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ANATOMY OF SEED DEVELOPMENT OF AMPHICARPAEA EDGEWORTHII BENTH

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ABSTRACT

Amphicarpaea edgeworthii Benth. is a wild legume species, with important value in the soybean breeding and research of plant developmental biology. The seed development of Amphicarpaea edgeworthii Benth. was observed using paraffin sectioning method by Nikon TE2000 light microscope. The ovule got fertilized and formed the zygote after flowering about 12 hours. The zygote was transversely divided into a terminal cell and a basal cell in two days after flowering. The proembryo continued cell division in directions forming globular embryo and then heart-shaped embryo, in which the cotyledons primordium began to occur. In 24 days after flowering, the embryo differentiated into matured embryo through Torpedo embryo (in 16 days after flowering). The endosperm cell formation began when the globular proembryo was formed, and the development of the endosperm was nuclear type. The mature seed of Amphicarpaea edgeworthii Benth. is devoid of endosperm. The outer integument developed into the seed coat, and the seed coat cells were the fence cells with corneum, that may be one of the important reasons of hard seeds for Amphicarpaea edgeworthii Benth.

Key words: Amphicarpaea edgeworthii Benth, Anatomy, Development, Seed, Legume.

INTRODUCTION

Amphicarpaea edgeworthii Benth. belongs genus of subtribe Glycininae (Phaseoleae, Papilionoideae, Leguminosae) comprising approximately ten species that are distributed in east Asia, north America and southeastern Africa, among these three species that are widely distributed in China are A. edgeworthii Benth., A. rufescens and A. linearis chun et T.chen. They are mainly found in provinces of Hebei, Shandong, Shanxi, Jiangsu and Anhui etc. (Wei Yuzong, 1995). Amphicarpaea edgeworthii Benth. is a slender, twining annual herb, and often occurs in disturbed habitats, e.g., grasslands, fringe of forests and roadside. This species is distinct from other legume by having aerial and subterranean flower and beans in the same individual, thus it is quite worth studying from the point of plant developmental biology.

Moreover, the seeds of A. edgeworthii Benth. could serve as medicine for gynecological diseases, also the stems could serve as forage (Hebei Flora,1998). In addition, isoflavones whose functions include antioxidant, anti-tumor, anti-inflammatory and antibacterial was founded in the seeds of A. edgeworthii Benth.

There are some reports about important characters in the Amphicarpaea edgeworthii Benth. elliot, such as geographic variations in the flowering and ripening behaviors (Teruo Arase et al., 1998a); relations among the environment of home habitat, taxonomic groups and seed production (Teruo Arase et al., 1998b); relations between the climbing growth and seed production (Teruo Arase et al., 1999); seed germination (Qiao Yake et al., 2000, 2002; Li Guilan et al., 2004); scanning electron microscope observation on seed coat, peel, pollen, leaf epidermis and flower (Guo Xuemin et al., 2002, 2003a, 2003b); anatomy on vegetative organs (Guo Xuemin et al., 2006); amino acid content of seed (Jiang Minghua et al., 2007); the aerial and subterranean flower development (Yi Zhang et al., 2006); comparative embryology of aerial and subterranean flowers (Shan Jinggang et al., 2009); Developmental Speciality and Cultural Practices (Qiao Yake et al., 2003); but there are few studies on the seed development of the amphicarpic species. The aims

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of the present study were: to observe the anatomical structure of aerial seeds and seed coat of *Amphicarpaea edgeworthii* Benth. in the different developmental stages, in order to understand the process of seed formation and development, and to explore the cause of hard seed information, and to provide the theoretical basis for future studies and exploitation on the resources of *Amphicarpaea edgeworthii* Benth.

**MATERIALS AND METHODS**

**Plant materials:** We used aerial seeds of *Amphicarpaea edgeworthii* Benth., which were initially subterranean seeds collected in 2008 at shady side of Laoban Hill in Yaan, Sichuan province, and then planted at March, 2009 in Farm of Sichuan Agricultural University. The sampling was performed at flowering and pod stage.

**Paraffin sectioning and observation with microscope:** The study was carried out during October and November in 2009. In the full blooming stage of *Amphicarpaea edgeworthii* Benth., the flowers bloomed at the same time were marked with tags, then they were collected from living plants each four hours for the first day after pollination, and since the second day after pollination, sampling was collected once a day. Each sampling was carried out by taken ten flowers and immediately fixed in FAA (90 parts 50% ethanol, five parts formalin, and five parts acetic acid), stored in 70% ethanol, embedded in paraffin, and sectioned at 5μm with a microtome. Staining was performed by safranin-fast green method. For each seed developmental stages, several sections were studied under a Nikon TE2000 light microscope. For each stage, at least 20 flowers were studied and photomicrographs were made from the best ones.

**RESULTS AND DISCUSSION**

**Embryo development:** The ovule of *Amphicarpaea edgeworthii* Benth. we observed was campylotropous ovule (Fig 1-a), same with most legumes. Fertilized zygote was formed 12 hours after flower (Fig 1-b), and it was obviously enlarged at 24 hours after flower (Fig 1-c). Two days after flower, the zygote had the initial horizontal cell division, and then proembryo was formed with one apical cell and one basilar cell. Cell close to micropyle was basilar cell and the one away from micropyle was apical cell, the apical cell was larger than the basilar cell (Fig 1-d). Five days after flower, the embryo was enlarged to form globular embryo, and entered the early stage of globular embryo (Fig 1-e), such enlargement was continued when six days after flower (Fig 1-f), and the globular embryo became the largest till eight days after flower, where the suspensor was already formed (Fig 1-g). At 10 days after flower, globular embryo was still developing and the tissues differentiation commenced, the apical site of embryo gradually became flat and formed two symmetrical bulges which were cotyletons primordium at both side, and then it was becoming heart shaped. By then, the suspensor was completely degraded (Fig 1-h). 12 to 14 days after flower, two cotyletons primordium of the heart embryo were still rising higher, the embryo differentiation entered the early stage of torpedo embryo (Fig 1-i). 16 days after flower, the germ began to differentiate, the torpedo embryo completed, and it was more obvious of tissues differentiation (Fig 1-j). 16 to 20 days after flower, the two cotyletons bulge of torpedo embryo gradually developed and formed two symmetrical cotyletons, germ primordium created clear bulges (Fig 1-k). 24 days after flower, the whole embryo became even larger, two cotyletons filled embryo sac, the germ, hypocotyl and radicle were also developed, and formed a ripe embryo.

**Endosperm development:** Ever since the two cells proembryo stage, the primary endosperm nucleus of *Amphicarpaea edgeworthii* Benth. started to divide (Fig 1-d) and formed endosperm free nucleus which closely attached to a thin layer of protoplasm on the embryo sac wall. During the interim of globular embryo, endosperm nucleus increased, by then, the free nucleus was spherical, comparatively similar in size and evenly attached to the embryo sac wall. In the late stage of globular embryo, endosperm nucleus vigorously divided and formed numerous endosperm cells that filled the whole embryo sac (Fig 1-g). As to the heart embryo stage, endosperm cells began to degrade and such degradation mainly took place around the embryo (Fig 1-h). While in the torpedo embryo stage, endosperm cells far away from embryo were also degraded. When two cotyletons initially formed, could endosperm cells neither close to nor far from the embryo be found but still rare cells distributed on the embryo sac wall (Fig 1-k). Endosperm was totally absorbed by the embryo when seed matured.
Fig. 1: Development of embryo and endosperm of Amphicarpaea edgeworthii Benth. (a) unfertilized ovule ×20; (b) zygote ×20; (c) zygote ×20; (d) proembryo with two cells ×20; (e) the early phase of globular embryo ×20; (f) the middle phase of globular embryo ×20; (g) the later phase of globular embryo with embryonic ×20; (h) the heart-shaped embryo ×20; (i) the early phase of Tropedo-shaped embryo ×20; (j) the later phase of Tropedo-shaped embryo ×20; (k) the early phase of cotyledon formation ×20; (l) mature embryo ×10
(Fig 1-l), thus the ripe seeds of *Amphicarpaea edgeworthii* Benth. were nonendospermic.

**Seeds coat development:** Four days after flower of *Amphicarpaea edgeworthii* Benth., parenchyma cells generally created every layer of seed coat, they were loosely arranged and no distinct differentiation was observed (Fig 2-a). Eight days after flower, cells of outer layer were elongated vertically, the palisade gradually formed, however below the palisade were still loosely arranged parenchyma cells without clear differentiation (Fig 2-b). 14 days after flower, with development process, obvious differentiation was found on seed coat, epidermal palisade cells were more and more compactly arranged, also the cuticle were thickened (Fig 2-c). Meanwhile, inner integument was differentiated, and formed tapetum layer on inner epidermis of inner integument where cells were orderly arranged and radially elongated (Fig 2-d). 16 days after flower, the cells of tapetum on inner integument became less orderly arranged and started to peel off the inner epidermis (Fig 2-e), the cells were disassembling. Up to 24 days after flower, each structure layer was completely differentiated (Fig 2-f), outer integument developed into seed coat whose cells were palisade shaped and coated by cuticular layer, there was one apparent line under the palisade cells and it could be one possible reason for the ground hard seeds formation of *Amphicarpaea edgeworthii* Benth..

Seeds are essential for flowering plant reproduction because they protect, nourish, and contain the developing embryo that represents the next sporophytic generation. In addition, seeds contain energy resources that sustain the young sporophyte during germination before photosynthesis begins. In legumes, food reserves stored in embryonic cotyledons make seeds important as a food source for both human and animal consumption. Research on legume seed development has led to direct applications (Brandon H. Le *et al.*, 2007). Here we present the survey on the anatomy of the aerial seeds of *Amphicarpaea edgeworthii*. The result provides a first description of the floral development of an amphicarpic species. The initial cell division of zygotes of *Amphicarpaea edgeworthii* Benth. ground seeds is unequal horizontal division which forms one apical cell and one basilar cell. With development process, the apical cell forms pro-embryo while the basilar cell

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**Fig. 2:** Development of seed coat of *Amphicarpaea edgeworthii* Benth. (a) integument cell in 4 DAF, ×10; (b) integument cell in 8 DAF, ×10; (c) the formation of fence cells in 14 DAF, ×10; (d) the initial differentiation of inner integument, ×10; (e) the disorganization of inner integument in 16 DAF, ×10; (f) episperm with fence cells from outer integument in 24 DAF, ×10
forms undeveloped and short suspensor, this result correspond to former study (Hu Shiyi, 2005) that *Amphicarpaea Elliot* plants only have the undeveloped suspensors which consist of three to four cells, and such suspensors would undergo degradation when seeds become ripe. Besides, *Amphicarpaea Elliot* plants have nuclear-type endosperm development. The embryogeny was of the Onagrad type (Johri, 1984), and endosperm development corresponded to the Nuclear type (Hu Shiyi, 2005). Through the embryo development of *A. edgeworthii* were compared with the embryological data available in Papilionoideae plants (Shen Jiaheng et al., 1991), it was found that *A. edgeworthii* was very similar to *Glycininae Elliot* in the embryological characteristics. So the relationship between *A. edgeworthii* and *Glycininae* were most close.

From zygote to tissues differentiation, there were basically three phases in embryos development of *Amphicarpaea edgeworthii* Benth. The first one was proembryos development phase that zygotes divide into globular embryos in about ten days. After the first phase, it took another ten days to differentiate cotyledons primordium from globular embryos and before the hypocotyls elongation and tissues formation, this was cotyledons differentiation phase. Finally, the tissues differentiation phase would take approximately five days to complete hypocotyls elongation and germs, hypocotyls, radicle formation.

Currently, there are several hypothesizes to explain the mechanisms and causes of hard seeds in legumes. It has been proved that structure of seeds coat is one of the important effects of the hard seeds formation. The study indicates that, with seeds development of *Amphicarpaea edgeworthii* Benth., a dense and water impermeable corneous layer forms at outmost of the seeds coat, also the palisade of inner cuticular layer forms water impermeable protection that keeps water enter the embryo, then seeds could not swell and germinate. Therefore, the ground seeds of *Amphicarpaea edgeworthii* Benth. are generally hard seeds.

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