layers of cells, the tangential walls of the outermost layer of cells are thin and the inner tangential wall and a part of anticlinal walls are lignified and thickened, which extend outward to form the cauliferous anchor-like trichomes with branches at the top (Fig. 1D, 2C1). The tangential walls of the other two layers of testa cells have columnar projections (Fig. 2C2). The seeds enclosed by gelatinous mass are suspended on the water with the fruit opening irregularly. The gelatinous mass are dissolved in about 2-5 days and seeds fall into the bottom of the water and the outer tangential wall of the outermost layer of testa cells are damaged and disappear (Fig. 2C2). The testa splits at the micropylar end (Fig. 1B2) and the egg-shaped embryo floats on the water (Fig. 1C1), and its parenchyma filled with starch grain has aerenchyma (Fig. 3A). The seed embryo contains a young leaf and plumule enveloped by a cotyledonary sheath (Fig. 3A). The radicle end is covered with several rectangular cells, which differ from the rest epidermal cells of the embryo (Fig. 3A).

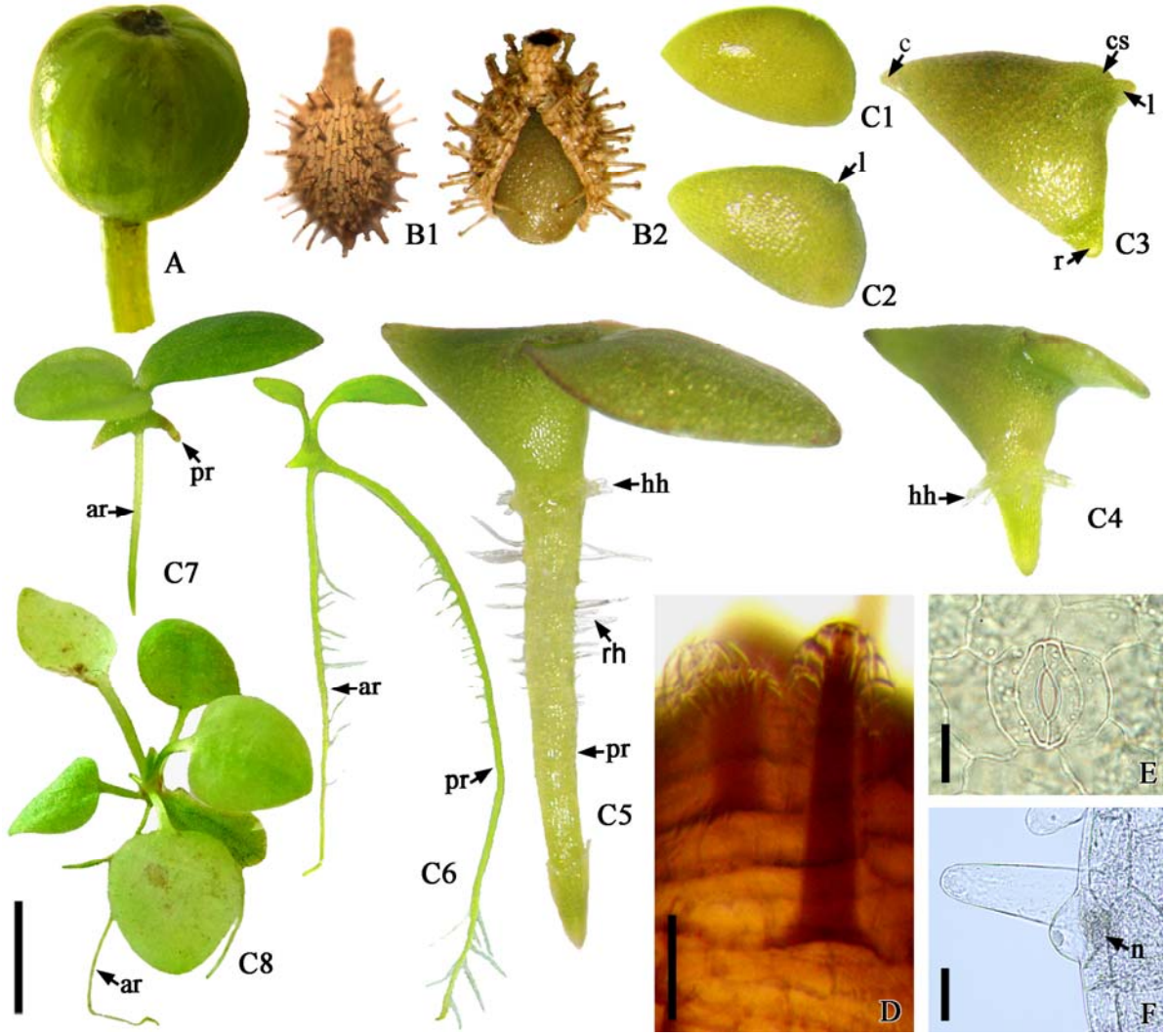

Fig. 1. The fruit, seed and seedling of *Hydrocharis dubia*. (A) Fruit shape. (B1, 2) Seed shape and seed coat. (C1) The egg-shaped embryo. (C2, 3) The triangle embryo with the cotyledon, leaf and radicle. (C4) Leaf and primary root with hypocotyl hairs. (C5) The elongated primary root with root hairs. (C6) The adventitious root. (C7) The weakly developed primary root. (C8) The seedling. (D) The trichome on the seed. (E) Paracytic stomata. (F) The hypocotyl hairs. Abbreviations: ar = adventitious root; c = cotyledon; cs = cotyledon sheath; hh = hypocotyl hair; l = leaf; n =nucleus; pr = primary root; r = radicle; rh = root hair. Scale bars = 5 mm in A, C6-8; 1 mm in B1-C5; 25 µm in D; 10 µm in E; 50 µm in F.

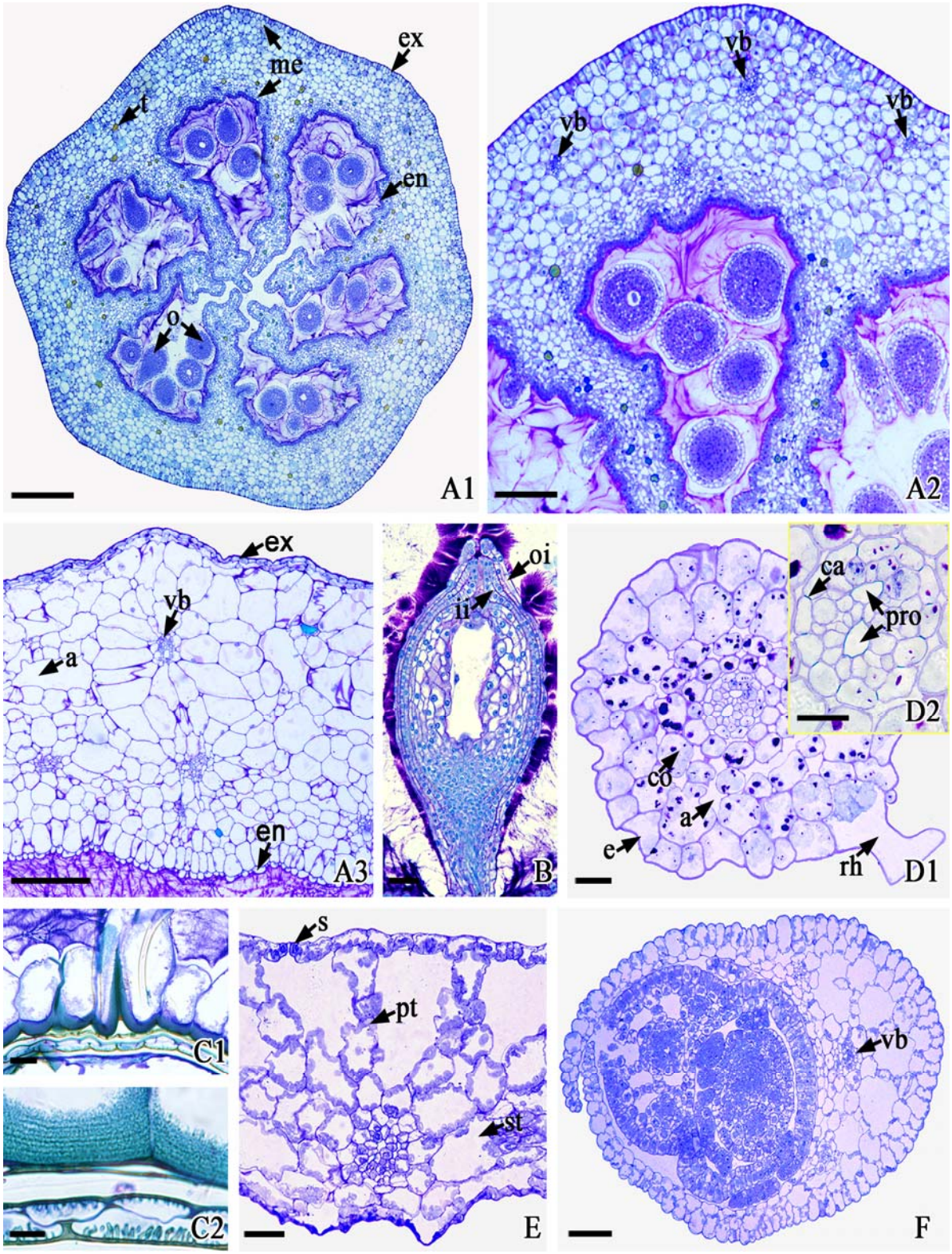

Fig. 2. The transvers sections of fruit, seed, primary root and leaf of *Hydrocharis dubia*. (A1) The young fruit. (A2) Vascular bundle in the mesocarp. (A3) The mature fruit. (B) Ovule. (C1, 2) Seed coat. (D1) Primary root with root hair. (D2) The casparian strip and protoxylem. (E) Leaf. (F) Petiole base. Abbreviations: a = aerenchyma; ca = casparian strip; co = cortex; e = epidermis: en = endocarp: ex = exocarp: ii = inner integument: me = mesocarp; o = ovule; oi = outer integument; pt = palisade tissue; pro = protoxylem; rh = root hair; s = stoma; st = spongy tissue; vb = vascular bundle. Scale bars = 500 μm in A1; 250 μm in A2, 3; 100 μm in B; 10 μm in C1, 2, D2; 20 μm in D1, E; 50 μm in F.

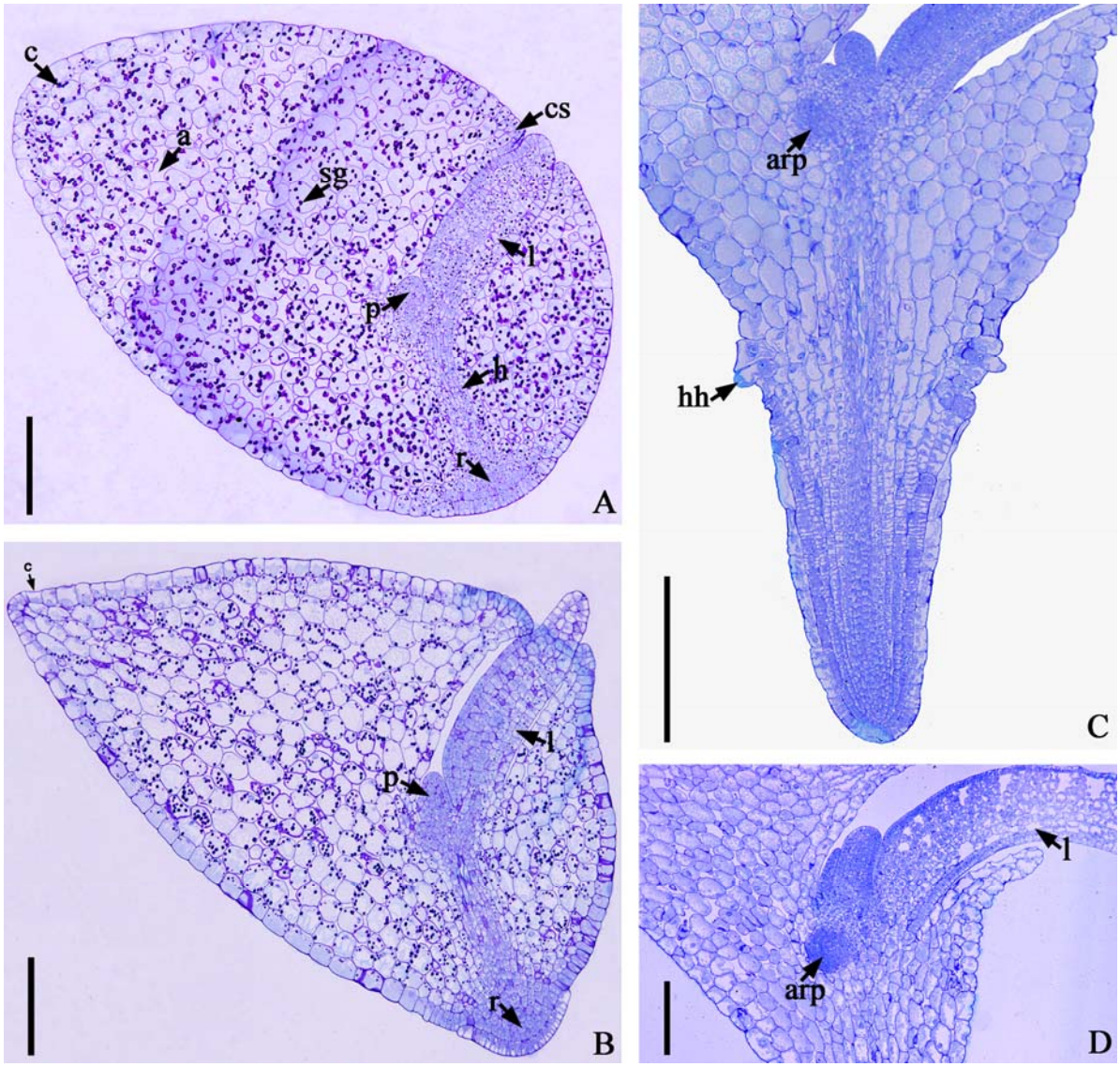

Fig. 3. The longitudinal sections of embryo and seedling of *Hydrocharis dubia*. (A) Egg-shaped embryo. (B) Triangle embryo. (C) Hypocotyl hairs, primary root and adventitious root primordium. (D) The adventitious root primordium and leaf. Abbreviations: a = aerenchyma; arp = adventitious root primordium; c = cotyledon; cs = cotyledonary sheath; h = hypocotyl; hh = hypocotyl hair; l = leaf; p = plumule; r = radicle; sg = starch grain. Scale bars = 100 µm in A, B, D; 500µm in C.

Seedling development and structure: The first leaf emerged from cotyledonary sheath develops parallel to the water surface as the seed embryo floats on the water for 1-2 days (Fig. 1C2). The rectangular cells on the tip of radicle form the paraboloid shell with the seedling formation (Fig. 1C3, 3B). The epidermal cells of the hypocotyl close to the root cap (developed from the paraboloid shell) develop into hairs (the hypocotyl hairs) in 2-5 days (Fig. 1C4) and their nuclei are located at the base of hairs (Fig. 1F, 3C). The primordium of the adventitious root is formed at the cotyledonary node (Fig. 3C, D) with the radicle developing into the primary root (Fig. 1C4, C5, 3C). The epidermal cells of the primary root are nearly square, and some cells develop into root hairs (Fig. 1C5, 2D1). The cortex composed of parenchyma cells is with large intercellular space and the casparian strips are visible in endodermis (Fig. 2D2). The vascular tissue is weakly developed and has only two protoxylem cavities (Fig. 2D2). The primary root stops growing when extending about 5 cm. The second leaf appears in 9-10 days, the adventitious root is developed from the cotyledonary node (Fig. 1C6). Whenever the new leaf is formed the adventitious root emerges from the node and the seedlings are usually with several leaves and adventitious roots (Fig. 1C8). Some seedlings may have no primary root developed (Fig. 1C7). The small leaves of the seeding, which are 6-8 mm in length, 3.5-5 mm in width, are cordiform and with short petioles (Fig. 1C8). The mesophyll of the blade is with palisade (1-3 layers of cell) and spongy tissues (2-3 layers of cell) and the air canals occur in both (Fig. 2E). The stoma is paracytic (Fig. 1E) and the guard cells are with cutinized horny

protrusions (Fig. 2E). The base of the petiole extends to forms a membranous sheath and three small vascular bundles close to the ventral side are observed (Fig. 2F). The seedling usually develops slowly when developing 5 or 6 leaves and die and only a few could develop into the mature plants.

Discussion

Reproductive organ: The *Hydrocharis morsus-ranae* has syncarpy (Scribailo & Posluszny, 1985b) similar to *H. dubia*. Li (2005) considers that the apocarpy is the primitive character of angiosperm, the apocarpy in Alismatales is the ancestral characters and syncarpy of Hydrocharitaceae may be originated from apocarpy. The gynoecium of *Butomus umbellatus* (Butomaceae) has 6 carpels which are connected only at the base. The ovary of it is with a large number of ovules are scattered on the inner wall of the ovary, and the superficial placentation (Singh, 1966a, 1966b; Singh & Sattler, 1974). *Ottelia alismoides* (Hydrocharitaceae) has laminar placentation (Ru *et al.*, 2013), and Butomaceae is with laminar or superficial placentation (Singh, 1966b; Dahlgren *et al*., 1985; Chen, 2012). Therefore, Hydrocharitaceae and Butomaceae have similar type of placentation (Fig. 2A1).

Hydrocharitaceae (*Vallisneria*, *Ottelia*, *Hydrocharis* and *Stratiotes*) fruits are opened irregularly probably due to the pressure of gelatinous mass (Sculthorpe, 1967; Kaul, 1978). Li *et al.* (2010) described that in Hydrocharitaceae the seeds enclosed by gelatinous mass are easy to form clustering deposition, which contribute to improve the germination rate and are adapted to the aquatic environment. The mature seeds of Alismataceae (*Sagittaria Sagittifolia*), Butomaceae and other taxa of Hydrocharitaceae (*Ottelia alismoides*, *Hydrilla verticillata*) have no endosperm (Cronquist, 1981; Shi & Xu, 1988; Ru, 2013; Ru *et al*., 2013), which is the same as that of *Hydrocharis dubia* observed. The seed without endosperm is considered to be one of the ancient and primitive characteristics in Monocotyledonous (Shi & Xu, 1988). Sun *et al*. (1997) described the tuberculate seed coat of *Hydrocharis dubia*. The authors consider these tuberculae are the structure of the trichomes at early stage. Shaffer-Fehre (1991a, 1991b) described the periclinal wall of endotegmen with tuberculae of *Najas* is similar to that of Hydrocharitaceae and placed *Najas* into Hydrocharitaceae. The pollen grains of Hydrocharitaceae have thick exine with well-developed tectum covered with spiny and weakly-developed columellae, which are the same as those of Butomaceae and Alismataceae (Sun *et al*., 2002).

Seedling: Our study showed the embryo in *Hydrocharis dubia* floating on the water develops the hypocotyl hairs before the primary root formation. Sculthorpe (1967) describes that it could help species spread in aquatic environment for a long distance. The hypocotyl hairs of *Hydrilla verticillata* and *Ottelia alismoides* appear before the formation of the primary root, which could anchor the seedlings into the substrate and make the seedlings erect (Kaul, 1978; Liu, 2007; Ru *et al.*, 2013). The seedling developments of Alimataceae (*Sagittaria Sagittifolia*, *Alisma orientale*), Butomaceae (*Butomus umbellatus*) and Najadaceae (*Najas marina*) at early stage are similar (Shi & Xu, 1988; Liu, 2002; Chen, 2012; Ye, 2002), which is also similar to that of *Hydrocharis dubia*. Shi & Xu (1988) described that the adventitious root formed early and grew rapidly could adapt to the degeneration of the primary root.

The taxa of Alismataceae, Butomaceae and Potamogetonaceae have paracytic stomata (Tomlinson, 1982, Shi & Xu, 1988), which also occur in *Hydrocharis dubia*. The Najadaceae has no stomata. However the stomata in Juncaginaceae and Scheuchzeriaceae are tetracytic (Cronquist, 1981). Matias (2007) indicated the vascular bundles in the petioles of *Echinodorus* (Alismataceae) are characteristically arranged in arc similar to their placement in Hydrocharitaceae. The main difference between the Hydrocharitaceae as a whole and the other two families is the absence of laticifer from the Hydrocharitaceae and their presence in most members of Alismataceae and Butomaceae (Stant, 1964, 1967; Ancibor, 1979).

Conclusions

The characteristics of *Hydrocharis dubia* such as connate carpel, superficial placentation, testa with thrichomes, vascular arrangement in petiole, seeds without endosperm, the hypocotyl hair appearing before the primary root, the primary root weakly-developed, and paracytic stomata, are similar with the taxa of Alismataceae and Butomaceae. This study provides the morphological evidence and strongly support the placement of Hydrocharitaceae in the order Alismatales.

Acknowledgements

We gratefully acknowledge support from the Chinese National Science Foundation (31070169 and 31270235), the Science and Technique Innovation Foundation of Heilongjiang Province and Harbin Normal University (KJTD2011-2).

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(Received for publication 20 July, 2014)