The morphological and anatomical studies of inflorescence, flower, embryo and fruit development in *Maclura pomifera* (Moraceae)

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**Abstract:** *Maclura pomifera*, a dioecious tree species, is recognized by its orange-like fruits and has a fascinating history and varied economic uses. The morphology and anatomy of female inflorescence, flower, embryo, fruit and seed development were surveyed by cyto-histological methods. The flowers and fruits were collected at different developmental stages, fixed in FAA fixative, embedded in the paraffin, sectioned using microtome, stained by hematoxylin and eosin and were photographed using light microscopy. There are 60–70 female flowers in each hairy inflorescence. Each young flower has pinkish and tetrameric perianth which surrounds the unicarpellate pistil. The perianth becomes fleshy and green during development and surrounds the ovary forming a drupe-like fruit with a permanent long style. The fleshy perianths and the drupes inside them produce a false multiple (accessory fruit or pseudocarp) and pomaceous fruit in which a few filled seeds are formed. Ovule primordium appears in an apical placation and its growth direction changes during development. The ovule is ana-campylotropous.

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**PUBLIC INTEREST STATEMENT**

*Maclura pomifera* is a dioecious tree from the Moraceae family. *Maclura pomifera* is recognized by its orange fruits and has a variety of economic and medicinal uses. Studies have shown that *Maclura pomifera* is used to treat many diseases, such as toothache and cancer. It was shown that fruit extract with two prenylated isoflavones (scandenone and auriculasin) isolated from it possess antinociceptive activity and anti-inflammatory activity on carrageenan-induced hind paw edema. So fruit is an important part of the *M. pomifera* tree. Study of the flower development until it reaches to mature fruit stage is necessary to understanding the valuable details of fruit growth. In this study, morphology and anatomy of female inflorescences, flowers, embryos, fruits and seeds were evaluated by cytohistological method. There are 60–70 female flowers in each hairy inflorescence. Each young flower has four pinkish peripheral perianths surrounding the unicarpellate pistil. The perianth becomes fleshy and green during development and surrounds the ovary forming a drupe-like fruit with a permanent long style.
suspended, bitegmic and tenuinucellate. The embryo passes globular, heart-shaped and cotyledonal steps. Finally, the curved embryo is formed whose cotyledons constitute massive volume of ovule (seed) and endosperm is eventually degenerated.

Suspensor is short and multi-celled. Ovule integument makes the membranous seed coat. The middle part of perianth contains secretory cells that is detached during development forming a soft spongy tissue. This study shows the characteristics of inflorescence and fruit architecture during development that can also clarify phylogenetic relationships among genera and in the family.

Subjects: Agriculture & Environmental Sciences; Soil Sciences; Food Chemistry

Keywords: endosperm; suspensor; stigma; curved embryo; apical placentation; perianth

1. Introduction

The monography of the Moraceae family is one of the most exciting chapters in angiosperms taxonomy. Corner (1962) suggested that the floral complexity of Moraceae has hindered the formulation of a well-supported hypothesis of morphological evolution in the family (Corner, 1962). Moraceae exhibits an amazing diversity of morphological and life-history traits, particularly inflorescence architectures, breeding systems and pollination syndromes (Clement & Weiblen, 2009; Zerega, Ragone, & Motley, 2004). The structural studies on mulberry family (Moraceae) allow addressing a variety of interesting questions about evolution, classification and biogeography. This family is composed of 39 genera and approximately 1100 species and has a global distribution, but its greatest diversity lies in the tropics. Moraceae has been divided into five tribes: Moreae, Artocarpeae, Dorstenieae, Castilleae and Ficeae. Osage orange (Maclura pomifera (Raf.) Schneid), is a unique tree found in deserted areas and unexpected places and is a stress-tolerant tree almost unknown and unused in communities (Berg, 2001; Cullina, 2002; Smith & Perino, 1981). It is native to the North America and of interest from fruit to root. Its fruit resembles a large orange that contains a sticky white latex sap. Various parts of the Maclura species are used in folkloric medicine worldwide (Kupeli, Orhan, Toker, & Yesilada, 2006). Compounds from several species have been investigated for medicinal purposes as treatments for conditions ranging from toothaches to cancer and (Gardner, Sarraf, Williams, & Zerega, 2017). Its wood is extremely dense, strong and reportedly the most decay-resistant wood in North America (Barnett & Burton, 1997). Its decay-resistant wood is still used for fence posts today, and recently, an oil produced from the seeds has become popular in the cosmetic industry (Gardner et al., 2017). It was shown that fruit extract with two prenylated isoflavones (scandenone and auriculasin) isolated from it possess antinociceptive activity and anti-inflammatory activity on carrageenan-induced hind paw edema (Kupeli et al., 2006). Various phytochemical studies carried out on Maclura pomifera showing that the plant contains lectins, triterpenes, xanthones and flavone-type compounds. Strong antioxidant capacity of Maclura pomifera has been attributed to flavonoid type components in particular isoflavones, osajin and pomiferin (Kupeli et al., 2006).

According to Clement and Weiblen (2009), Maclura is transferred from Moreae to Maclureae. The species in Moraceae family are dioecious or monocious. Flowers arrange in either unisexual or bisexual inflorescences, and the structure of unisexual staminate and pistillate inflorescences within a species can vary. Characters that are present in both staminate and pistillate inflorescences, such as perianth merosity and perianth connection, were coded independently for each sex (Specht & Bartlett, 2009). The Moreae species are characterized by simple inflorescences, such as racemes or spikes and flower parts tetramerous. Many species have inflexed stamens, which are structurally supported by pistillodes (Berg, 2001; Clement & Weiblen, 2009). In Maclura tinctoria, each flower presents 6–8 green tepals and a pistil with unicellular ovary and long style/stigma. The ovary has only one ovule with apical placentation (Oyama & Souza, 2011). Flower structure of M. pomifera has been studied morphologically but there is no anatomical and developmental survey. Flowers are dioecious and show characteristics of true unisexuality by lacking vestiges of
the pistil in male flowers and androecium in female flowers at all stages in the development. The pistillate flowers are located axillary to the leaves in dense and globular clusters. These flowers have a 4-lobed calyx that encloses the ovoid, upper and compressed ovary with filiform and long styles (Bonner & Karrfalt, 2008; Stephens, 1969). Inflexed stamens are associated with explosive release of pollen and presumed wind pollination (Gardner et al., 2017). Dioecy was found to be the ancestral condition of the family, and repeated shifts to monoecy suggested that the shift from unisexual to bisexual inflorescences occurred more than once. (Monoecious species can have bisexual inflorescences or unisexual inflorescences of both kinds on the same individual) (Clement & Weiblen, 2009). Further, there were no rudimentary gyroecia found in the male flowers or rudimentary androecia in the female flowers of Maclura pomifera at any stage of floral development (Maier, Chapman, & Smith, 1997).

There are some diversities in embryo shape of Moracea, the straight embryo of M. tinctoria diverges from the curved embryo found in other species of Moraceae. Moraceae family presents different types of fruits as pseudodrupe, an anthocarpous fruit with an undifferentiated indurate pericarp surrounded by a fleshy or coriaceous exocarp (Spjut, 1994). A study on fruit development in M. tinctoria and M. brasiliensis shows that the fruitlets are drupes consisting of epicarp and mesocarp. The ovule is ana-campylotropous, suspended, bitegmic and crassinucellate (Barroso, Morim, Peixoto, & Ichaso, 1999; Kaasra, 1972; Oyama & Souza, 2011). Our studies in literature showed that there is not any published study on different developmental stages of flower, fruit and embryo in M. pomifera. Thus, in this research, these cited processes were studied by cyto- and histological methods.

2. Materials and methods
The flowers and fruits of M. pomifera were collected at different developmental stages from Kerman city (30° 17’ 35.999’ and 57° 5’ 12.851’), Kerman province, Iran. For morphological studies, the specimens were freshly investigated by Stereo Microscope (Olympus Tl2) or naked eye and photographed (canon IXY digital camera). In addition, the buds, flowers and fruits at different stages of development were fixed in FAA70 fixative (formalin/acetic acid/alcohol70%, 5/5/90 ml), rinsed with distilled water, dehydrated in alcohol series 30%, 50% and 70% respectively, embedded in paraffin, sectioned by rotary microtome at 3–4 μm thickness and placed on glass slides. After clarification and staining by hematoxylin and eosin, the sections were investigated by light microscope (Olympus Model BH-2) and photographed by canon digital camera (IXY model) (Xue & Li, 2005).

3. Results
3.1. Morphological structure and development of inflorescence, flower and fruit
Small trees of M. pomifera have a height of more than 5 m in the area of sampling (Figure 1(a)), though, in some sites, trees are may grow as tall as 21 m (Burton, 1990). The species is dioecious and 60–70 female flowers are formed in each hairy inflorescence (Figure 1(b–g)). There are membranous scales or bracts containing trichomes at the base of the inflorescence peduncle which are dried during flower development and then shed (Figure 1(b)). Each young flower has four pinkish peripheral perianths surrounding the unicarpellate pistil. The pistil has a unilocular ovary, a style and a stigma. The style and the stigma are cylindrical in shape and indistinguishable from each other. During development, the styles become long, filamentous and thread-like, covering the inflorescence surface as a white felt-like coating. They become upright and needle-like gradually and finally are dried after pollination but their basal remnants persist and even is seen around mature seeds (Figure 1(c.g–i)). During maturity, the perianth of each flower becomes fleshy and green surrounding ovary. The ovary develops into a simple drupe-like fruit with a permanent long style. The fleshy perianths and fruits inside them (simple drupe-like fruits) produce a multiple (compound) or accessory fruit (pome-like false fruit) in which, a few filled seeds (about 18–25) are formed (Figure 1(h)).
3.2. The structure and development of inflorescence, flower and ovule

The longitudinal and transverse sections of inflorescence show that 60–70 female flowers with very short peduncles (without pedicle) are placed on a grown receptacle in each hairy inflorescence. Therefore, dense clusters of sessile flowers are grouped in globular inflorescence described as dense head (capitulum) (Figure 2(a,b)).

In the transverse view, secretory structures are seen in the center of the receptacle. In each growing flower on the inflorescence receptacle, tetrameric perianth and style are growing (Figures 2(a,b) and 3(a,b)). The cylindrical outgrowth is observed at the end of the ovary, but no obvious distinction is seen between stigma and style as separate structures (Figure 3(b,e)). In the upper part of the unilocular ovary, the ovule primordium appears on ovary wall indicating apical placentation (Figure 3(b–e)). During downward growth of ovule, two-layered integument differentiates forming micropylar pore towards flower base (Figure 3(c–e)). In the continuation of growth, the growth direction of ovule is changed and the growth of right integument is increased. During development and at the end of...
growth, ovule changes as ana-campylotropous, so that micropyle is placed towards ovule funicle and style (Figure 3(f–h)). As it can be seen in the figures, the integuments constitute the major volume of ovule and as a result, ovule has a small and thin nucellus (tenuinucellate) (Figure 3(c–h)). In this stage, the growth of perianth and their secretory and alveolar structures is observed (Figure 3(g,h)). In the center of nucellus, megaspore mother cell is differentiated and functional megaspore is formed by meiosis (Figure 3(c–e)).

Functional megaspore produces embryonic sac by division and differentiation, expanding in a pear-shaped way (Figure 4(a,b)). After fertilization, the embryo begins to grow and differentiate and passes the globular, heart-shaped and cotyledonary stages (Figure 4(c–e)). At the basal part of the embryo, there is a short multicellular suspensor (Figure 4(e)).

As the embryo and ovule tissues continue to grow and develop, cotyledonary embryo and then curved embryo (Bent cotyledon stage embryo) is formed at the end of the embryonic growth (Figure 5(a–d)). Cotyledons form the major volume of growing ovule that is differentiating into a seed at this point. During seed development, endosperm is consumed by the growing embryo especially its cotyledons and hence, a thin layer of endosperm remnants is seen around embryo (Figure 5(a–g)). During embryo development, embryonic structures including embryonic axis, shoot apical meristem (SAM), root apical meristem (RAM) and cotyledons are formed (Figure 5(a–g)). During development, ovary tissues are differentiated into fruit pericarp with fleshy and thin exo-mesocarpe and stony and lignified endocarp showing drupe fruit (Figure 5(g)).

Similarly, morphological study of fruit and seeds with stereo microscope shows that the embryo is curved and massive endosperm is reducing, the exo-mesocarp is thin and fleshy and endocarp is stony and lignified. Also, the separation of pericarp from seed coat (brown membranous structure) is clear (Figure 6(a–d)). Therefore, it is concluded from the anatomical and morphological studies that the true fruit is a drupe or drupelet.
At the final stage of development, perianth is enlarged and connected to pericarp forming a part of fruit pericarp (true exo-mesocarp is thin) that is a drupe-like fruit. From the superficial and central part of each drupe-like fruit, permanent style extends outwards (Figure 7(a)). As seen in this figure, the upper (outer) part of each perianth, forming the surface of fruit, has more growth, as a result, it seems prominent and globular (Figure 7(b,c)). Anatomical structure of pome like and false fruit (whole fruit) shows that in middle part of each perianth, the cells are larger and detached from each other and in result, in the final stages of maturity, perianth is seen as a soft spongy tissue (Figure 7(b–e)). In the transverse section of a mature flower, developed perianth with central secretory cells is seen. True simple fruit (drupelet) is also seen in the center of mature flower that its perianth is surrounding these simple fruits (Figure 7(f)).

4. Discussion

*M. pomifera* as an imported species is distributed in some parts of Iran, including several areas in Kerman province. It is a dioecious species which pistillate inflorescences are arranged in condensed heads. Due to significant growth of style and stigma which are indistinguishable from each
other, the surface of young inflorescences has a felt-like appearance. The species has considerable features such as very long style and stigma, one row and tetrameric perianth (perigon), the unicarpellate pistil with one ovule, superior ovary, apical placentation, and then 90 degrees rotation and formation of an ana-campylotropous ovule. According to Berg (2001), Moraceae family is categorized into five tribes including Moreae, Artocarpeae, Dorstenieae, Castilleae and Ficeae. The Moreae tribe is characterized with simple inflorescences like raceme or spike and tetrameric flower components. This tribe is widely distributed in temperate regions and some
species such as *Morus alba* and *M. pomifera* with having characteristics such as spike inflorescences to cymose (glomerules) ones, numerous interfloral bracts and complete or partial continuity of testa, show a different architecture from the rest of Moraceae. Dorstenieae also has variable features, although most of its genera have bisexual flowers. Castilleae is determined by bisexual inflorescences with collar, disc-shaped to cup-shaped receptacle, and variable habitat characteristics. The genus *Ficus* is the only genus of Ficeae tribe and the largest taxon in Moraceae family with over 800 species and global distribution. This genus is characterized by having synconium, which is an enclosed inflorescence, in which the inner flowers are organized only for certain pollinators and parasites. This genus has different vegetative forms such as shrubs and trees to hemiepiphytes and stranglers. Its habitats and phylogenic features are also different. According to new classifications, some previous genera of Ficeae are found in other tribes. Some researchers presented other classifications and classified this family into six and even seven tribes with different phylogenetic and morphological properties. These researchers introduced characteristics such as bisexual flowers, unisexual flowers with residues of the other sex in the form of staminode or pistilode, completely unisexual flowers, thick cotyledons, the presence or absence of perianth and different types of inflorescences in different taxa (Clement & Weiblen, 2009; Oyama & Souza, 2011). *M. pomifera* has some of the above-mentioned characteristics, such as thick cotyledons, dense glomerule inflorescences and completely unisexual female flowers. Because of some properties such as the tetrameric flower components and distribution in temperate regions, the genus *Maclura* is located in the Moreae tribe, as Berg (2001) reported. However, further studies on the inflorescence, pistil and fruit structure showed different characteristics. For this reason, based on the characteristics of inflorescence and fruit, Clement and Weiblen (2009) separated the genus *Maclura* and several other genera from the Moreae tribe. These researchers placed this genus in a monotypic tribe called Maclureae due to certain genetic and morphological features, such as yellow glands in inflorescences, glomerule pistilate inflorescence, tetrameric flower components, porous vessels, right and curved stamens and also distribution in temperate regions. They also mentioned that female flowers typically have two stigmas of varying size, and fruits are often fleshy. Maclureae includes climbers, trees, or shrubs armed with axillary thorns and mostly fleshy fruits. All species are dioecious that staminate inflorescences vary from spicate or racemose to globose-capitate, and have both inflexed and straight stamens. Pistillate inflorescences are globose typically with two stigmas that are often unequal in length (Clement & Weiblen, 2009).

In this study, the perianth is tetrameric and the pistil is unicarpellate and unilocular. In addition, a single stigma was seen and no difference was observed between stigma and style. Sytsma et al. (2002) mentioned that features such as latex, anatropous ovules, apical placement and fleshy compound fruits are common in the berries family (Sytsma et al., 2002). Although some researchers have noted that some species have characteristics different from the above-mentioned features, but general characteristics are confirmed in *M. pomifera*. Compound pomaceous fruits are included fruitlets surrounded by fleshy perianth tissues. These fruitlets with fleshy perianth are introduced as drupe (Clement & Weiblen, 2009; Gardner et al., 2017; Oyama & Souza, 2011; Spjut, 1994). However, some others introduced the fruitlets of this genus as nutlet (Barroso et al., 1999) or achene (Judd, Campbell, Kellogg, Stevens, & Donoghue, 2002). A study on the fruit development of *M. tinctoria* showed that the fruitlets are drupes with fleshy pericarp, mesocarp and a stone part (pyrene) surrounding the single seed. Pyrene develops from an internal epidermis of the ovary, which differentiates into macrosclereids and may be multi-layered (Oyama & Souza, 2011). Jacomassi, Moscheta, and Machado (2010) introduced pyrene in *Brosimum gaudichaudii* (Moraceae) without defining the type of fruit. However, pyrene was not seen in *Soroceae bonplandii* (Moraceae), whose fruit is pomaceous. Studies of Oyama and Souza (2011) in *M. tinctoria* showed that the seeds had an unorganized coating and hence the embryo was protected by pyrene. Studies in *M. pomifera* on the development of perianth, real fruit and seed coat showed that pericarp differentiates to real fruitlet or drupelet with thin and fleshy exo-mesocarp and stony endocarp. The perianth becomes grown, fleshy and secretory during development and surrounds drupelet making it thicker. However, although they are connected together apparently but perianth is detached of true (real) fruitlet. Further, according to the stability of style in pericarp apex, which remains until final maturation and even after falling fruit and study of
pericarp structure in this stage, it is also recognizable that fruitlet pericarp is differentiated to drupelet. The fruitlets (drupelets) together form a compound, pomaceous and false fruit. Synorganization that is an evolutionary feature and is seen in flower components (Endress, 2015) is also observed in M. pomifera., synorganization of the growing flowers (florets) in inflorescence and formation of compound fruit as well as the connection of the perianth components together and perianth to pericarp during maturation is resulted in more protection of flowers and fruits, more evolution of the plant in attracting pollinators as well as transfer of fruits and seeds. The study of embryonic stages showed that similar to Polygonum type, the embryonic stages including globular, heart-shaped, cotyledonary and curved embryo is observed in M. pomifera. Similarly, embryological studies in the Moraceae family showed that mature embryos are curved in some species such as Soroecea bonplandii, Broussonetiapa pyifera, Cudrania tricuspidata and M. pomifera (Oyama & Souza, 2011; Souza & da Rosa, 2005). On the contrary, embryological studies in M. tinctoria showed that the embryo is straight, which is considered to be advanced compared to the curved form. It seems that more detailed studies should be done on this species. The cotyledons grow up during embryo development and form massive volume of seed; as a result, the amount of endosperm decreases and is limited to peripheral areas. Hickey and King (1988) reported that species of Moraceae family may or may not have endosperm (Hickey & King, 1988). However, Oyama and Souza (2011) reported that in M. tinctoria, endosperm was parenchymal and oily and did not mention its reduction. The number of mature seeds in compound fruits is low and there are only about 18–25 seeds in each compound fruit with about 60–70 carpels. Due to the lack of male trees in the vicinity of female trees, it seems that the lack of pollination is the cause of seed reduction. It is also probable that the parthenocarpy causes the formation of these limited seeds that should be studied more detailed. There are varieties of common fig that normally produce an economic summer product that does not require pollen, although also pollination exists. Pollination leads to larger fruit size, greener skin and darker color inside the compound fruit and the process of ripening slows down compared to fruits without pollination (Flaishman, Rodov, & Stover, 2008; Rosianskey et al., 2016).

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