"Standardization of nursery technology in two endemic and endangered species: *Flemingia tuberosa* Dalzell and *Merremia rhyncorhiza* Hallier"

Tungenwar A G, Baig R S, Khan K Z and Surwase B S
DST-FIST and UGC-SAP Supported School of Life Sciences, Swami Ramanand Teerth Marathwada University, Vishnupuri, Nanded – 431606, Maharashtra, India

Corresponding author- Dr Surwase B S bsurwase@rediffmail.com

Abstract

Flemingia tuberosa Dalz. of family Fabaceae has edible tubers and are eaten by locals fresh or cooked. It is also useful in case of dysentery and vaginal discharges. Because of it's over exploitation, it has become endemic and endangered. *Merremia rhyncorhiza* Hallier of Convolvulaceae flowers and fruits in October. It bears a bunch of edible tuberous roots, which are eaten fresh or cooked. Leaves are utilized as vegetable by locals. It is also reported as an endemic and endangered species. Sprouting response of horizontally cut upper $1/4^{th}$ tuber segments was similar to that of whole tubers in both the selected species. Also, seed germination obtained with needle scarification gave 80% germination within 8-10 days for both the species. Half strength MS alone was the best for *in vitro* seed germination with 70 % result for *Flemingia tuberosa* and 80 % result for *Merremia rhyncorhiza* species respectively. These standardized protocols may be utilized for the conservation of these selected endemic and endangered species.

KEYWORDS: Nursery technology, seed germination, sprouting response, endemic and endangered plants, conservation

INTRODUCTION

Industrialization, urbanization and use of land for agricultural crop production is the major reason for habitat destruction of most of the plant species to make them endemic and endangered, during last few centuries of industrial revolution in the world. Therefore, proper management of traditional biodiversity has become a matter of utmost urgency. Generally, most of the medicinal plants are slow growing trees in forests, bulbous and tuberous plants. Underground parts of these plants are being mainly utilized or directly consumed. Therefore, effective strategies which would satisfy the requirements of sustainable harvesting of these and endemic and endangered plant species are needed (Tasheva and Kosturkova, 2013; Pauchard *et al*., 2006).The Western Ghats constitute 27 % of Indian plants with 4000 species. Out of which, 1500 are endemic (Dudani *et al*., 2012).

Western Ghats and Eastern Himalayas are the two mega biodiversity hotspots in India, out of which Western Ghats are recently given the status of world heritage by UNESCO. Western Ghats are rich in flora and fauna and 1/3rd of endemic plants are on the boundary of rare and threatened status. Even, most of them get extinct before they are identified (Stohlgren and Kumar, 2013).

***Flemingia tuberosa* Dalzell:** *Flemingia* genus is chiefly native of tropical Asia and Australia. A few species are also found in tropical Africa. There are about 26 species (Cooke, 1908). In India, *Flemingia tuberosa* Dalz. is found in Maharashtra, Kerala, Karnataka, Gujarat, Goa and Tamilnadu. In Maharashtra, it is found in Raigad, Ratnagiri and Thane district (Prasad *et al.*, 2011; Laxminarsimham and Prasanna, 1983; Singh *et al.*, 2000). *Flemingia tuberosa* of Fabaceae is an herb. Locally, it is known as Birmova/ Khaparkudi in Marathi and Jaambula Gadde in Kannada. Its synonym is *Moghania tuberosa* Dalz. Talbot (1909) considered it as apparently endemic to Konkan and North Kanara. Bhat (2003) reported it as 'very rare' in Udupi district of Karnataka. Kothari and Moorthy (2000) reported it as rare in moist deciduous forests of Raigad, Ratnagiri and Thane district of Maharashtra. Datar and Lakshminarasimham (2013) reported this species as endemic. Singh and Divakar (2013) reported it as endemic to Manmanhara plateau of Karnataka.

It is an herb, stem 0.9-1.2 cm long, trailing, branches terete, roots tuberous, leaves oblong-elliptic or lanceolate, flowers solitary, in few flowered axillary racemes, corolla lilac, seeds oval, flowering and fruiting during October to December (Prasad *et al*., 2011).

Most of the medicinal plant species bear specific medicinal significance along with nutritional role in the human diet. *Flemingia tuberosa* Dalz., which is an endemic and endangered plant species, possesses edible tubers which are nutritional (Cooke, 1908; Nadkarni, 1982). The tubers are eaten by locals fresh as well as cooked (Cooke, 1908). The tuberous roots of *Flemingia* are sweet and astringent and are useful in dysentery and vaginal discharges (leucorrhoea). Tubers contain yellow resin, sugars, gum, asparagin, starch and tannins (Nadkarni, 1982). This species has become endangered mainly due to vigorous exploitation of its edible tubers by the local population (Surwase and Kulkarni, 1998).

***Merremia rhyncorhiza* Hallier:** There are about 40 species of *Merremia* distributed in warmer parts of the world. In India, it is found in Western Ghats of Maharashtra and adjoining parts of Cannara of Karnataka (Cooke, 1908). *Merremia rhyncorhiza* of Convolvulaceae is an endangered, endemic, perennial and spreading hirsute herb. It bears a bunch of robust tuberous roots. Locally, tuberous roots known as Ran ratale or Nangarvel in Marathi, are consumed raw/cooked mainly by cowherds. This is apparently the main reason for this taxon to have reached endangered status (Surwase, 2000). It is reported to be rare. The tubers are eaten and greedily sought by the natives. The leaves are also used as vegetable (Cooke, 1908). Watve (2013) reported this species as endemic to Maharashtra.

Each plant bears a bunch of 15 nepiform tubers. Short aerial erect stem bears prostate spreading branches. Leaves tri lobed, inflorescences are axillary cymes with yellow flowers. Flowering and fruiting occurs in October.

Merremia rhyncorhiza is used as food because tubers are eaten and greedily sought by the natives. The leaves are also used as vegetable (Cooke, 1908).

Merremia rhyncorhiza is reported as one of the endemic and endangered species which is not studied much.

Since these species are rare and endangered, there is a call for urgent attention for conservation of these rare palatable and medicinally important plants. Nursery protocols need to be developed for their multiplication. Therefore, considering this *Flemingia tuberosa* and *Merremia rhyncorhiza* were selected for the current research work

MATERIAL AND METHODS

Collection, identification and authentication of plant material:

***Flemingia tuberosa* Dalzell:** *F. tuberosa* Dalzell plant material was collected from Utthan Hills near Dongri village of Mumbai, Maharashtra, India. It was authenticated by Dr. R. M. Mulani in Botany Division using Regional Flora and Voucher specimen (No. SRTMU/SLS/2012-111) is deposited in the herbarium of the School of Life Sciences, S.R.T.M. University, Nanded (MS). Collected seeds and tubers were planted in Botanical Garden of S.R.T.M. University, Nanded (MS).

***Merremia rhyncorhiza* Hallier:** *Merremia rhyncorhiza* Hallier plant material was collected from its natural habitat – Amboli Ghats situated near Sawantwadi, Sindhudurg District, Maharashtra, India, which is a famous tourist spot. It was also authenticated by Dr. R. M. Mulani in Botany Division using Regional Flora and Voucher specimen (No. SRTMU/SLS/2012-112) is deposited in the herbarium of the School of Life Sciences, S.R.T.M. University, Nanded (MS). Collected seeds and tubers were planted in Botanical Garden of S.R.T.M. University, Nanded (MS).

Autoecological studies in *Flemingia tuberosa* Dalzell and *Merremia rhyncorhiza* Hallier

Species performance: During the present investigation, population of *Flemingia tuberosa* Dalzell found in the nature at Utthan Hills near Dongri village of Mumbai, Maharashtra, India was utilized for autoecological studies. For auto ecological studies in *Merremia rhyncorhiza* Hallier, population at Amboli Ghats, Near Sawantwadi, District Sindhudurg was utilized.

The morphological parameters used for autoecological studies were average height of main stem, average number of branches, average length of branches, average number of leaves, buds, seeds, branches/plant, fresh weight and dry weight parameters, mean leaf area, average weight of seeds etc.

The plant observations are based on an average of minimum fifty and maximum 100 plants. An average dry weight of pods was based on an average of 5 lots. The weight of pods was determined using 0.001 gm sensitivity of Shimadzu electronic balance.

Nursery Technology: Sprouting response of tubers and seed germination in *Flemingia tuberosa* Dalzell

Sprouting response of tubers in *Flemingia tuberosa* Dalzell:

Sprouting response of tubers in *F. tuberosa* was studied at pot level containing garden soil. Horizontally and vertically cut ¼th tubers were used for sprouting response. This sprouting response of tubers was compared with its whole tubers.

Seed germination studies in *F. tuberosa* Dalz.: Initially, seeds of *F. tuberosa* were collected from uniform population from its natural habitat. Seeds were air dried for 15-20 days in laboratory conditions and used for seed germination studies.

The seed germination percentage was calculated with seedling emerged out of total number of seeds tried for germination. This experiment was carried out in petridish (diameter 10 cm) with moist discs of blotting papers. Five replicates were used for each treatment and the mean values were given in text.

Concentrated $H_2SO_4$was used for chemical scarification. To remove the traces of acid, seeds were washed under running tap water and at last with double distilled water. For mechanical scarification, seed coat was broken with the help of sand paper or by removal of pericarp with the help of needle. Physical scarification was done with the help of washing seeds under running tap water for about 12 hours or by hot water treatment.

Nursery Technology: Sprouting response of tubers and seed germination in *Merremia rhyncorhiza* Hallier:

Sprouting response of tubers in *Merremia rhyncorhiza*: Sprouting response of tubers in *M. rhyncorhiza* was studied at pot level containing garden soil. Horizontally and vertically cut ¼th tubers were used for sprouting response. This sprouting response of tubers was compared with its whole tubers.

Seed germination studies in *Merremia rhyncorhiza*: Initially, seeds of *M. rhyncorhiza* were collected from uniform population from its natural habitat. Seeds were air dried for 15-20 days in laboratory conditions and used for seed germination studies.

***In vitro* seed germination studies:**

***In vitro* seed germination studies in *Flemingia tuberosa* Dalzell and *Merremia rhyncorhiza* Hallier:**
F. tuberosa seeds were randomly collected from uniform population and stored in glass bottles. Air dried seeds were used for *in vitro* seed germination. Seeds were washed under running tap water for half an hour. Thereafter, the seeds were rewashed with double distilled water. Then, these seeds were surface sterilized with 1 % (w/v) $HgCl_2$ solution (Hi-Media) for 7-10 minutes. Finally, the seeds were given 4 or 5 times wash with sterile double distilled water under laminar flow cabinet to remove the traces of mercuric chloride prior to placing onto MS, ½ MS and Knudson medium alone.

RESULTS

A. *Flemingia tuberosa*: Autoecological studies in *Flemingia tuberosa* Dalz.: *Flemingia tuberosa* Dalz. of Convolvulaceae is an annual, scandent or trailing and tuberous herb (Plate I, Fig. a). It grows well in well drained moist places especially on grassy and sloppy hilly soil. The plants flower during September to October. Flowers are light pink in color. Seeds mature in November and plants dry up by the end of November. The plant material was collected from Utthan hills near Dongri in Bhayander area of Mumbai district of Maharashtra (Pate I, Fig. b and c). The analysis of growth parameters of plants growing at Utthan hills is given in Table No.1.

Table No 1: Autoecological studies in *Flemingia tuberosa* Dalz.:

Sr. No	Parameter	Average ± SE
1	Length of main stem (cm)	50.5 ± 0.6
2	Diameter of main stem (cm)	0.9 ± 0.0
3	Total number of branches / plants	5.3 ± 0.6
4	Total fresh weight of stem and branches / plant (gm)	12.6 ± 0.7
5	Total dry weight of stem and branches / plant (gm)	5.38 ± 0.4
6	Total number of leaves / plants	43.0 ± 0.1
7	Total fresh weight of all leaves / plant (gm)	4.2 ± 0.1
8	Total dry weight of all leaves / plant (gm)	2.2 ± 0.0
9	Total number of pods per plant	16.0 ± 1.5
10	Total fresh weight of pods / plant (gm)	1.4 ± 0.1
11	Total dry weight of pods / plant (gm)	0.7 ± 0.0
12	Average number of seeds per pod	1.00 ± 0.0
13	Length of tuber / plant (cm)	3.6 ± 0.3
14	Fresh weight of tuber / plant (gm)	7.2 ± 0.1
15	Dry weight of tuber / plant (gm)	4.1 ± 0.4
16	Total dry biomass / plant (gm)	12.38 ± 0.5

Nursery Technology: Sprouting response of tubers in *Flemingia tuberosa* Dalz. : Vertically and horizontally cut 1/4th tubers were used for sprouting response. It was observed that only horizontally cut upper 1/4th tubers sprouted within 10-12 days, indicating that there is only one bud on the top of tuber. This response was similar to like that whole tubers (Plate I, Fig. b).Vertical cut 1/4th tubers did not sprout indicating that the bud on the top of tuber has been damaged during its cutting.

Seed germination studies in *Flemingia tuberosa* Dalz.: Mature seeds of *F. tuberosa* have poor seed germination capacity due to presence of hard seed coat (Plate I, Fig. c). The seeds harvested from the nature were subjected to mechanical scarification with needle, chemical scarification with H_2SO_4, hot water treatment, washing seeds under running tap water for 12 hrs and removal of seed coat treatment with a view to break the dormancy.

Unscarified seeds gave just 10% germination. The mechanical scarification gave 80% germination. However, hot water treatment and chemical scarification did not enhance seed germination.

Washing seeds under running tap water for 12 hours gave 50% germination within 8-10 days. The removal of seed coat enhanced seed germination to 70%. The results are summarized in Table No 2.

Table No 2: Seed germination studies in *Flemingia tuberosa* Dalz.:

Sr. No.	Treatment	% of Germination
1	Unscarified seeds	10
2	**Scarification with needle**	**80**
3	Hot water treatment (10-15 min)	70
4	Chemical scarification with H_2SO_4 (95-98 %) for 2 min	70
5	Washing seeds under running tap water for 12hrs	50
6	Removal of seed coat	70

***In vitro* seed germination studies in *Flemingia tuberosa* Dalz. :** The seeds were surface sterilized and inoculated on seed germination media like full strength MS medium, ½ strength MS medium and Knudson medium. The highest germination percentage was recorded after 30 days of inoculation.

Effect of ½ MS, full MS and Knudson medium on seed germination of *F. tuberosa*:

The seeds inoculated on half strength MS medium showed 70 % germination (Plate I, Fig. d). Whereas, seeds inoculated on full strength MS medium showed 40 % germination. Use of specific seed germination medium like Knudson medium and MS medium containing other hormones or charcoal did not have any positive effect over seed germination. The results are summarized in Table No.3.

Table No.3: Effect of ½ MS, full MS and Knudson medium on seed germination of *F. tuberosa*:

Sr. No.	Treatment	% of Germination
1	½ MS Medium	70
2	Full MS Medium	40
3	Knudson Medium	10

B. *Merremia rhyncorhiza* Hallier: Autoecological studies in *Merremia rhyncorhiza* Hallier: *Merremia rhyncorhiza* Hallier of Convolvulaceae is a perennial spreading herb (Plate I, Fig a), which grows on grassy hills of Amboli Ghats, Near Sawantwadi of Sindhudurg district of Maharashtra. It bears a bunch of tuberous roots. These tubers (Plate I, Fig b) are edible and are eaten by locals raw or cooked. This is apparently the main reason for this taxon to have reached endangered status. Its leaves are trifoliate, inflorescences are axillary cymes with yellow flowers. Seeds are small and black colored (Plate I, Fig c) It flowers and fruits in October. The leaves are also used as vegetable. The Analysis of growth parameters of plants growing at Amboli Ghats is given in Table No. 4.

Table No.4: Autoecological studies in *Merremia rhyncorhiza* Hallier

Sr. No	Parameter	Average ± SE
1	Length of plant (cm)	100.03±1.29
2	Diameter of main stem (cm)	1.50 ± 0.0
3	Total No of branches / plants	12.2 ± 0.6
4	Total fresh weight of stem and all branches/plant (gm)	40.7 ± 1.2
5	Total dry weight of stem and branches / plant (gm)	8.5 ± 0.4
6	Total number of leaves / plants	312.2 ± 0.2
7	Total fresh weight of all leaves / plant (gm)	8.7 ± 0.8
8	Total dry weight of all leaves / plant (gm)	1.3 ± 0.4
9	Total number of pods per plant	266.1 ± 1.4
10	Total fresh weight of pods / plant (gm)	10.9 ± 0.2

11	Total dry weight of pods / plant (gm)	1.1 ± 0.1
12	Average number of seeds per pod / plant	4.00 ± 0.2
13	Length of main root / plant (cm)	11.0 ± 0.5
14	Fresh weight of roots / plant (gm)	13.7 ± 0.3
15	Dry weight of roots / plant (gm)	2.2 ± 0.2
16	Total dry biomass / plant (gm)	13.1 ± 0.3

Nursery technology in *M. rhyncorhiza* Hallier: Sprouting response of tubers in *M. rhyncorhiza*:

Vertically and horizontally cut 1/4th tubers were used for sprouting response. It was observed that only horizontally cut upper 1/4th tubers sprouted within 10-12 days, indicating that there is only one bud on the top of tuber. This response was similar to like that of whole tubers. Vertical cut 1/4th tubers did not sprout indicating that the bud on the top of tuber has been damaged during its cutting.

Seed germination studies in *Merremia rhyncorhiza*: Mature seeds of *Merremia rhyncorhiza* Hallier have poor seed germination capacity due to presence of hard seed coat. Unscarified seeds gave 30% germination. Scarification with needle gave 80% germination and was found to be the best among different treatments tried. However, other treatments like hot water and chemical scarification did not increase the seed germination. The results are summarized in Table No.5.

Table No. 5: Seed germination studies in *Merremia rhyncorhiza* Hallier:

Sr. No.	**Treatment**	**% of germination**
1	Unscarified seeds	30
2	Scarification with needle	80
3	Hot water treatment (10 min)	60
4	Chemical scarification with H_2SO_4 (95-98%) for 2 min	70
5	Washing seeds under running tap water for 12 hrs	70

***In vitro* seed germination studies in *M. rhyncorhiza*:** The seeds were surface sterilized with 1.0 % $HgCl_2$ and inoculated on seed germination media like full strength MS medium, ½ strength MS medium alone and Knudson medium. The highest germination percentage was recorded after 30 days of inoculation.

Effect of Full MS, ½ MS and Knudson medium alone on *in vitro* seed germination of *M. rhyncorhiza*: The seeds were inoculated on full strength MS medium, ½ strength MS

medium and Knudson medium. In Knudson medium, the 30 % seeds and on full MS alone 40 % seeds germinate within 30 days. Seeds inoculated on ½ MS alone showed highest of 80 % germination within 30 days (Plate I, Fig. h). This was found to be the best medium for seed germination. The results are summarized in Table No. 6.

PLATE I

Fig.a) *Flemingia tuberosa* Dalz. :Plant growing in a pot

Fig. b) Normal and sprouted one forth upper tubers of *Flemingia tuberosa* Dalz.

Fig. c) Seeds of *Flemingia tuberosa* Dalz.

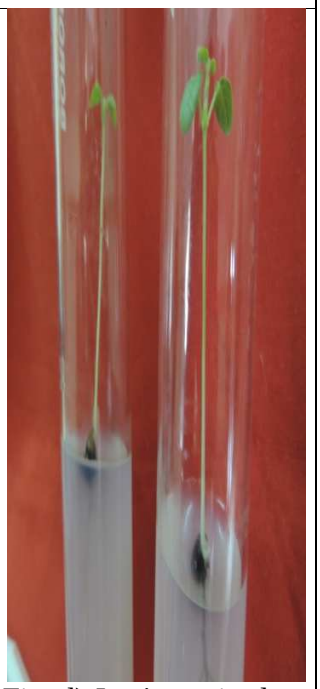

Fig. d) *In vitro* raised seedlings of *Flemingia tuberosa* Dalz. on ½ MS alone

***Flemingia tuberosa* Dalz.**

 Fig. e) *Merremia rhyncorhiza* Hallier: Plant growing in pot	 Fig. f) Tubers of *Merremia rhyncorhiza* Hallier	 Fig. g) Seeds of *Merremia rhyncorhiza* Hallier	 Fig. h) *In vitro* raised seedlings of *M. rhyncorhiza* on½ MS alone.
***Merremia rhyncorhiza* Hallier**			

Table No. 6: Effect of Full MS, ½ MS and Knudson medium alone on *in vitro* seed germination in *M. rhyncorhiza*:

Sr. No.	Treatment	% of Germination
1	Full MS Medium	40
2	**½ MS Medium**	**80**
3	Knudson Medium	30

DISCUSSION

A. *Flemingia tuberosa* Dalz.: Autoecological studies in *Flemingia tuberosa*: *Flemingia tuberosa* is an herb which grows on open moist grassy slopes preferably in loose soil. The plants flower during September to October. Seeds mature by November and plants dry up by December (Hooker, 1876).

F. tuberosa species number is speedily declining in that particular geographical natural habitat due to its various medicinal and neutraceutical properties. Due to human anthropological actions and over grazing, population at Uttan hills area near Dongri is almost depleted to endemism. The tubers are sweet and are eaten by locals fresh or cooked. This has resulted in depletion of its population.

The present investigation supports their contention that the due to its nutritional tubers and various anthropological activities this species has become endemic and endangered (Surwase and Kulkarni, 1998).

Nursery Technology: Sprouting response of tubers in *Flemingia tuberosa*: Nursery technology is a consequently the basic need. Plant propagation techniques are the cores of the nursery. There are many methods of vegetative propagation like budding, grafting, planting tubers and planting suckers. Horizontally cut upper ¼ th tubers sprouted with 100% result, indicating that there is only one bud on the top of tuber. This response is similar to like that of whole tubers.

Similar observations are also made in *Discorea esculenta* (Surwase, 2000).

Seed germination studies in *Flemingia tuberosa*: Because of poor seed germination characteristics, cultivation of *Flemingia tuberosa* as a field crop could potentially be not affordable. Mature seeds do not germinate naturally or *in vitro* in large percentage due to presence of hard seed coat. Besides, seed viability assessments via germination tests are not possible in the absence of an appropriate dormancy release protocol.

To overcome this issue, the seeds were subjected to physical scarification with needle with a view to develop appropriate dormancy release procedure.

Seeds which are not given scarification treatment gave only 10% germination. The physical scarification or mechanical scarification processes have favorable outcome on seed germination with 80% result. Mechanical scarification significantly improved seed germination in *Pterocarpus angolensis* (Chisha-Kasumu *et al*., 2007).

However, washing seeds under running tap water for 10-12 hours gives 50 % germination within 7-8 days at laboratory level. Similar observations were also made in *V. anthelmintica*. The removal of seed coat enhanced percentage of germination (Bhattacharya and Khuspe, 2001; Pipinis *et al.*, 2012).

On the other hand, it was reported that seeds of *Calotropis persica* after soaking in water did not improve the seed germination effectively (Farajollahi *et al*., 2014).

Dormancy of seeds in some plants species is controlled by a balance of endogenous and exogenous inhibitors for growth and also caused by physical as well as chemical barriers (Liu *et al*., 2014).

Bentsink and Koornneef (2008) found that exogenous dormancy occurs because of unavailability of essential germination components as like water, oxygen and light. Softening, mechanical or chemical removal of the seed coat, scarification and leaching is required for effectiveness of germination.

***In vitro* seed germination studies in *Flemingia tuberosa*:** There is tremendous scope for propagation of plants by modern tissue culture techniques as there is need to conserve the plant biodiversity (Gamborg, 2002).

Also, there is an increased need to alter commercially important plants to make them endure in changed environment. If this is delayed, there is a risk of losing commercially viable plant species which will become diminished due to unfavorable climate. Therefore, this study was undertaken to develop efficient *in vitro* seed germination protocol of *F. tuberosa*.

Seeds of *F. tuberosa* show dormancy. The seeds inoculated on full MS alone showed just 40% response. Similarly, it was found that full MS was inhibitory and showed lowest percentage of seed germination in *Pterocarpus marsupium* (Mishra *et al*., 2013).

When the strength of MS medium was reduced to half, it increased germination response to 70%. Similarly, there was highest % of seed germination in ½ MS alone in *Givotia rottleriformis* Griff. (Samuel *et al*., 2009), *Pterocarpus marsupium* Roxb. (Mishra *et al*., 2013) and *Taverniera cuneifolia* (Jamdhade *et al*., 2012).

B. *Merremia rhyncorhiza* Hallier: Autoecological studies in *M. rhyncorhiza*- *M. rhyncorhiza* is a tuberous plant of about 30-40 cm height. It grows on rocky and grassy hills. Tubers are eaten by locals. Leaves are also used as vegetables.

Each plant bears a bunch of 15 nepiform tubers. Short aerial erect stem bears prostate spreading branches. Leaves trilobed, inflorescences are axillary cymes with yellow flowers. Flowering and fruiting occurs in October.

M. rhyncorhiza species number is speedily declining in that particular geographical natural habitat due to its various nutritional and medicinal properties. Due to human anthropological actions and over grazing, population at Ambolighat area Near Sawantwadi, Maharashtra is almost depleted to endemism. This supports their contention that due to over exploitation of the tubers, this species has become endemic and endangered (Surwase and Kulkarni, 1998).

Nursery Technology: Sprouting response of tubers in *M. rhyncorhiza*: Nursery is consequently the basic need. Plant propagation techniques and practices are the core of nurseries. The planting materials for plantations are raised from seeds and vegetative parts. Vegetative propagation is the production of new plants without the use of seeds. There are many different methods of vegetative propagation including rooting, cuttings, grafting, budding, air layering, planting tubers, planting suckers, separating plants and tissue culture.

In the present study it was found that tuber showed 100 % sprouting response using upper ¼th tubers. Similar result was also obtained in *Discorea esculenta* (Surwase, 2000).Cultivation practices of tuberous plants have also been standardized by Surwase and Kulkarni (1998).

Seed germination studies in *Merremia rhyncorhiza*: *Merremia rhyncorhiza* was reported as a disappearing species in India (Surwase, 2000). Plants are endemic to India (Watve, 2013) and thus prioritized for conservation.

Mature seeds of *M. rhyncorhiza* have poor seed germination capacity due to presence of hard seed coat. The unscarified seeds gave just 30% germination. Scarification of seeds

with needle gave 80% germination and was found to be the best amongst hot water, chemical and washing seeds under running tap water treatments. Similarly, it was reported that mechanical scarification has favorable effect on seed germination in *Petrocarpus angolensis* (Chisha-Kasumu *et al.*, 2007) and *Taverniera cuneifolia* (Jamdhade *et al.*, 2012).

In contrast to this, washing seeds in running tap water treatment has favourable effect and was the best for seed germination in *Calatropis persica* (Farajollahi *et al.*, 2014).

***In vitro* seed germination studies in *M. rhyncorhiza*:** Seeds of *M. rhyncorhiza* show seed dormancy. Seeds inoculated on ½ MS medium were found to be the best for *in vitro* seed germination with 80% result. Similar results were also reported in *Pterocarpus marsupium* (Mishra*et al.*, 2013), *Taverniera cuneifolia* (Jamdhade *et al.*, 2012) and *Momordica cymbalaria* (Balkhande *et al.*, 2013).

Significance of the work: These standardized nursery protocols may be utilized for the multiplication of these selected species, which are reported to be endemic and endangered. So that, these species will not become extinct.

ACKNOWLEDGEMENT: Amol Tungenwar is thankful to Rajiv Gandhi Science and Technology Commission, Govt. of Maharashtra and Swami Ramanand Teerth Marathwada University, Nanded providing financial support during the present work

BIBLIOGRAPHY

1. Balkhande, S., Kure, S. and Surwase, B. (2013) Influence of silver nitrate on shoot regeneration from excised meristems of *Momordica cymbalaria* Hook; a diminishing species. *Research Journal of Biotechnology*, 8: 42-47.
2. Bentsink, L. and Koornneef, M. (2008) Seed dormancy and germination. *The Arabidopsis Book*, 6: 1-18.
3. Bhat, K. G. (2003) Flora of Udupi, Indian Naturalist (R), Udupi.
4. Bhattacharya, J. and Khuspe, S. S. (2001) *In vitro* and *in vivo* germination of papaya (*Carica papaya* L.) seeds. *Scientia Horticulturae*, 91(1): 39-49.
5. Chisha-Kasumu, E., Woodward, S., and Price, A. (2007) Comparison of the effect of mechanical scarification and gibberellic acid treatment on seed germination in *Pterocarpus angolensis*. *Southern Hemisphere Forestry Journal*, 69(1):63-70.
6. Cooke, T. (1908) The flora of the Presidency of Bombay, Vol. 1, Taylor and Francis, London.
7. Datar, M. N. and Lakshminarsimham, P. (2013) Check listof wild angiosperms of Bhagwan Mahavir (Molem) National Park, Goa, India. *Check List*, 9 (2) : 186-207.
8. Dudani, S., Chandran, S. M. D., Mahesh, M. and Ramachandra, T. V. (2012) Pteridophytes of Western Ghats. *Biodiversity Documentation and Taxonomy*, 343-351.
9. Farajollahi, A., Gholinejad, B. and Jonaidi Jafari, H. (2014) Effect of different treatments on seed germination improvement of *Calotropis persica*. *Advances in Agriculture*, 2014: 1-5.
10. Gamborg, O. L. (2002) Plant tissue culture. Biotechnology. Milestones, *In Vitro Cellular and Developmental Biology-Plant* 38: 84–92.
11. Hooker, J. D. (1876) The Flora of British India, Vol II. Reeve and Company Ltd.,London.

12. Jamdhade, V. C., Balkhande, S. V. and Surwase, B. S. (2012) Micropropagation of *Taverniera cuneifolia* (Roth) Arn: a substitute for commercial liquorice. *International Journal of Pharma and Bio Sciences*, 3(4): 204-212.
13. Kothari, M. J. and Moorthy, S. (2000) Flora of Raigad District, Maharashtra State Vol.1, Dicots Vol.I Edited : N. P. Singh and S. Karthikayan, Botanical Survey of India, Pune.
14. Lakshminarasimhan, P. and Prasanna, P. V. (1983) Flora of Maharashtra State. Botanical Survey of India, Culcutta.
15. Liu, X., Zhang, H., Zhao, Y., Feng, Z., Li, Q., Yang, H. Q., Luan, S., Li, J. and He, Z. H. (2013) Auxin controls seed dormancy through stimulation of abscisic acid signaling by inducing ARF-mediated ABI3 activation in *Arabidopsis*. *Proceedings of the National Academy of Sciences of the United States of America*, 110(38): 15485–15490.
16. Mishra, Y., Rawat, R., Brajesh, N. E. M. A. and Shirin, F. (2013) Effect of seed orientation and medium strength on *in vitro* germination of *Pterocarpus marsupium* Roxb. *Notulae Scientia Biologicae*, 5(4): 476-479.
17. Murashige, T. and Skoog, F. (1962) A revised medium for rapid growth and bio assays with tobacco tissue cultures. *Physiologia Plantarum*, 15(3): 473-497.
18. Nadkarni, K. M. (1982) Indian Materia Medica Vol.2, Popular Prakashan, New Delhi.
19. Pauchard, A., Aguayo, M., Pena, E. and Urrutia, R. (2006) Multiple effects of urbanization on the biodiversity of developing countries: The case of a fast-growing metropolitan area (Concepcion, Chile). *Biological Conservation*, 127: 272-281.
20. Pipinis, E., Milios, E., Mavrokordopoulou, O., Gkanatsiou, C., Aslanidou, M. and Smiris, P. (2012) Effect of pretreatments on seed germination of *Prunus mahaleb* L. *Notulae Botanicae Horti Agrobotanici Cluj-Napoca*, 40: 183-189.
21. Prasad, B. P., Biju, P. and Ravindran, K. (2011) *Flemingia tuberosa* Dalz. (*Fabaceae*)- a new addition to the Flora of Kerala, India, *Journal of Threatened Taxa* , 3(2): 1548-1549.
22. Samuel, K., Debashish, D., Madhumita, B., Padmaja, G., Prasad, S., Murthy, V. and Rao, P. (2009) *In vitro* germination and micropropagation of *Givotia rottleriformis* Griff. *In Vitro Cellular and Developmental Biology- Plant*, 45(4): 466-473.
23. Singh, N. P., Karthikeyan, S., Lakshminarasimhan, P. and Prasanna, P. V. (2000) Flora of Maharashtra State-Dicotyledons-Vol. 2. Botanical Survey of India, Calcatta.
24. Singh, R. K. and Divakar, P. G. (2013) Discovery of Manmanhara, the largest flattened plateau of Karnataka and second largest in India , *Current Science*, 105 (1): 12-13.
25. Stohlgren, T. J. and Kumar, S. (2013) Endangered plants In: Lewin S. A.(ed) Encyclopedia of Biodiversity, Second edition,.Waltham, MA: Academia Press.
26. Surwase, B. S. (2000) Productivity studies on tuberous starchy wild angiosperms. A Ph. D. thesis submitted to the University of Mumbai, Mumbai.
27. Surwase, B. S. and Kulkarni, A. R. (1998) Physiochemical properties of tuber starch and cultivation practices of *Flemingia tuberosa* Dalz. *Journal of Indian Botanical Society*, 77 (1-4): 189-194.
28. Talbot, W. A. (1909) Forest Flora of Bombay Presidency and Sind. Vol.1, Printed by Govt. at the Photozincographic Dept., Poona.
29. Tasheva, K. and Kosturkova, G. (2013) Role of biotechnology for protection of endangered medicinal plants. *Environmental Biotechnology-New Approaches and Prospective Applications*, 235-285.
30. Watve, A. (2013) Status review of rocky plateaus in the Northern Western Ghats and Konkan Region of Maharashtra, India with recommendations for conservation and management. *Journal of Threatened Taxa*, 5(5): 3935-3962.