RESEARCH ARTICLE

In-vivo study of morphology of embryo in the medicinal plant, *Basella alba* Linn. (Basellaceae)

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ABSTRACT

The present paper deals with morphology of freshly dissected out live embryos of medicinally important plant, Basella alba Linn. The various part of the plant is used to cure dysentery, diarrhea, anemia, cancer etc. It is also has a known antioxidant, anti mutagenic, infertility, central nervous system depressant and anti inflammatory activity. The study of embryo at maturity in its native form is very significant, since its normal development is related to the viability of the seed. The young and mature embryo can be distinguished with the help of presence of chlorophyll. The viability of seeds is determined by the presence of green embryo at maturity, while nonviable seeds contains white coloured embryo. The embryo is coiled twice at young stage, while at maturity it is coiled one and half times, with the growing tip situated at the centre and the radicle on the outer side. Considering the medicinal value of B. alba, study the reproductive biology with respect to structure of mature embryo is very pertinent. The study of embryo in its native form also shows the manner in which the growing tip is protected by coiling so as to ensure that the species is protected against adverse condition.

Keywords: Endosperm, viability, seeds, In-vivo, viability

INTRODUCTION

Basella alba belonging to family Basellaceae is a native of tropical Asia and is widely cultivated. It is a succulent twiner with red or green stem, simple broad-ovate fleshy leaves. It shows the presence of small pinkish white flowers borne on peduncled spike and subglobose purple fleshy utricles (Daniel, 2006). Adhikari *et al*. (2012) mentioned the use of *Basella alba* in various parts of the world such as Thailand, Bangladesh, Nigeria, Nepal, India etc. Rahmatullah *et al*. (2010) mentioned the use of *Basella alba* for the treatment of Anemia in women, coughs, cold and cold related infections. Focho *et al*. (2009) found that the maceration is taken orally for infertility, pelvic inflammatory disease, threatened abortion and spurious labour. Joseph *et al*. (1999) and Phadungkit *et al*. (2012) detected flavonoids and phenolic compounds in *Basella alba*, which are known antioxidant and antimutagenic agents, respectively.

The apparent antioxidant and antimutagenic activities of the plant further suggests their potential usefulness in cancer prevention**.** Glassgen *et al*. (1993) had shown the presence of betacyanin, gomphrenin I, II and III. Anandarajagopal *et al*. (2011) reported the central nervous system depressant activity from the aerial parts of the *Basella* plant. Ayurvedic treatment in India uses leaves and stem of *Basella alba* for anticancer such as melanoma, leukaemia and oral cancer (Premalatha and Rajgopal, 2005).

The seed is an important stage in the higher plant life cycle with respect to the survival as a species (Bewley and Black**,** 1994). The development of seed comprises of two major phases namely embryo development and seed maturation. Embryogenesis, which is a post fertilization product, starts with the formation of a single-cell zygote and ends in the heart stage when all embryo structures have been formed (Mayer *et al*., 1991). At the end of the embryo growth phase, cell division in the embryo arrests (Raz *et al*., 2001). Hereafter, the seed, containing a full sized embryo, undergoes maturation during which food reserves accumulate and dormancy and desiccation tolerance develops (Goldberg *et al*., 1994). The process by which the embryo emerges from the seed to complete germination and how embryo emergence is blocked in dormant seeds is still less understood (Bewley, 1997). The traditional method of studying viability of the seeds is by taking into account its germination percentage. However, the study of morphology of developing embryo at the stage of maturity can also give a conclusive idea regarding the viability of seeds. The classical way of studying embryology is by doing laborious microtome studies but the biggest drawback is that it is highly impossible to understand the embryo morphology in its native form at maturity.

Looking at the medicinal potential of *Basella alba* and its usage throughout the world, the study of reproductive biology is very significant for its conservation and sustainable utilization. The study of reproductive biology of medicinal and RET (rare, endangered and threatened) plants will help in the development of various conservation strategies. The study of morphology of embryo at maturity will helps in knowing the form and structure of embryo and also the percentage of seed viability (Labhane, 2011). The study of embryo in its native form also shows the manner in which the embryo undergoes modification by the different plant species so as to ensure that the species is protected against adverse condition (Labhane and Dongarwar, 2011, 2012, 2014).

MATERIALS AND METHODS

The material selected for the present investigation is *Basella alba* Linn. The taxon selected for the present investigation was collected mostly from the Nagpur district and Mumbai. 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^{0}C, while during summer it goes up to 40-45^{0}C. The Mumbai is the coastal city present on the western part of India, which is considered to be more polluted as compared to Nagpur. Mumbai shows more or less moderate temperature of about 20-25^{0}C and heavy rainfall. The plant material was identified with the help of standard floras, namely the Flora of Maharashtra (Singh *et al*., 2001), Flora of Marathwada (Naik, 1998), Flora of Nagpur (Ugemuge, 1986) and Flora of British India (Hooker, 1885). The taxon 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/501 and NML/502 for specimens collected from Nagpur and Mumbai regions respectively.

Young and mature fruits of *Basella alba* Linn. were collected every year from 2011-2013, during the months of August to March from various locations, so as to nullify the localised effect on the development of the seed and the embryo. The fruits were fixed in formalin-acetic-alcohol and stored in 70 % alcohol. Under dissecting microscope, the fresh and fixed fruits were dissected to study the morphology of the seed and the embryo at maturity. Six locations from Nagpur and four locations from Mumbai were selected (Table-1). Twenty fruits of each location were dissected and presences of well developed and poorly developed embryos were co-related with the viability of the seed.

RESULTS AND DISCUSSION

The flowers are borne at the end of the spike inflorescence. The flowers are small and pink in colour which turns to light greenish pink after fertilization. Slightly mature fruits become green in colour but show the presence of prominent pink tip, but as the fruits

mature further it becomes green. The further advancement in the maturation results in the formation of deep black colour fruits (Table-2). At the advance stage of development the fruit shrivels and becomes brown colour, due to loss of the water from the fruits mesocarp region which contains deep blue colour dye (Fig-1).

The fresh fruits are more or less spherical and green coloured at young stage, showing distinct ridges and furrows. As the fruit matures the fruit wall turns dark purplish to black coloured. The mature fruit is approximately 1(± 0.1) cm in diameter. The fruit contains single seed, which is 0.5(± 0.1) cm in diameter. The seed is protected by a hard seed coat and the seed coat become harder as the fruit and the seed attains maturity. However the size of embryo at maturity is 1(± 0.1) cm (Table-1). No endosperm is found when the young and mature fruits were dissected. Thus the seed is non albuminous in nature.

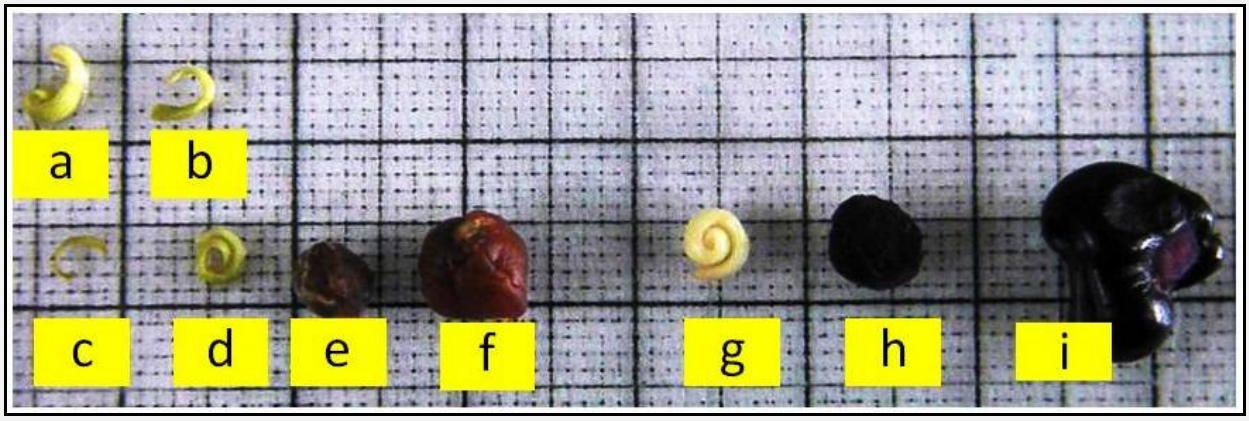

Fig. 1: Structure of the fruit, seed and embryo in *B. alba* in mature and young fruits.

a, b and **d** Mature and viable embryo.

c- Unviable and weak embryo dissected from mature fruit.

g, h and **i-** structure of embryo, seed and fruit in young stage respectively.

Table 1: Size of fruit, seed and embryo at maturity in *Basella alba* with respect to various locations in Mumbai and Nagpur.

S. No	Location	Size of fruit	Size of seed	Size of embryo
1	Nagpur, Dhantoli	1(± 0.25) cm	0.5(± 0.1) cm	1(± 0.25) cm
2	Nagpur, PDKV Agriculture college	1(± 0.20) cm	0.4(± 0.1) cm	1(± 0.20) cm
3	Nagpur, Bajaj nagar	1(± 0.20) cm	0.5(± 0.1) cm	1(± 0.25) cm
4	Nagpur, Bhandara road	1(± 0.25) cm	0.4(± 0.1) cm	1(± 0.25) cm
5	Nagpur, Gorewada	1(± 0.25) cm	0.6(± 0.1) cm	1(± 0.25) cm
6	Nagpur, Telankhedi garden	1(± 0.20) cm	0.5(± 0.1) cm	1(± 0.20) cm
7	Mumbai, Andheri-west	1(± 0.15) cm	0.5(± 0.1) cm	1(± 0.15) cm
8	Mumbai, Virar	1(± 0.20) cm	0.5(± 0.1) cm	1(± 0.20) cm
9	Mumbai, Borivali	1(± 0.25) cm	0.5(± 0.1) cm	1(± 0.25) cm
10	Mumbai, Palghar	1(± 0.20) cm	0.5(± 0.1) cm	1(± 0.20) cm

Table-2: Table showing relative growth of radicle and cotyledon at young, medium and mature stage of embryo development inside the seed.

S. No	Stage of seed and colour	Length of radicle	Length of cotyledon	Radicle/cotyledon ratio
1	Young green	0.5(± 0.1) cm	0.4(± 0.1) cm	1.25(± 0.1) cm
2	Medium purple	0.5(± 0.1) cm	0.6(± 0.1) cm	0.83(± 0.1) cm
3	Mature Black	0.5(± 0.1) cm	0.7(± 0.1) cm	0.71 (± 0.1) cm

Table No-3 Showing the abortion percentage

Characters	Nagpur	Mumbai
Number of ovules in ovary	1(120)	1(80)
Number of seeds at young stage	1(120)	1(80)
Abortion percentage of ovules	Nil	Nil
Number of seeds at maturity	1(114)	1(76)
Abortion Percentage of embryo	5%	5%

(N=20; Fruits were collected from various location and the embryo abortion was uniformly noticed in all the taxa investigated). The figure in the parenthesis indicates the total number of ovaries and seeds studied from two regions of Maharashtra state.

Young embryos are white in colour (Fig-1 g), while the mature seed shows the presence of green coloured embryos (Fig-1 a, b, c, d). Thus, the young and mature embryo can be distinguished with the help of presence of chlorophyll. The viability of seeds is determined by the presence of green embryo at maturity, while nonviable seeds contains white coloured embryo (Fig-1 c) in mature fruits. The embryo is coiled twice at young stage (Fig- 1 g), while at maturity it is coiled one and half times, with the growing tip situated at the centre and the radicle on the outer side (Fig- 1 a, b, d). The young fruits also show the presence of white coloured embryo, which is highly coiled, but as the fruit matures the coiling is slightly relaxed and the embryo tends to become phototrophic by the synthesis of chlorophyll pigment, which is completely or partially absent in unviable embryo**.**

The fruit containing young seeds showed the embryo with higher radicle growth as compared to the cotyledon, where as mature fruits showed more growth of cotyledons as compared to the radicle (Fig-1a, b, c, d & g; Table-2). The percentage of embryo abortion is found to be nearly 5%, since the mature embryos were seen in nearly 95% seeds (Table-3). The structure of the mature embryo at both Nagpur and Mumbai region shows no variation with respect to percentage of embryo abortion. The abortive embryo is found to be very weak, since the radicle and the cotyledons are very poorly developed (Fig-1 c).

The reproductive capacity or potential of plants is a critical aspect of plant reproduction. Reproductive capacity of many plants is extremely great and that there are large differences between species (Salisbury, 1942). These differences may be the result of different selective pressures and are related to the ability of a species to persist in time and in space (Harper et al., 1970; Harper and White, 1974). The percentage of embryo abortion in *B. alba* was found to be nearly 5%, since the mature embryos were seen in nearly 95% seeds. The embryo abortion percentage in both Nagpur and Mumbai region is found to similar. The similarity in the percentage of abortion and high viability may be due to the typical coiling of the embryo, so as to protect the growing tip i.e. plumule. Labhane and Dongarwar (2012) had studied the reproductive capacity of some plants of Acanthaceae with respect to embryo abortion. The study of embryo abortion in wild and ornamental plants shows that the embryo abortion is very much prevalent (Labhane and Dongarwar, 2012). The reproductive capacity is studied in relation to embryo abortion has been reported in *Epilobium angustifolium* (Wiens et al., 1987), *Dalbergia sissoo* (Ganeshaiah & Uma Shaanker, 1988), *Nelumbo nucifera* (Wang et al, 2012), *Phaseolus vulgaris* (Nakamura, 1988), *Schima wallichii* (Goswami & Pandey, 2012), *Asclepias speciosa* (Bookman, 1984), *Quercus* (Craft et al, 2009), *Tecoma stans* (Labhane & Dongarwar, 2014) etc.

CONCLUSION

The study of mature embryo of *B. alba* had revealed the coiled morphology of mature embryo. The embryo is coiled twice at young stage, while at maturity it is coiled one and half times, with the growing tip situated at the centre and the radicle on the outer side. The coiling of the embryo can be attributed as a strategy of the plant to protect the growing tip, so as to enable the perpetuation of the species during the young stages, since at maturity the coiling is reduced. The percentage of embryo abortion in *B. alba* from both Nagpur and Mumbai region is more or less same, which suggest that pollution has very less effect on the reproductive capacity of this plant. The minimal effect on the reproductive capacity on *B. alba* may be due to the super coiling of the embryo so as to protect the growing tip of the embryo and also by the strong seed coat and fruit wall. The viability of the seed in mature fruits can be correlated with the presence of green coloured embryo, since in case of nonviable embryos the embryo remains white coloured most probably due to degradation of chlorophyll or inhibition of the genes involved in chlorophyll synthesis. This degeneration is evident from the light green colour in degenerating embryo. Thus the study of embryo In-vivo at maturity can give us clue regarding the viability of the seed and many other aspects including embryo abortion.

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