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SEED GERMINATION STUDIES OF *BUTEA MONOSPERMA* (LAM.) TAUB. VAR. *LUTEA* (WITT.) MAHESHWARI : A RARE AND ENDANGERED MEDICINAL PLANT

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Butea monosperma (Lam.)Taub.var. *lutea* (Witt.) Maheshwari is an endemic and less populated plant in the Deccan plateau of India and belongs to Fabaceae family. The medicinal value of this plant is immense. The plant is used in folk medicine. It has been recently incorporated into Red Data Book of India as it is a very rare medicinal plant and also declared as globally endangered that needs immediate conservation. Some of the reasons contributing to its near extinction are expanding agricultural fields, illicit felling of trees for fire wood and also lack of knowledge about its rarity. To aid conservation measures, intensive studies were taken up to improve the efficiency of germination of seeds of *Butea monosperma* (Lam.) Taub. var. *lutea* (Witt.) Maheshwari as a prelude to plant tissue culture. By adopting novel methods involving use of filter-paper –bridges, germination of seeds was achieved both *ex situ* and *in vitro* thus proving that the seeds available from the herbal sellers were viable. However, it was also found that the viability of seeds is more when the seeds are immediately sown in the month of May as soon as they ripen.

Key Words : *Butea monosperma*, seed germination, rare medicinal plant.

Butea monosperma (Lam.) Taub. var. *lutea* (Witt.) Maheshwari is an endemic and less populated plant (Reddy *et al.* 2001, 2008) in the Deccan plateau of India. It is an over looked taxon, because of expanding agricultural fields, illicit felling of trees for fire wood and also lack of knowledge about its rarity which is contributing to its near extinction. It has been recently incorporated into Red Data Book of India as it is very rare medicinal plant and also declared as globally endangered that needs immediate conservation (Molur and Walker 1998, CAMP workshop on Medicinal Plants India 1998, Reddy *et al.* 2001, 2008). The medicinal value of this plant is immense. The plant is used in folk medicine. Its bark and wood scrapes are used as ethno gynecological medicine, extraction of the stem bark along with jeera powder is used to cure Jaundice and skin diseases etc (Borkar *et al.* 2010, Reddy *et al.* 2008). Chemically it was proved that the plant contains flavonoides, chalcones, linoleic acid and unsaturated fatty acids. Besides these cyclitols are also present which can cure bronchitis and asthma (Reddy *et al.* 2008).

Butea monosperma (Lam.) *Taub.* var. *lutea* (Witt.) Maheshwari belongs to Fabaceae family (Tandon *et al.* 2003). It is a medium sized deciduous tree seldom rises beyond 6 meter height, very conspicuous during periods of flowering. The leaves are trifoliate, petiolate, leaflets coriaceous, margin entire, obtuse, and glabrous above when old, finely silky and conspicuously veined underneath. Flowers are large, pale yellow to striking yellow coloured variety and produced in rigid racemes. The base of the petals is primrose yellow and they shade off into a creamy tinge on the edges and on their reverse sides. The corolla contracts very beautifully with its rich olive blossom downy calyx. This tree resembles *Butea monosperma* in all respects except for the colour of the corolla. *Butea monosperma* (Lam.) Taub. var. *lutea*, (Witt.) Maheshwari is significant for its striking beauty of the flowers and the rarity of the plant (CAMP workshop 1998). The flowering and fruiting could be observed once in two years. Flower buds appear after January and pale yellow to striking yellow coloured blossoms appear during March - April when the tree is leafless. The fruits are pods with a

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single seed in each. Pods ripen in May-June. Seeds sown in the experimental garden were found to be non- viable (Reddy *et al.* 2001, 2008). Lack of seed viability may be one of the reasons for its rarity and vulnerability for endangerment (Ramoliya *et al.* 2004, Hirpara *et al.* 2005, Chang *et al.* 2007, Yadav and Gupta 2009, Vaghela *et al.* 2010), Kulkarni and DSouza (2000) tried to grow the plants *in vitro* without much success. To aid conservation measures, intensive studies were taken up to improve the efficiency of germination of seeds of *Butea monosperma* (Lam.) Taub. var. *lutea* (Witt.) Maheshwari as a prelude to plant tissue culture.

MATERIALS AND METHODS

The seeds of *Butea monosperma* (Lam.) Taub. var. *lutea* (Witt.) Maheshwari ripen in the month of May. The germination studies were carried out with seeds soon after ripening to study if there was any dormancy period and also after several periods of time after ripening which is when they are usually available with herbal sellers and Ayurvedic practitioners. The seeds were harvested directly from the trees in the months of May-June growing in the wild and some were procured (3, 5, 7, 10 and 12-month-old seeds) from an Ayurvedic Practitioner, Hanamkonda, Warangal District, A.P, India. The seeds were surface sterilized and were treated with Bavistin (fungicide) for about 30 min. They were further washed 3-4 times with normal tap water and soaked for 24 hrs in the tap water. The seeds were germinated in two different ways *Ex situ* germination in the natural way in a garden and *in vitro* germination in the tissue culture laboratory.

***Ex situ* germination :** Hundred each of freshly harvested seeds and 3, 5, 7, 10 and 12-month-old seeds were used for the study. The poly bags were filled with the normal soil which was rich in organic material. The soaked seeds were sown in the soil rich in organic matter after 24 hrs and left for *ex situ* germination in the garden under normal environmental conditions watering regularly.

***In vitro* germination :** Hundred each of freshly harvested seeds and 3, 5, 7, 10 and 12-month-old seeds were used for the study. After 24 hours of soaking, the seeds were again surface sterilized with tween 20 and rinsed 2-3 times with sterile water. They were further treated with 0.1% mercuric chloride for 6-8 min and later rinsed thoroughly 3-4 times with sterile distilled water. The seeds were transferred on to the filter paper bridges placed over sterile water taken in culture bottles for germination (Reddy 2008).

RESULTS AND DISCUSSION

The seeds of *Butea monosperma* (Lam.) Taub. var. *lutea* (Witt.) Maheshwari were germinated in two different ways: *Ex situ* germination in the natural way in a garden and *in vitro* germination in the tissue culture laboratory to find the best suited method that can produce 100% germination.

***Ex situ* germination :** Out of the hundred freshly harvested seeds sown in the poly bags (*ex situ* germination), 90% of the seeds germinated after a week (between 9-10 days) (Plate-1) and the rest germinated by the second week. The seedlings were healthy although the growth was very slow. New leaves were observed to grow after a month from the date of development of the first leaf. However, after 20 days,10 of the seedlings developed a fungal disease and were killed. It was also observed that environmental conditions especially rainfall and humidity encourage the *ex-situ* germination and probably were also the reasons for the fungal disease.

Out of the hundred 3-month-old seeds, sown in the poly bags, 70% seeds germinated after ten days (Plate-1) after which, the other 20% germinated (Table-1). The rest of the seeds did not germinate indicating the seed viability may be reduced with time. In case of the 5 and 7-month-old seeds, germination was further reduced to 80% and was also delayed (after 15-20 days). Further, the number of seedlings dying due to fungal disease increased to almost 50% with increase in the age of the seeds. The germination percentage decreased drastically to 50% in the 10-month-old seeds and in the 12-month-old seeds was further decreased to 10%, indicating that the

Plate-1 : Stages of ex-situ seed germination of *Butea monosperma* (Lam). Taub. Var *lutea* (Witt.) a. Germination of seed after 3rd day, **b.** Seedling after 7th day, **c.** Seedling after 12 day, **d.** Plant after 20 days, **e.** Plant transplanted to the field after 45 days, **f.** Conservation through *ex situ* germination.

freshly harvested seeds had the highest germination percentage and could be used for up to 10 months although the older seeds (more than 10-month-old seeds) continued to germinate albeit with a very low percentage.

***In vitro* germination :** Out of the hundred seeds kept in the culture bottles (*in vitro* germination), 60% of them germinated after 3 days (Plate-2), followed by the rest by the end of the first week to record a 100% germination (Table-1). Similar results were obtained for the 3, 5, 7, 10 and 12-month-old seeds which were used for the study, except for the fact that the germination percentage

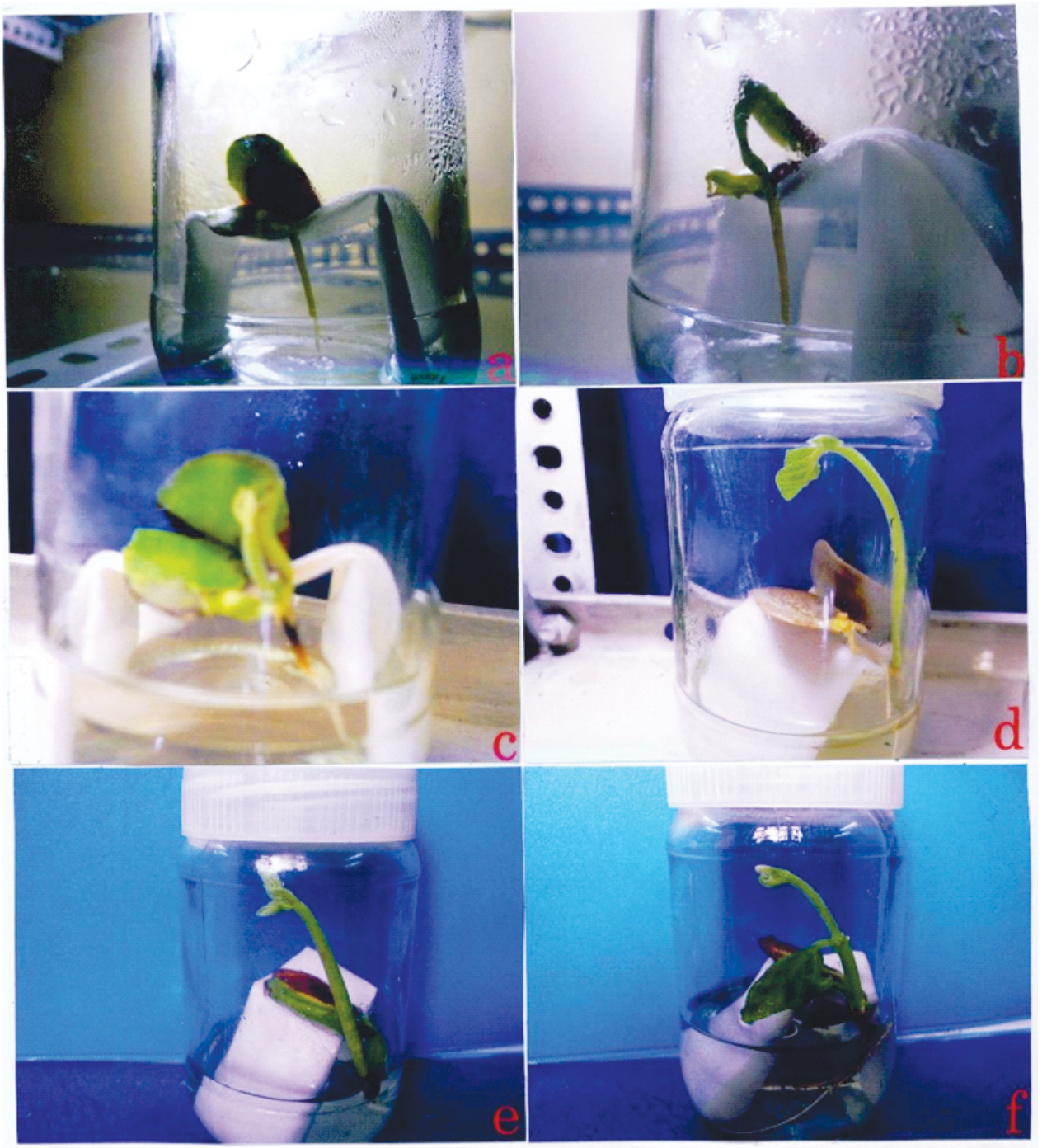

Plate-2 : Stages of *in vitro* seed germination of *Butea monosperma* (Lam). Taub. Var *lutea* (Witt.) a. Germination of seed after 3rd day, **b.** Growth of seedling after 7th day, **c.** Growth of seedling after 10 days, **d.** Formation of new leaves after 15th days, **e.** Formation of new leaves after 20 days, **f.** In vitro seedling after 1 month of germination in culture bottle.

decreased with the age of the seeds (95, 80, 80, 75 and 60% respectively). The further growth and development of these seedlings was observed in terms of development of new leaves. The first leaf was developed by the first week and the new leaves were observed to grow after the second week. Although the initial growth was very slow, the growth increased by the fourth week. Further, on testing, the 12-month-old seeds also germinated with a modest percentage of 60%. This shows that seeds are viable even after 10 months and when compared with the *ex-situ* germination method, they could be successfully germinated *in vitro* with a modest percentage.

Table-1 : Germination of seeds of *Buteamonosperma* (Lam.) Taub. var. *lutea* (Witt.) Maheshwari undertaken by *ex situ* and *in vitro* methods.

S.No	Age of seeds*	Germination percentage	% Seedlings killed due to fungal disease	% Surviving plants
EX SITU method				
1	Freshly harvested	100	10	90
2	3 months	90	15	75
3	5 months	80	12	68
4	7 months	80	10	70
5	10 months	50	18	32
6	12 months	10	8	02
IN VITRO method				
1	Freshly harvested	100	-	98*
2	3 months	90	-	89*
3	5 months	80	-	77*
4	7 months	80	-	79*
5	10 months	50	-	48*
6	12 months	10	-	9*

* Surviving plants after transplantation into garden

Jaiswal and Chaudhary (2005) and Ramoliya *et al.* (2004) stated that the germination rate of seeds of several forest trees has been decreasing due to various reasons especially those of medicinal trees due to over-collection. According to Reddy *et al.* (2008) the seeds were found to be non-viable in experimental garden. However, in our study the seeds were treated with the fungicide and sown in the soil rich in organic matter which has actually encouraged the germination of the seeds and thus proved that the seeds were very much viable in the experimental garden.

As far as the *in vitro* germination is concerned, there are no previous reports available and therefore we applied our time-tested technique for the study (Reddy 2008). Our present findings and successful *in vitro* technique adopted to induce germination by the use of filter paper bridges which resulted in 100% germination is the very first report on the rare and endangered medicinal plant. This technique will go a long way in improving the conservation of *Butea monosperma* (Lam.) Taub. var. *lutea* (Witt.) Maheshwari.

CONCLUSION

The results of the present germination studies in the endangered plant *Butea monosperma* (Lam.) Taub. var. *lutea* (Witt.) Maheshwari which needs urgent conservation and multiplication indicate that the freshly harvested seeds had the highest germination percentage and could be used in *ex situ* method for upto 10 months although the older seeds continued to germinate albeit with a very low percentage. The *in vitro* germination method proved that the seeds (both freshly harvested and the 12-month-old seeds) could be successfully germinated with a much higher percentage when compared to the *ex-situ* germination method. It was also observed that environmental conditions especially rainfall and humidity encourage the *ex-situ* germination and probably were also the reasons for the fungal disease. Further, the reason for previous reports claiming low germination and regeneration in forests may be due to the non-availabilityof seeds for self-regeneration on the forest soils due to over collection for commercial sale. This is the very first report on the rare and endangered

medicinal plant and this technique will go a long way in improving its conservation.

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