

## A RUMINATE EMBRYO IN *BLEPHARIS REPENS* (VAHL.) ROTH. (ACANTHACEAE)

Nitin M. LABHANE<sup>1\*</sup>, Nitin M. DONGARWAR<sup>1</sup>

**Abstract:** The study of morphology of embryo is very significant considering the fact that the embryo represents the important step in the determination of the viability of the seed. Ruminant endosperm has been reported in about 58 families of angiosperms. The rumination caused by the activity of the seed coat or by the endosperm itself is quite recurrent in angiosperm. Ruminant endosperm due to seed coat is reported from the family Acanthaceae in *Andrographis paniculata*. The rumination of endosperm is also considered as phylogenetically important. Rumination of endosperm is very common, however very little is known about rumination in embryo. The present papers reports the *de novo* development of ruminant embryo in *Blepharis repens*. The development of ruminant embryo is seen as an adaptation to ensure proper aeration and optimum germination for survival of the species.

**Keywords:** rumination, seed, ruminant embryo, endosperm

### Introduction

Angiosperms are characterized by double fertilization, which initiates the development of two intimately interconnected multicellular structures, the embryo and the endosperm, which are derived from the zygote and the fertilized central cell, respectively [VIJAYRAGHVAN & PRABHAKAR, 1984]. The post fertilization product of ovule is seed, which harbor embryo and the endosperm [MAHESHWARI, 1950, 1963]. Embryo survives on the nourishment provided by the endosperm in case of albuminous or endospermic seed. However, in exalbuminous or nonendospermic seed, the nourishment to the developing embryo is provided by the surrounding tissues, since the endosperm whatsoever present is utilized during the early developmental stages of the embryo. Thus the role of endosperm and surrounding tissues for nourishment, leading to the normal growth and development of the embryo is very decisive for the survival of the species [RAGHAVAN, 1997]. The first developmental study on ruminant structure has been reported from family Araliaceae on taxon *Hedera helix* [HEGELMAIER, 1886]. The term ruminant is defined as an uneven endosperm surface that is often highly enlarged by ingrowths or in folding of surrounding tissues [PERIASAMY, 1962b]. The term ruminant endosperm is pertinent only when the endosperm is present, however many earlier reports were from exalbuminous taxa [PERIASAMY, 1962a; DAHLGREN, 1991]. Under the rubric of rumination those seeds are also included that at maturity showed a folded or ruminant structures on embryo [DAHLGREN, 1991]. Thus the term rumination is applicable to the embryo as well as the endosperm. WERKER (1997) considered the seed as ruminant if the surface of any part of

<sup>1</sup> Department of Botany, Rashtrasant Tukadoji Maharaj Nagpur University, Nagpur 440033 – India

\* Corresponding author. E-mail: nlabhane@yahoo.com

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the seed is irregular or uneven. JACOBS & al. (2008) considered three types of rumination one resulting from the shape and structure of the surrounding endocarp, while the last two types of ruminations are due to uneven growth of specific tissues of the seed, i.e., seed coat and endosperm, and which develops independently of the adjacent endocarp. The rumination or ornamentation in the seed is either by the activity of the seed coat or the endosperm of the seed itself. Ruminant nature of the seed has been reported in about 58 families of angiosperms [BAYER & APPEL, 1996]. However, *de novo* development of ruminant embryo is not reported in any angiosperms.

### Materials and methods

The material selected for the present investigation *Blepharis repens* (Vahl) Roth. is collected mostly from the Nagpur district. Nagpur falls within the tropical to sub-tropical region of central India in the state of Maharashtra, India. Temperature of Nagpur during rainy and winter season varies from 10-30 °C, while during summer it goes up to 40-45 °C. The plant material was identified with the help of standard floras, viz., the Flora of Maharashtra [SINGH & al. 2001], Flora of Marathwada [NAIK, 1998], Flora of Nagpur [UGEMUGE, 1986] and Flora of British India [HOOKER, 1885]. The taxa under investigation were preserved in the form of herbarium specimen and deposited in the Department of Botany, Rashtrasant Tukadoji Maharaj Nagpur University, Nagpur with the accession numbers NML/201.

Young and mature fruits of *B. repens* (Vahl) Roth. were collected every year from 2006-2011, during the months of October to March from various locations, so as to nullify the localized effect on the morphology and development of the seed and the embryo. The fresh fruits, as well as fruits fixed in formalin-acetic-alcohol were collected and stored in 70% alcohol for carrying out further investigation. Under dissecting microscope, the fresh and preserved fruits were dissected to study the morphology of the seed and the embryo at maturity.

### Results and discussion

The capsular fruit is more or less compressed (Fig. 1A) with a central partition wall, showing the presence of one seed in each chamber. The mature fruit of *B. repens* is green, before turning brown on drying and shows the presence of two seeds (Fig. 1B). The mature fruit is 0.8 cm long and 0.6 cm broad. The mature seed on dissection shows the presence of seed coat which encloses the embryo. The seed is 0.6 cm long and slightly triangular in shape. The seed coat is covered profusely with of long and hairy outgrowth, which becomes mucilaginous on hydration (Fig. 1C, D). Endosperm is completely absent at maturity, thus the seed is non-endospermic at maturity [LABHANE, 2011]. Thus, the formation of the ruminant structures due to endosperm seems to be distant reality. The endosperm was absent but still ruminant structures is seen on the mature embryo. The mature embryo is more or less triangular, dorsio-ventrally compressed showing the presence of two distinct cotyledons and radicle (Fig. 1E, F). The embryo is upright with radicle, cotyledon and plumule lying in one straight line. The mature embryo is 0.4-0.5 cm in length along the long axis of the embryo. The cotyledons show the presence of distinct ridges and furrows on its surface. The ornamentation on the surface of the cotyledon is more or less circular in outline and appears

as small craters or pits. Thus the mature embryo shows distinct ruminant structure (Fig. 1E, F). The ornamentation is also present on the cotyledons of the embryo, however ornamentation on stalk is less pronounced as compared to the cotyledons. The presence of ruminant structure in family Acanthaceae has also been reported in *Andrographis serpyllifolia* [MOHANRAM, 1960] and *Elytraria acaulis* [JOHRI & SINGH, 1959]. However in both the cases, the origin of ruminant structure is attributed to the seed coat or the irregular development of the endosperm. In the present investigation, the endosperm is completely absent in the mature seed and the seed coat is not having any kind of irregular outline or in growth inside the seed [LABHANE, 2011]. Thus the development of ruminant structure on the embryo in *B. repens* seems to be *de novo*.

The study of ruminant nature of embryo and endosperm is the pioneer work of PERIASAMY (1962a, 1962b, 1966, 1990), however the first developmental work on ruminant structures in angiosperms was done on *Hedera helix* [HEGELMAIER, 1886]. The number of families of angiosperms showing ruminant structure varies according to different authors. PERIASAMY (1962a, b, 1966) reported ruminant structure in 26 families, DAHLGREN (1991) 25 families, BHOJWANI & BHATNAGAR (1999) 32 families, whereas BAYER & APPEL (1996) reported in 58 families.

The numbers of ovules per ovary and the number of seeds per fruit vary considerably in different members of the family Acanthaceae [RENDLE, 1938]. LABHANE & DONGARWAR (2012) reported about 50% embryo abortion in *B. repens*, hence at maturity the fruits in most of the cases showed the presence of only one seed, since one of the seed is aborted. At early stages and till maturity the fruit shows the presence of two seeds (Fig. 1B), but when the mature fruits were dissected it showed the presence of one fully developed embryo and other embryo was found to be rudimentary and aborted. Embryo abortion has also been reported in some other members of family Acanthaceae such as *Justicia procumbens* L., *Rungia repens* (L.) Nees and *Haplanthodes verticillata* (Roxb.) Majumdar [LABHANE & DONGARWAR, 2012]. Embryo of *B. repens* seems to have evolved ruminant morphology, with distinct ornamentation all over the cotyledons to facilitate proper aeration and optimum germination, the same is also reported by GOEBEL (1933) and ARNDT (1967). As the fruit contains one or two seeds, the *B. repens* seems to have evolved ruminant embryo to ensure its own survival. The ingrowths seen in ruminant structure often contain phenolic substances or ethereal oils which led to the assumption that ruminant might make the seeds less attractive to predators [MULLER, 1887; OSENBRUG, 1894; GOEBEL, 1933]. The various substances which provides chemical defense in ruminant structures have been detected in the ingrowths, but not in the endosperm [MULLER, 1887; MEYER, 1891].

The presence of ruminant structures in family Araliaceae showed graded variation in various taxa of that family, suggesting the importance of ruminant structures in angiosperms [HARMS, 1894]. GURKE (1890) was most probably the first to use the variation in the structure of ruminant endosperm for taxonomic segregation of the various species of the genus *Diospyros*. CHUANG & CONSTANCE (1992) considered the ruminant character to be very useful, while studying the seeds and systematics in *Hydrophyllaceae*. JACOBS & al. (2008) found the seed character as phylogenetically very important while classifying 31 species of *Viburnum* belonging to *Caprifoliaceae*.

The presence of ruminant structure in angiosperms both in the primitive as well as the advanced taxa suggest that ruminant character seems to have evolved several time

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independently [NETOLITZKY, 1926]. PERIASAMY & SWAMY (1961) reported ruminant structure in family Annonaceae. PERIASAMY (1963, 1964, 1990) reported ruminant structure from related families such as *Rubiaceae* and *Apocynaceae*. The presence of rumination was considered as a primitive trait of angiosperms [TAMAMSHJAN, 1951]. The occurrence of ruminant endosperm might represent an ancestral feature but still present in both primitive and advanced taxa of the angiosperms [VIJAYARAGHAVAN & PRABHAKAR, 1984]. In fact, the occurrence of ruminant endosperm in several families of the *Magnolianaes* and *Monocotyledoneae* further strengthens the assumption that ruminant structure might have evolved several times in angiosperms, independently. It seems that the presence of ruminant morphology in angiosperms needs to be further exploited so as to assess its viability in phylogenetic consideration.

MOHANRAM (1960), JOHRI & SINGH (1959), PERIASAMY (1962b), DAHLGREN (1991), BAYER & APPEL (1996) reported ruminant morphology in some of the members of the family Acanthaceae, which is due to the uneven activity of endosperm or the seed coat; however *de novo* development of ruminant embryo as seen in *B. repens*, is not reported till date. Endosperm is absent at maturity (Fig. 1D), since it is completely utilized by the developing embryo at early stages of seed development [LABHANE, 2011]. Thus the seed is non-endospermic at maturity. Seeds being devoid of endosperm, the outgrowths present on the seed coat which form a mucilaginous mass might function as the reserve food material for the developing embryo. The development of such embryo with outgrowths on the seed can be attributed to the ecological conditions in which the species is growing. At maturity even the hairy outgrowths are consumed by the voracious developing embryo, since the number of hairy outgrowths is very less in dried seeds as compared to seeds collected before drying [LABHANE, 2011]. The seed character in *Silene* L. has also been used for identification of various species [IFRIM, 2011]. The two species of *Blepharis* namely *B. repens* and *B. maderaspatensis* were found to be phonologically and reproductively distinct [DABGAR & MALI, 2010]. The flower of *B. repens* is found to be covered by one bract and seven bracteoles, so the fruit in case of *B. repens* is found to be strongly attached to the mother plant, making it difficult for the seeds dispersal. Hence the seed in most of the cases germinates while the fruit is still attached to the mother plant under favorable conditions.

*De novo* development of ruminant embryo in *B. repens* is obvious, as the seed is exalbuminous and there is complete absence of uneven growth of specific tissues of the seed, i.e., seed coat and endosperm. The adaptation of the plant species for survival in order to have maximum aeration and germination of the seed is by the development of the rumination of the embryo.

#### Conclusions

The presence of ruminant embryo in *B. repens* seems to have evolved ruminant morphology, with distinct ornamentation all over the cotyledons to facilitate proper aeration and optimum germination. Thus the development of rumination in some plant species, such as *B. repens* appears to be a step towards its own conservation during the process of evolution, since it also shows the embryo abortion.

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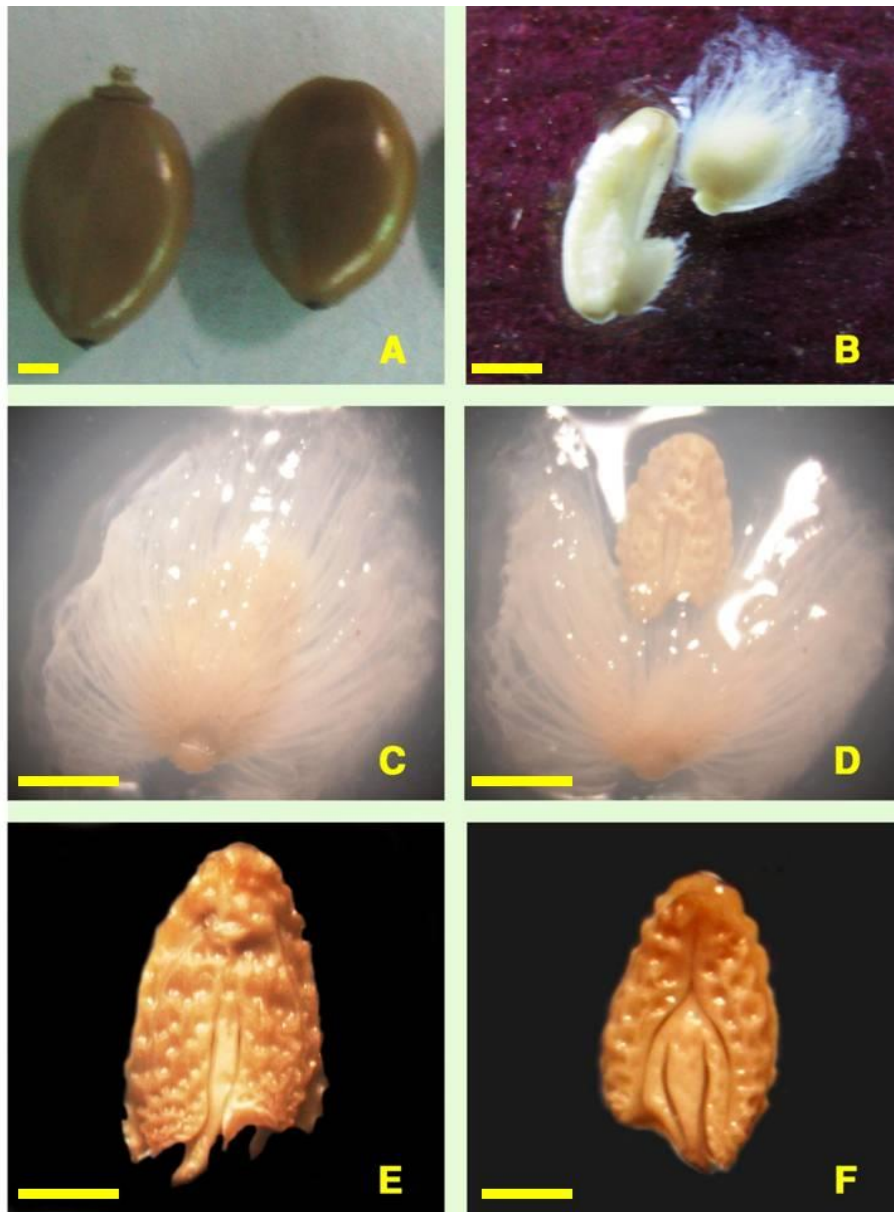
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**Fig. 1.** A. Mature fruit; B. Seeds dissected out of the fruit; C. Seed at maturity; D. Seed dissected with mature embryo; E. Adaxial view of embryo; F. Abaxial view of embryo (Scale = 100  $\mu$ m)