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Seed Behavior of *Clerodendrum colebrokianum* Walp., a Vulnerable Medicinal Shrub of Mizoram, North-East India

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Abstract *Clerodendrum colebrokianum* Walp., East Indian Glory Bower locally known as Phuihnam in Mizoram, North east India is one of the most important medicinal plant. The present investigation was carried out during 2015-17 to study the germplasm variability with respect to the physical properties of the seeds of *Clerodendrum colebrokianum* collected from different locations. The study reveals that there was wide range of variability with respect to seed weight, seed length, seed diameter. There was significant variation among the seeds collected from different locations with respect to moisture loss, seed volume and water uptake (imbibition). The seeds losses viability after 6 months if stored in ambient conditions and by 8 months if stored in refrigerated conditions. The germination was initially low irrespective of the locations and storage conditions and it reaches the maximum by 3rd months and thereafter again decreases gradually. In refrigerated storage, at 3rd month after storage, the maximum germination was obtained in the seeds collected from Reiek (96.67 ± 5.77%) at 3 months after storage, while in ambient conditions, the maximum germination was obtained from the seeds collected from Reiek (90.00 ± 10.00%) at 2 months after storage.

Keywords *Clerodendrum colebrokianum* Walp., germination, North east India, Mizoram, Seeds.

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Introduction

The genus *Clerodendrum* is flowering plants belongs to the family Verbenaceae and is widely distributed in the tropical and warm temperat regions of the world, with most of the species occurring in tropical and Northern Africa, Asia, Egypt and Madagascar. *Clerodendrum* is a very large and diverse genus with about 580 identified species is distributed throughout the world [1]. It is the largest genus of the tribe Teucrieae. In India 23 species were recorded by [2], of

Table 1. Different locations and their geographical coordinates.

Sl. No.	Location	Latitude	Longitude	Elevation (m)
1	Durtlang	N 23°46´30.0´´	E092°44´00.3´´	1254
2	Reiek	N 23°41´45.7´´	E092°36´44.0´´	1148
3	Luangmual	N23°45´39.1´´	E092°43´19.4´´	1041
4	Lungdai	N 23°53´11.9´´	E092°44´34.5´´	1132
5	Serkhan	N 23°54´25.2´´	E092°44´27.6´´	1028
6	Zonuam	N 23°44´12.9´´	E092°42´11.1´´	1109
7	Chawlhh-mun	N 23°44´39.6´´	E092°41´32.1´´	942
8	Tanhril	N 23°44´21.1´´	E092°40´59.5´´	901
9.	Sakawrtui-chhun	N 23°76´11	E92°67´11´´5	440
10	Lengpui	n 23°84´05.99´´	E92°61´96.89´´	426.11

which 16 were recorded from Arunachal Pradesh by [3]. *Clerodendrum colebrookianum* Walp. commonly known as East Indian Glory Bower is a flowering shrub or small tree, characterized by a foetid smell. The species is found in tropical and subtropical regions of Asia including India, Myanmar, Bangladesh, Malaysia, Indonesia, Thailand, Bhutan and Nepal; and also in temperate China. It is erect reaches up to 1.5–3.5 m in height and is evergreen. Branchlets are usually 4-angled when young. Leaves are simple, opposite or rarely whorled. Leaf base is wedge-shaped to heart-shaped, margin entire to slightly wavy, tip long-pointed to pointed. Flowers are white and borne in 4-6-branched corymbose cymes, at the end of branches. Inflorescences loosely cymose orcapitate, in terminal or rarely axillary paniculatethyrses. Calyx is campanulate or cup-shaped, densely pubescent. Corolla with a slender tube; lobes 5, spreading. Fruit is a drupe with 4 1-seeded pyrenes, sometimes separating into 2 2-loculed or 4 1-locular mericarps. It flowers during post-monsoon, from August to December. East Indian Glory Bower is found in NE India, and parts of China and SE Asia. *C. colebrookianum*, has been reported to have antidiabetic, antihypertensive and sedative properties [4—7]. In Mizoram, it is considered as anti-cancer, used to increase breast milk [8]. Besides, it is one of the delicious vegetable recipes of local people.

The species *Clerodendrum colebrokianum* being important medicinal plants as well as vegetables is a native plant of Mizoram, North-east India. The plant is usually consumed for effective control of high blood pressure, hypertension and diabetes and found to possessed antimicrobial activity also. Local people consumed as vegetables instead of medicinal values. Unlike other vegetables, this plant is available throughout the year and also gives continuous income to the farmers. Once the plant has established, it can be last long as it is a perennial shrub. *Clerodendrum colebrokianum* is a shallow rooted crop and lack tap root, grows horizontally. From these roots a new plants arises and becomes difficult to maintain the proper spacing. Once we uproot the whole plant, a new plant has come up from the intact root which is left remaining. Leaf are the used part and is inhibited by ants and somewhat bitter in taste. The leaf boiled with water is proved to increase breast milk. The viability test showed that the seeds of *Clerodendron colebrokianum* are recalcitrant seeds and it cannot be stored for a long period.

Seed is a unit of life developing from fertilized ovule. They represent the mostcritical phase of a plant's life cycle and are responsible for the evolutionary continuum of plant species. Seed characteristics, germination preferences and seed dormancy patterns have been proposed as tools for understanding evolutionary patterns [9]. The time duration for which the seeds retain their viability varies with species and to some extent with the prevailing environmental conditions. There are seeds which remain viable for hundreds of years and those which lose their viability within a week or a month. What brings about the state of non-viability in a seed has been the subject of extensive investigation.

Materials and Methods

The investigation was carried out in the laboratory of Department of Horticulture, Aromatic and Medicinal Plants, Mizoram University, Aizawl during 2015–2017.

Table 2. Physical parameters of the seeds from different locations.

Sl. No.	Location	Weight (g)	Length (mm)	Diameter (mm)	Moisture loss (%) 30 days	60 days	90 days	Seed volume (cc)	Imbibition (%)
1	Durtlang	0.22±0.02	6.43±0.20	5.02±0.30	17.01±0.02	17.92±0.02	19.13±0.02	0.55±0.07	34.82±3.79
2	Reiek	0.40±0.02	7.38±0.46	5.86±0.48	15.22±0.02	15.92±0.01	15.92±0.01	0.86±0.12	30.70±2.67
3	Luangmual	0.26±0.02	6.81±0.15	5.45±0.18	15.41±0.01	16.76±0.01	16.77±0.01	0.69±0.11	39.71±6.94
4	Lungdai	0.24±0.02	6.84±0.45	5.47±0.21	13.95±0.03	14.32±0.15	14.25±0.01	0.61±0.08	43.91±1.98
5	Serkhan	0.27±0.04	6.64±0.24	5.15±0.18	10.05±0.03	13.51±0.01	18.70±0.01	0.70±0.12	34.79±2.42
6	Zonuam	0.38±0.03	7.02±0.28	5.65±0.13	14.08±0.02	14.80±0.01	15.47±0.01	0.73±0.08	38.98±7.60
7	Chawlhhmun	0.21±0.01	6.92±0.41	5.50±0.19	13.06±0.01	14.31±0.02	16.04±0.01	0.68±0.08	36.29±9.82
8	Tanhril	0.25±0.01	6.71±0.38	5.22±0.20	17.65±0.01	18.26±0.02	18.28±0.01	0.64±0.11	33.83±5.11
9	Sakawrtui-chhun	0.24±0.03	6.46±0.24	5.10±0.35	12.63±0.02	14.12±0.01	14.13±0.00	0.76±0.08	32.48±1.48
10	Lengpui	0.23±0.02	6.66±0.21	5.37±0.24	14.17±0.01	15.08±0.02	15.85±0.01	0.74±0.12	31.09±2.10
SEd (±)		0.02	0.23	0.19	0.012	0.041	0.008	0.07	3.42
$CD_{0.05}$		0.04	0.49	0.40	0.026	0.087	0.019	0.15	7.19

Seeds of *Clerodendron colebrokianum* were harvested after ripening during the month of November, 2015 and 2016 from ten natural populations having altitudes between 426.11–1254 m asl. These populations include Durtlang, Reiek, Luangmual, Lungdai, Serkhan, Zonuam, Chawlhhmun, Tanhril, Sakawrtuichhunand Lengpui. The geographical co-ordinates of different locations are presented in Table 1. The seeds were collected to study the physical properties of the seeds in terms of seed weight, diameter, length, volume of the seeds, moisture content, imbibition, viability and germination percentage. After harvesting of the fruits, the seeds were air dried for one week and kept in a perforated polythene bags for further observations.

Immediately after collection of seeds, 20 seeds from each replication were randomly selected for recording the physical parameters of the seeds. The weight of the seeds was measured with the help of digital balance and expressed in gram (g). Similarly, the length and diameter of the seeds were measured with the help of verniercallipers and the result was expressed in mm.

The initial weight of the seeds was first measured by using digital weighing balance and recorded. The moisture loss of the seeds were measured at every 30 days interval till 90 days. After completion of this experiment, the moisture loss percentage at each level was calculated by using this formula:

$$\text{Moisture loss \%} = \frac{\text{Moisture loss}}{\text{Fresh weight}} \times 100$$

Seed volume was measured by water displacement method for which seeds were dipped in a known volume of water in a measuring cylinder and after immersing the seeds, the rise in water level was noted. Ten replicates with 10 seeds in each replicate was used for this purpose. Seed volume (V) was calculated using following formula:

$$V = V_2 - V_1$$

Where V_1 is initial water level and V_2 is final level after dipping the seeds. Mean seed volume with standard deviation was calculated.

To determine the water uptake by the seeds, five replicates of five seeds each was randomly taken and weighed individually for their initial weight using the electric balance. Seeds was then soaked in distilled water and kept at room temperature (25+2°C). Weight of these seeds was taken after every 24 h till the constant weight.

Water imbibition was estimated as percent increase in weight of seed using following formula:

$$\text{Imbibition rate} = \frac{\text{Imbibed weight - Initial weight}}{\text{Initial weight}} \times 100$$

Table 3. Monthwise germination per cent of seeds at refrigerated condition.

Sl. No.	Location	December	January	February	March	April
1	Durtlang	63.33 ± 5.77	76.67 ± 11.55	86.67 ± 5.77	73.33 ± 11.55	66.67 ± 5.77
2	Reiek	66.67 ± 5.77	66.67 ± 5.77	96.67 ±5.77	76.67 ± 15.28	56.67 ± 5.77
3	Luangmual	46.67 ± 5.77	93.33 ± 5.77	76.67 ± 5.77	66.67 ± 5.77	83.33 ± 15.28
4	Lungdai	40.00 ± 14.14	90.00 ± 10.00	73.33 ± 11.55	83.33 ± 15.28	76.67 ± 5.77
5	Serkhan	53.33 ± 5.77	83.33 ± 15.28	86.67 ± 5.77	63.33 ± 15.28	83.33 ± 11.55
6	Zonuam	43.33 ± 15.28	83.33 ± 5.77	76.67 ± 15.28	70.00 ± 20.00	70.00 ± 10.00
7	Chawlhhmun	50.0 ± 10.00	73.33 ± 11.55	63.33 ± 11.55	66.67 ± 5.77	70.00 ± 10.00
8	Tanhril	53.3 ± 15.28	76.67 ± 11.55	83.33 ± 15.28	80.00 ± 10.00	73.33 ± 15.28
9	Sakawrtuichhun	56.7 ±5.77	86.67 ± 5.77	86.67 ± 15.28	66.67 ± 15.28	63.33 ± 25.17
10	Lengpui	60.0 ± 20.00	80.00 ± 10.00	66.67 ± 5.77	66.67 ± 30.55	56.67 ± 5.77
	SEd (±)	7.22	6.83	6.50	5.74	7.95
	$CD_{0.05}$	15.16	14.35	13.65	12.06	16.71

Table 3. Continued.

Sl. No.	Location	May	June	July	August
1	Durtlang	43.33 ± 15.28	43.33 ± 5.77	3.33 ± 5.77	3.33 ± 5.77
2	Reiek	30.00 ± 10.00	40.00 ± 17.32	6.67 ± 5.77	0.00 ± 0.00
3	Luangmual	36.67 ± 15.28	26.67 ± 5.77	0.00 ± 0.00	0.00 ± 0.00
4	Lungdai	40.00 ± 17.32	30.00 ± 20.00	3.33 ± 5.77	0.00 ± 0.00
5	Serkhan	30.00 ± 10.00	30.00 ± 17.32	0.00 ± 0.00	3.33 ± 5.77
6	Zonuam	26.67 ± 5.77	40.00 ± 10.00	6.67 ± 11.55	0.00 ± 0.00
7	Chawlhhmun	33.33 ± 25.17	26.67 ± 5.77	3.33 ± 5.77	0.00 ± 0.00
8	Tanhril	40.00 ± 20.00	23.33 ± 5.77	3.35 ± 5.77	0.00 ± 0.00
9	Sakawrtuichhun	30.00 ± 20.00	26.67 ± 11.55	3.33 ± 5.77	3.33 ± 5.77
10	Lengpui	33.33 ± 20.82	20.00 ± 10.00	0.00 ± 0.00	0.00 ± 0.00
	SEd (±)	4.65	6.02	0.08	0.08
	$CD_{0.05}$	9.77	12.64	0.16	0.16

Germination test was conducted at monthly interval in refrigerated condition and ambient temperature. The seeds collected from different locations were kept in perforated polythene bags inside the refrigerator to study the effect of germination at monthly interval in refrigerated condition and ambient temperature.

Results and Discussion

The results of the present study as depicted in Table 2 revealed that among the various locations, significant to highly significant variation was observed among the seeds. The maximum seed wight was obtained from the seeds collected from Reiek (0.40±0.02 g) followed by the seeds collected from Zonuam (0.38±0.03 g). Among all the locations the lowest seed weight was obtained in Chawlhhmun (0.21±0.01 g). Similarly, among the different locations, the significantly maximum seed length was obtained from the seeds collected from Reiek (7.38±0.46 mm) followed by Zonuam (7.02±0.28 mm). Among the different seeds collected from various locations, the significantly lowest length was obtained from the seeds collected from Sakawrtuichhun (6.46±0.24 mm). Similarly, there was significant variation among the germplasms with respect to diameter of the seeds. The maximum was obtained from the seeds collected from Reiek (5.86±0.48 mm) and the minimum was obtained from the seeds collected from Durtlang (5.02±0.30 mm). The variation in physical properties of the seeds might be due to different genetic make-up of the individual seeds.

Seed moisture is a critical factor determining the

Table 4. Monthwise germination per cent of seeds at ambient condition.

Sl. No.	Location	December	January	February	March	April
1	Durtlang	50.00 ± 10.00	70.00 ± 10.00	86.67 ± 5.77	70.00 ± 26.46	66.67 ± 11.55
2	Reiek	50.00 ± 10.00	90.00 ± 10.00	80.00 ± 0.00	66.67 ± 15.28	60.00 ± 10.00
3	Luangmual	46.67 ± 15.28	66.67 ± 5.77	70.00 ± 10.00	63.33 ± 5.77	53.33 ± 5.77
4	Lungdai	43.33 ± 15.28	56.67 ± 5.77	63.33 ± 5.77	56.67 ± 5.77	50.00 ± 10.00
5	Serkhan	43.33 ± 5.77	76.67 ± 5.77	60.00 ± 10.00	86.67 ± 15.28	70.00 ± 10.00
6	Zonuam	33.33 ± 5.77	70.00 ± 10.00	70.00 ± 10.00	80.00 ± 20.00	50.00 ± 10.00
7	Chawlhhmun	46.67 ± 15.28	56.67 ± 15.28	86.67 ± 5.77	63.33 ± 15.28	43.33 ± 15.28
8	Tanhril	53.33 ± 15.28	76.67 ± 15.28	86.67 ± 23.09	70.00 ± 10.00	40.00 ± 20.00
9	Sakawrtuichhun	46.67 ± 5.77	53.33 ± 11.55	73.33 ± 5.77	70.00 ± 17.32	36.67 ± 11.55
10	Lengpui	36.67 ± 5.77	46.67 ± 15.28	73.33 ± 15.28	76.67 ± 5.77	40.00 ± 20.00
	SEd (±)	4.77	6.61	6.71	7.08	8.66
	$CD_{0.05}$	10.01	13.89	14.10	14.87	18.20

Table 4. Continued.

Sl. No.	Location	May	June	July	August
1	Durtlang	26.67 ± 5.77	0.00 ± 0.00	0.0 ± 0.0	0.0 ± 0.0
2	Reiek	23.33 ± 15.28	3.33 ± 5.77	0.0 ± 0.0	0.0 ± 0.0
3	Luangmual	16.67 ± 5.77	6.67 ± 11.55	0.0 ± 0.0	0.0 ± 0.0
4	Lungdai	23.33 ± 20.82	0.00 ± 0.00	0.0 ± 0.0	0.0 ± 0.0
5	Serkhan	23.33 ± 15.28	3.33 ± 5.77	0.0 ± 0.0	0.0 ± 0.0
6	Zonuam	13.33 ± 5.77	6.67 ± 11.55	0.0 ± 0.0	0.0 ± 0.0
7	Chawlhhmun	26.67 ± 23.09	0.00 ± 0.00	0.0 ± 0.0	0.0 ± 0.0
8	Tanhril	13.33 ± 11.55	0.00 ± 0.00	0.0 ± 0.0	0.0 ± 0.0
9	Sakawrtuichhun	26.67 ± 15.28	6.67 ± 11.55	0.0 ± 0.0	0.0 ± 0.0
10	Lengpui	30.00 ± 26.46	0.00 ± 0.00	0.0 ± 0.0	0.0 ± 0.0
	SEd (±)	4.86	2.58		
	$CD_{0.05}$	10.22	5.42		

viability and longevity of both recalcitrant and orthodox seeds. In our present investigation, the moisture loss was increased with the storage period right from 30 days after storage till 90 days. Among the seeds collected from various locations, the moisture loss was also varied significantly throughout the observation periods. It is clear from the data presented in Table 2, that at 30 days after storage, the maximum moisture loss was observed in found from the seeds collected from Tanhril (17.65 ± 0.01%), while the lowest was reported from the seeds collected from Serkhan (10.05 ± 0.03%). At 60 days after storage, the moisture loss followed the similar pattern as that of 30 days. The maximum was in Tanhril (18.26±0.02%), and the lowest was in Serkhan (13.50 ± 0.01%). At 90 days after storage of the seeds, the maximum moisture loss was observed in found from the seeds collected from Durtlang (19.13±0.02%), while the lowest was from the seeds collected from Sakawrtuichhun (14.13±0.00%). The variation in moisture content of this plant might be due to different ripening stage of the fruits as well as different soil and climatic condition and inherent character associated with species.

The seeds varied significantly with respect to the volume of seeds also (Table 2). The highest seed volume of was found in the seeds collected from Reiek (0.86±0.12 cc) and lowest was in Durtlang (0.55±0.07 cc). The variation in seed volume might be due to the size of the seed coat, in addition embryo of the seeds also increases the volume of the seed.

The imbibition result presented in Table 2 showed that percentage imbibition was found to be the high-

est in Lungdai (43.91±1.98) and the lowest was observed in Reiek (30.70±2.67). Claimed that porous seed coats are usually permeable and non-porous ones impermeable. The seeds of *Clerodendrum* have small opening or pore on the ventral side of the seeds. Water can pass through easily so that the imbibition speed can be enhanced by this opening. But there should be limitations for entering the water into the seeds. It also depends upon the size of the embryo and the hardness of the seed coat.

There are two types of seeds in nature. One is orthodox and another one is recalcitrant seeds. Orthodox seeds dry up-to low moisture content and can tolerate freezing temperature and can be stored for many years. But recalcitrant seeds are dessicated sensitive, loss viability in a short period and cannot be stored for prolonged. Attempted has been made on the storage of recalcitrant seeds, such as moist storage, partially dry storage and cryostorage, but all these methods have their own limitations. The seeds *Clerodendrum colebrokianum* comes under the latter. The seeds were collected in the month of November and germination test conducted from December till it losses viability. The seeds we stored in ambient temperature (room temperature) losses viability after 6 months of stored in ambient conditions and by 8 months if stored in refrigerated conditions. The germination was initially low irrespective of the locations and storage conditions and it reaches the maximum by 3rd months and thereafter again decreases gradually and finally loss the viability by 6 months in ambient condition and 8 months in refrigerated condition. In the present investigation, in refrigerated storage, at 3rd month after storage, the maximum germination was obtained in the seeds collected from Reiek (96.67 ± 5.77) at 3 months after storage, while in ambient conditions, the maximum germination was obtained from the seeds collected from Reiek (90.00 ± 10.00%) at 2 months after storage (Tables 3 and 4). Viability test indicated that seeds of *C. colebrokianum* may not remain viable for extended periods at room temperature and even storage at low temperature (4^{o}C – 6^{o}C) for long time. Loss of viability in stored seeds is a common phenomenon, and it increased with storage duration with storage condition as another factor. However, it was also observed that seed moisture content did not played key role as population with low seed moisture at the time of harvesting had high viability in *C. colebrokianum*, Further, seed moisture was slightly decreased after one year storage. Loss of viability as well as variation in seed viability among different natural populations may have the relation with growth and development of embryo which caused morpho-physiological dormancy as suggested earlier [10] in another Apiaceae species *Osmorhiza depauperata*.

Conclusion

Clerodendrum colebrokianum seeds are recalcitrant so they loss the viability very soon. In the present investigation, observations reveals variation in seed weight, seed length, seed diameter and seed volume in addition to moisture content as well as imbibition rate of the seeds. Viability among the different populations irrespective of moisture content which may suggests morpho-physiological type of dormancy in this species. On the basis of present observations, it is further suggested that seed storage at low temperature remain viable for eight months. Further observations are needed to overcome morpho-physiological dormancy and bringing uniformity in germination behavior of *C. colebrokianum*.

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