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Studies on reproductive and seed biology of *Dioscorea villosa* Linnaeus (Dioscoreaceae): a rare medicinal plant in NE India

Tabitha Langhu and **Chitta Ranjan Deb**[1]

Department of Botany, Nagaland University, Lumami 798 627, Nagaland, India
[1]*Corresponding Author: E-mail*: debchitta@rediffmail.com and debchitta@gmail.com

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Abstract

Dioscorea villosa Linnaeus (Dioscoreaceae) is an important medicinal plant. Present study records certain reproductive behaviors and seed biology of this species. The male and female flowers of *Dioscorea villosa* were arranged in 2 whorls of 3, bracteates, anthers bilobed, didymous. Though there was high fruit setting, but the mean seed production per plant was quite low. The major pollinators were wasps, flies, ants and beetles. The pollens were orange and sticky. The time of radical emergence, germination rate, seedling morphology and seedling establishment are influenced by various factors. Effective dormancy break was occurred with the cold-stratification at 4ºC for 96 hrs suggests that *D. villosa* seeds are morphologically dormant. While the cold treated seeds were moved in an incubator at 30º C where ~25 % seeds were germinated after 35 days by breaking the dormancy against only ~10 % germination in the seed bed.

Key words: *Dioscorea villosa,* Reproductive behaviors, Seed dormancy, Cold stratification

INTRODUCTION

The Dioscoreaceae is represented by about 650 species with its ten genera**.** They possess rhizomes and many species bear bulbils, commonly referred as aerial potatoes, which are rich in carbohydrate. The tubers and bulbils (yams) of many species of *Dioscorea* Linnaeus constitute the staple food in some parts of the world (Dweck 2002)**.** Tubers of many species contain high amount of diosgenin, which is the precursor for the commercial production of sex hormones and corticosteroids (Dweck 2002). In India, yams are cultivated as garden crop or as a subordinate crop in some places. They are propagated through tuber cuttings and bulbils. The crop generally matures in 7 – 10 months. They are grown on well drained deep soil. Some species are used as a source of saponins for the preparation of steroids in the pharmaceutical. The tuber extract of these medicinal herbs are used to treat symptoms of rheumatoid arthritis and menopause colic (Kaimal & Kemper 1999; Wojcikowski 2008). Diosgenin as a source of natural hormone used as skin cream (antifungal); used in urinary tract problems in Chinese traditional medicine, treatment of asthma and cardiovascular problems (Kaimal & Kemper 1999). They also possess anti carcinogenic potential. To understand the reproductive behaviours of one of the important species of *Dioscorea* i.e. *Dioscorea villosa* Linnaeus, the present work was undertaken and its findings might be helpful in framing the conservation strategies for wild yams.

Dioscorea villosa Linnaeus (Dioscoreaceae) is a perennial dioecious herbaceous vine climb clockwise with an underground ~20-30 cm long tuberous root, but do not produce any bulbil. The plants sprout from the tuber during March-April and branches climb up to 3.5 m.

Leaves alternate, 7 – 9 veined, ovate-cordate, entire, glabrous, with reticulate venation. The species is use as a source of saponins for the preparation of steroids in pharmaceutical industry (Dweck 2002). The tuber extract is used to treat symptoms of rheumatoid arthritis, menopause and colic (Kaimal & Kemper 1999; Wojcikowski 2008). The species also possess anti-carcinogenic properties. Due to its many fold uses, the plant is now highly exploited and is under threat in the natural habitat (Chao-chin *et al*. 2007).

MATERIAL AND METHODS

Plant material

The plants were collected from Mopunchuket village [26º24'02.1" N and 94º31'17.6" E at 1044 m AMSL] of Mokokchung District, and Lumami village [26º12'37" N and 95º29'28" E] of Zunheboto District, Nagaland, India. The plants with the tubers were grown in the Botanical Garden, Department of Botany, Nagaland University, Lumami located in Zunheboto district, Nagaland (26º12'37" N and 95º29'28" E at 1150-1200 m AMSL) for the study of reproductive behaviors, seed biology etc.

Floral phenology and morphology

The reproductive phenology *viz*, time of tuber sprouting, time of budding, flowering of male and female plants, fruit and seed setting were studied. The female and male plants were maintained side by side for the present study. In order to estimate flower production, total number of flower per plant was counted manually in the selected plants. Pods per plant and seeds per pod were counted to understand their reproductive potential.

Pollination

The pollination of *Dioscorea villosa* was studied by identifying the visitors to the flowering plants. The frequency of visit per flower was observed during the flowering period. Maximum visitation rate of the insects during the day time was also recorded.

Seed biology

Seed collection: The mature fruits were harvested from the plants in the Botanical garden. For the present study, the intact matured fruits of about 16 weeks old, when the fruits turned yellowish green, were harvested randomly irrespective of their size from plants during the years 2011 – 2013 (**Fig. 1a**). The harvested fruits were air dried inside the laboratory by spreading on the old newsprints immediately after collection. The dried fruits were stored in plastic bags at 25º C till used (**Fig. 1b)**.

***Preparation of potting mix*:** The potting mix for the experiment was prepared by mixing finely crushed garden soil, sand and coconut coir at 1:1:1 ratio and put in a plastic pots and transparent poly-bags. The poly-bags and plastic pots were perforated for better aeration. The pots were moistened before the transfer of seeds. To study the emergence, survival and growth of seedlings of *Dioscorea villosa* under the above mentioned conditions, 4 replicates of 10 seeds (N=40/test) were used. In each potting mix 10 seeds were sowed.

***Experimental process*:** A part of the seeds were sowed in the potting mix as mentioned above and 10 seeds were sowed per pot, while another lot of seeds were treated differentially at 4º C in a refrigerator for 0, 24, 48, 72, 96 hours and sowed as described below:

1. A set of stratified seeds as described above were used for filter paper germination. Seeds were sowed in filter paper in a humidity chamber of 90 mm in diameter. The filter paper was moistened with 5 ml of water and kept in a laboratory at room temperature (25º C).
2. Another set of stratified seeds were sowed in a poly bag and kept in an incubator at constant temperature of 30º C.

3. While, the third set of processed seeds were sowed on different PGRs containing Murashige and Skoog (MS) medium (Murashige & Skoog, 1962) fortified with sucrose (3 %, w/v) and gelled with agar (0.8%, w/v). The medium was dispensed in a borosilicate test tube (25 x 150 mm) and kept under florescence light (40 µmol m^2 s^{-1} illumination) of 12/12 light/ dark period.

In filter paper germination test, 4 replicate of 10 seeds were sowed in a humidity chamber of 90 mm in diameter. The filter paper was moistened with 5 ml of water on regular basis. The experimental design was completely randomized. The data was collected at regular basis for seed germination and seedling morphology. Once the seedlings showed normal functioning like formation of seedling growth, normal leaves etc., the seedling were transferred to the poly house and seedlings mortality were observed.

RESULTS

Plant morphology and floral phenology

Dioscorea villosa Linnaeus is a dioecious plant. The tubers of the species sprouts during March-April, bud initiation in September, and flowering in October (Table 1). Seeds set in November and matures during February-March. The tubers mature during March and become ready to harvest. While most of the species of *Dioscorea* produce bulbils or air potato, *D. villosa* does not produce it. Flowers of both sexes are pedicellate, perianth fleshy, 3 + 3, polytepalous, pistillate flowers yellowish green, tepals in two whorls of three, stigma is wet, bifid, epigynous, ovary trilocular with axial placentation (Fig. 1c & d**)**. Staminate inflorescence bracteolate. The inflorescence is single and in the axils of upper leaves. The filaments are straight, separated, didynamous anther lobes and ovate tepals. In male flower, anther bilobed, staminoids six in two whorls of three with short filaments, basally attached to tepals, anthers

Table 1: Phenology of *D. villosa* plant in the experimental garden [Data collected from 2011 – 2013]

Parameter	Female plant	Male plant
Sprouting of tuber	March-April	March-April
Floral bud initiation	September-October	September-October
Flowering	October	October
Seed maturity	February-March	-
Tuber maturity	March	March
Tuber splitting	March-April	March-April

bilobed. Both male and female flowers are mildly scented of a jasmine like odour. Ovary is trilocular, axial placentation, fruits loculicidal. In the cross section of ovaries and or young fruits, in most of the cases the locules were found to be empty/ aborted (Fig. 1e, f). However, it is a highly polymorphic species.

It was found that many of the flowers were reproductively non-functional i.e., do not produce any fruits or fruit setting is very poor. There was an average of 7.2 fruit formation per inflorescence of which 50 % of the fruits bearing seeds while remaining 50 % of the fruits were seedless (Table 2). Wherever seeds formed were very light, winged and brown.

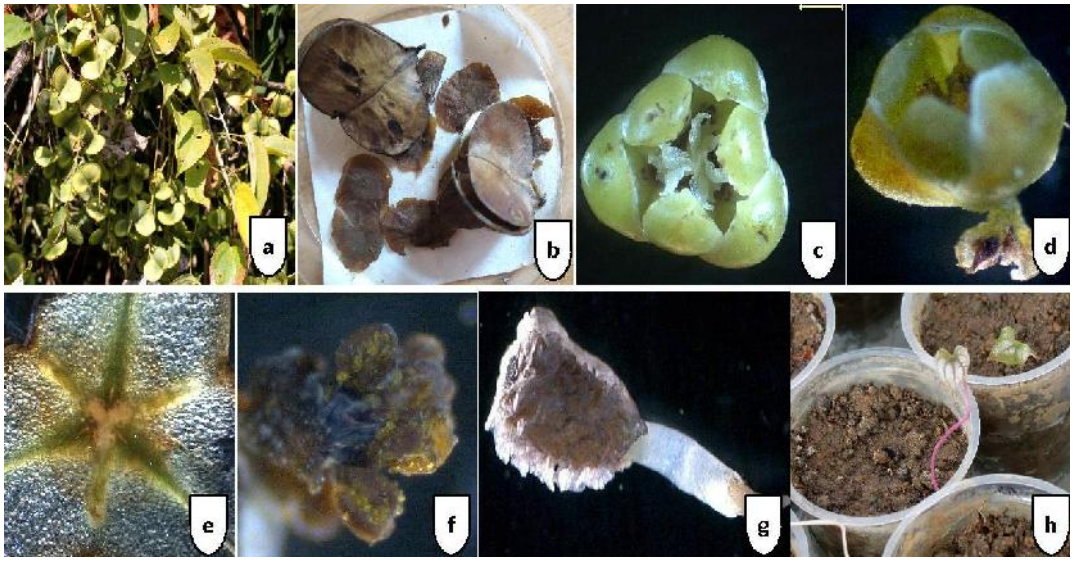

Figure 1: a. Mature fruits of *Dioscorea villosa* Linnaeus; **b.** Dry fruits ready for storage; **c.** Female flower (enlarged); **d.** Male flower (enlarged); **e.** Cross section of ovary showing empty locule; **f.** Bilobed anther; **g.** Germinated seed showing radical on filter paper in incubator; and **h.** Germinated seed formed seedling in seed bed.

Table 2. Enumeration of fruit setting of *D. villosa*

Sl. No.	No. of fruit per inflorescence	Healthy fruit formed per inflorescence	Aborted fruits per inflorescence
1	7	3	4
2	5	3	2
3	10	3	7
4	6	4	2
5	3	2	1
6	7	3	4
7	8	6	2
8	5	2	3
9	11	5	6
10	12	6	6
11	10	5	5
12	5	2	3
13	5	5	0
14	8	4	4
15	6	3	3
16	8	3	5
17	5	3	2
18	6	2	4
19	10	4	6
20	7	4	3
Mean	**7.2**	**3.6 (50%)**	**3.6 (50%)**

Table 3. Effects of cold stratification (4° C) on the seed germination of *D. villosa* on seed bed and incubator

Stratification duration (in hour)	% response (±SE)* in the seed bed	% response (±SE)* in the incubator
0	2.5 (0.2)	-
24	2.5 (0.2)	-
48	7.5 (0.3)	-
72	3.5 (0.2)	-
96	10.2 (0.2)	25.5 (1.5)

Pollination

The pollination of *Dioscorea villosa* was found to be xenogamous. There is the presence of olfactory in the form of floral scents. The flowers were visited by different insects includpng beetles, flies, wasps etc. Maximum visitation rate was observed during morning from 78.30 A.M to 9.30 A.M. and in the afternoon evening from 1.30 P.M to 4.30 P.M but less visitation of insect was observed during afternoon. Highest frequency of visit was recorded with beetles. The collections of pollinators are now in the process of identification.

Seed biology

During the present study attempt was made to sow the freshly harvested seeds (greenish) on filter paper to test the post harvest dormancy and it was found that seeds failed to show any sign of germination but seeds sowed in the incubator at a constant temperature of 30° C showed emergence of radical and shoot formation and germination started within 35 days (Fig. 1g) indicating that the species exhibit post harvest thermal dormancy and can grow well and adopted to germinate in comparatively high temperature. Besides, the finding of the present study was greatly influence by pre-treatments of the seeds (Table 3) as far as germination rate and seedling formation is concerned. The seeds were stratified at 4° C for 96 hrs exhibited optimum germination (10.2 %) under the given conditions in the seed-bed compared to non-stratified seeds (2.5 %). While seeds incubated in the incubator at 30° C exhibited 25.5 % germination from the seeds stratified for 96 hrs (Table 3 and Fig. 1h). There was significant difference in the germination period, germination rate with the pre-treated seeds. It was observed that though the seed germination was very low, but seedling mortality was very low. This finding suggests that once the seed germination is achieved, and seedlings are established easily.

DISCUSSION

Dioscorea villosa is one medicinally important plant and are generally collected from the wild due non-cultivation of the species in commercial scale in India. One of the means of vegetative propagation of many crops through the secondary propagules as bulbils or air potato in many species of *Dioscorea*. During the present investigation it was observed that *D. villosa* does not produce bulbils or air potato which makes the species different from the other species of the genus *Dioscorea*. Due to non-production of bulbils the species to a great extend depend for propagation on tuber splitting. But tubers are collected for food and medicines and do not get much opportunity propagating through this propagule. This could one of the reasons. Another alternative means of propagation is through seeds. Though there was high flowering and fruiting, seed production per plant was quite low. Further it was found that wherever there was fruiting, most of the fruits were either seedless or aborted. This could be one region why the plants are rare because seeds are also an important factor for maintaining the plant from generation to generation. This

result supports the previous work done on *Crataeva religiosa* and *Evolvulus alsinoides* (Singh *et al.* 2003, 2010).

The seed preservation practice is as old as agricultural practices but systematic collection and storage facilities have seen a development in the 20th century (Onan 2006). There are numbers of seed gene banks throughout the world engaged in studied on seed biology (Dosmann 2002; Albrecht 2006). Reports from the past studies reveal that some plant species, using relatively fresh seeds gives superior germination over stored seeds.

The time for emergence of radicals from the germinated seeds, germination rate, seedling morphology and seedling establishment is influenced by various factors. Plant species differ greatly in their preference to their seed bed characters, temperature requirements, post harvest storage, specific pre-treatment for seed germination, seedling emergence and survival. According to Onen (2006) germination of mugword seed was greatly influenced from temperature, but light condition was almost independent. Dosmann (2002) also reports that seed stratification improves germination of seeds. In the present study, freshly harvested seeds (green) of *Dioscorea villosa* when sowed on seed beds failed to germinate indicating that seeds were dormant at maturity. Effective seed germination was achieved when seed dormancy was broken by cold stratification at 4°C for 96 hrs suggest that *Dioscorea villosa* seeds are morphologically dormant. Findings in the present investigation is in agreement with the previous results that species with underdeveloped embryo require some post harvest period for embryo to grow to some critical threshold before germination occurs, and require some special treatment before they break morphological dormancy and exhibit germination (Albrecht 2006).

During the present study, study was carried out on the relationship among rate of germination with pre-treated seeds and non-treated seeds. When seeds were given a constant cold stratification for a period of 96 hr (4º C) and then moved into warmer temperature (30ºC, incubator), dormancy was broken and seeds germinated to high rate of 40% after 35 days. This suggest that once the critical time period of cold stratification demanded by *Dioscorea villosa* seeds is achieved, seeds are capable of germinating in temperature e™ 15ºC. This breaking of dormancy by cold stratification without prior warm stratification treatment, indicates that *Dioscorea villosa* seeds has non-deep simple morphologically dormancy seeds (Albercht 2006). The seeds which were not treated with cold stratification show less percent of germination ~10% only with delayed in the germination period.

The finding in the present study reveals that the seeds of *Dioscorea villosa* are physiologically dormant. Since cold stratification breaks dormancy which suggest that effective dormancy could be broken with 96 hr of cold stratification at 4º C followed by sowing at warmer temperature of 30ºC. Conversely, this alternating temperature was ineffective at breaking dormancy in *C. canadensis* suggesting that temperature higher than 4ºC is ineffective at breaking physiological dormancy (Albercht 2006). *Dioscorea villosa* seeds is therefore capable of germinating at a high constant temperature of 30º C in an incubator, once the critical time period of cold stratification (4º C) demanded by the *Dioscorea villosa* seeds was achieved. The dispersal of seed was observed to be mediated through wind in nature. Few seeds which shaded on the forest soil germinated during favourable conditions. Seeds which fall on the forest leaf failed to germinate due to deficiency of nutrients.

The outcome of the present study can guide in establishment of seedling in the field by following the temperature requirements for dormancy break identified, thereby, facilitating the conservation management strategies which often limit the seed dispersal and scarcity of the plant in nature.

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