Leaflet No- 16/2020

The Republic of the Union of Myanmar
Ministry of Natural Resources and Environmental Conservation
Forest Department

Study on Acclimatization Potential of Tissue -Culture Eucalypt Plantlets during Transplanting

Shwe Lone, Research Assistant-2
Aung Zaw Moe, Research Officer
Forest Research Institute

December 2020

Table of Contents

Page

Study on Acclimatization Potential of Tissue Culture Eucalypt Plantlets during Transplanting

Shwe Lone, Research Assistant-2, Forest Research Institute, Forest Department

Aung Zaw Moe, Research Officer, Forest Research Institute, Forest Department

Abstract

Tissue Culture is an ideal method to produce the mass production of good genetic planting materials. Hybrid seedlings of (*Eucalyptus grandis*) × (*E. urophylla*) have been propagated by mean of tissue culture method in Forest Research Institute, Forest Department. They are successfully grown in *in vitro* culture but it is difficult in out planting to raise the tissue plantlets which were acclimatized by planting media. In this paper, the hardening-off (*Eucalyptus grandis)* × (*E. urophylla)* tissue cultured plantlets were tested with different planting media treatments; (A) compost: sand(8 : 2), (B) compost: sand: FYM(Farm yard manure) (3 : 2 : 1), (C) compost, (D) Soil (Ottara thiri) (E) Clay soil and sand (8 : 2). According to our experiments, Treatment (D) Soil (Ottara thiri) & Treatment (E) Clay soil and sand (8: 2) showed that the best performance by means of survival rates (96.8%) and (92.4%) respectively for Eucalypt tissue culture.

Keywords: In vitro, Hybrid Eucalyptus, Hardening, Survival rate, Planting media

အမြစ်ထွက် ယူကလစ်တစ်သျှူးပင်များအား ပျိုးဘောင်သို့ချစိုက်ရာတွင် ဒဏ်ခံနိုင်ရည်ကို လေ့လာဆန်းစစ်ခြင်း

ဒေါ်ရွှေလုံး (သုတေသနလက်ထောက်-၂)
ဦးအောင်ဇော်မိုး (သုတေသနအရာရှိ)၊
သစ်တောသုတေသနဌာန၊ သစ်တောဦးစီးဌာန

စာတမ်းအကျဉ်း

တစ်သျှူးမျိုးပွားခြင်းသည် မျိုးရိုးဗီဇကောင်းမွန်သည့် မျိုးကောင်းမျိုးသန့် ပျိုးပင်အမြောက်အမြား ထုတ်လုပ်ရန်အတွက် ထိရောက်စွာ အသုံးပြုနိုင်သော လုပ်ငန်းစဉ်တစ်ရပ် ဖြစ်ပါသည်။ သစ်တောဦးစီးဌာန၊ သစ်တောသုတေသနဌာနတွင် (*Eucalyptus grandis*)× (*E. urophylla*) ယူကလစ်စပ်မျိုး တစ်သျှူးနည်းဖြင့် ပွားများ ထုတ်လုပ်ရာ၌ ဓါတ်ခွဲခန်းအတွင်း ဆောင်ရွက်ရာတွင် အောင်မြင်မှုရှိသော်လည်း အမြစ်ထွက်ပုလင်းများမှ ထွက်ရှိလာသော ယူကလစ်တစ်သျှူးပင်များအား ပျိုးဥယျာဉ်၌ ဒဏ်ခံနိုင်ရည်ရှိစေရန် စိုက်ပျိုးပြုစုထိန်းသိမ်းရာတွင် စပ်မြေအမျိုး အစားနှင့်အချိုးအစား အသုံးပြုမှုတွင် အခက်အခဲများနှင့် ရင်ဆိုင်ခဲ့ရပါသည်။ ဤစာတမ်းတွင် အမြစ်ထွက်နေသည့် (*Eucalyptus grandis*) × (*E. urophylla*) ယူကလစ်စပ်မျိုး တစ်သျှူးပုလင်းများမှရရှိသော တစ်သျှူးမျိုးပွားပင်ငယ်များကို (က) မြေဆွေးနှင့်သဲ ၈:၂ အချိုး၊ (ခ) မြေဆွေး၊ သဲနှင့် နွားချေး ၃:၂:၁ အချိုး၊ (ဂ) မြေဆွေး၊ (ဃ)မြေ (ဥတ္တရသီရိ) နှင့် (င) မြေစေးနှင့်သဲ ၈:၂ အချိုး အမျိုးမျိုး ရောစပ်ထားသော ပျိုးသေတ္တာများတွင် ပျိုးထောင်၍ ရေငွေ့ထိန်းအိမ်ငယ်အတွင်း အပင်များ၏ ရှင်သန်မှုအား လေ့လာဆန်းစစ်မှုများ ပြုလုပ်ခဲ့ရာ စပ်မြေအမျိုးအစား (ဃ) မြေ (ဥတ္တရသီရိ) နှင့် စပ်မြေအမျိုးအစား (င) မြေစေး နှင့်သဲ ၈:၂ တို့ဖြင့် စိုက်ပျိုးခြင်းသည် ယူကလစ်တစ်သျှူးပင်များ ဒဏ်ခံနိုင်ရည်ရှိစေခြင်း အတွက် ရှင်သန်မှု (၉၆.၈%)နှင့် (၉၂.၄%) အကောင်းဆုံးဖြစ်ကြောင်း တွေ့ရှိရပါ သည်။

Study on Acclimatization Potential of Tissue Culture Eucalypt Plantlets during Transplanting

1. Introduction

The increasing number of plant species is being micro propagated throughout the world (Pierik, 1988), but the production cost of micropropagation is still high (KIRDMANEE, KITAYA, & KOZAI, 1994). Micro propagated plants of most species cannot survive well if transplanted from *in vitro* conditions to greenhouse or field environment (POSPÍŠILOVÁ, TICHÁ, KADLEČEK, & HAISEL, 1999). Acclimatization losses and labor costs have limited the profitability of the tissue culture processes in many species, (Kozai, 1991), that is, during transfer from a tissue culture to the soil. Due to the special environment *in vitro*, it is difficult to produce plants which can be acclimatized to the outside environment.

The term acclimatization is defined as the climatic adaptation of an organism, especially a plant that has been moved to a new environment (KOZAI, 1991). Plantlets with succulent characteristics are particularly susceptible to excessive desiccation during ex vitro culture (Donnelly and Vidaver, 1984; Cheliak, 1993). Therefore, irreversible tissue damage may result (Kirdmanee, Kitaya, & Kozai, 1995). Most species grown *in vitro* require an acclimatization process in order to ensure that sufficient numbers of plants survive and grow vigorously when transferred to soil (Preece & Sutter, 1991). Hardening of the plants is necessary to develop resistance against physical, chemical and biological factors at the time of transferring *in vitro* raised plantlets to field conditions.

Eucalypt is one of the species of the family Myrtaceae, and the most widely planted tree species in tropical and subtropical regions (Shengjian Ma et al). Eucalypt are the important raw materials, mainly for pulp and paper production. Moreover, Eucalypt is one of the most productive forest crops especially in tropical area (Khaing, Mu, & Thent, 2015).

(*Eucalyptus grandis*) × (*E. urophylla*) clones were identified by the South African forestry industry as being of superior vigour and as demonstrating desirable pulping qualities (Jones & Staden, 2016). It is a hybrid variety with good adaptation to Guangdong provincial area, China and elite wood quality and fast growth and speed (Ma, Cai, & Liu, 2016) and *E.urophylla* × *E. grandis* has great potential for plantation highly resistant of diseases such as strain, gall, ranks of fascicled symptom (Yonghuai et al., 2014).

Hybrid seedlings of *E.urophylla* × *E.grandis* have been propagated by mean of tissue culture method in Forest Research Institute, Forest Department. They are successfully grown in *in vitro* culture but it is difficult to produce plants which can be acclimatized to the outside environment.

There are some studies on the micropropagation techniques, especially different media for Eucalyptus tissue cultured. However, there is limited study of different hormones

The objective of this study is to examine the survival rate of the hardening of Eucalyptus tissue cultured plantlets by different media proportion.

3. Basic Steps of Tissue Culture

There are six steps in plant tissue culture. The followings are basic steps of plant tissue culture (Fig.1).

i. Collection of explants from known genotypic superior quality tree, removing uninfected part from meristem and sterilization.
ii. Induction of tissue in test tube on medium and nurse in the culture room where light and temperature are controlled.
iii. Multiplication of shoot-lets and replication of sub-culture.
iv. To transfer into different media (gel-like or sterilized sand) for root formation.
v. To acclimatize rooted plantlets in the chamber under the justification of light and temperature
vi. To perform the out planting of acclimatized plants and observe the performance


i. Explants Collection from hedge garden & Sterilization


ii. Inoculation or Induction

iii. Multiplication

iv. Rooting

v. Acclimatization

vi. Out Planting

Figure 1. Basic Steps of Tissue Culture

4. Materials and methods

4.1 Preparation of Materials

(*Eucalyptus grandis*) × (*E. urophylla*) is a hybrid variety with good adaptation to Guangdong provincial area, China. It was planted in the hedge garden of Forest Research Institute as a mother plant. Shoots were collected as explants and these explants were used for doing the tissue culture method such as induction, multiplication and rooting step by step. Finally, 90% successful in vitro rooted plantlets transfer to potting mixtures after taken to avoid breaking of roots while washing away traces of agar (Fig. 2 - a and b)

4.2 Preparation of the Hardening Medium

To study the survival rate of eucalypt tissue culture, rooting plantlets transferred to plastic containers having different sterilized soil and mixture ratio. The soil (red color) was collected from Ottara Thiri township Nay Pyi Taw and clay soil from Pobba Thiri township Nay Pyi Taw and compost/ forest top soil (under bamboo forest) Zayar Thiri township Nay Pyi Taw. Experiment were carried out at FRI tissue culture lab and tested five treatments are as follows; -

NO.	Treatments	soil mixture
1-	A	compost: sand (8: 2),
2-	B	compost: sand: Farm yard manure (3: 2: 1)
3-	C	compost
4-	D	soil (red color)
5-	E	clay soil: sand (8: 2)

Soil chemical analysis of soil mixtures are conducted in FRI soil lab.

4.3 Acclimatization

The plants were watered regularly and covered by 60%-80% light plastic and 70% nursery net to maintain the mist chamber having the relative humidity of 60-80% with a temperature of 35 -40°C (Fig. 2-e).

4.4 Data collection and Statistical Analysis

Five different treatments were performed, using 100 plantlets for each method in one time and five replications. The plantlets were put into different boxes of each treatment Fig. 2-(d). The objectives of these treatments are to find the observations for the survival rate in five different treatments.

The survival rate was assessed during 1 month of transfer to the mist chamber. Data preparations were done by Microsoft Office Excel 2010. The data was analyzed for significance of mean survival rate by using Analysis of variance (ANOVA), and Tukey post hoc test was use to find out pairwise comparison within five treatments.

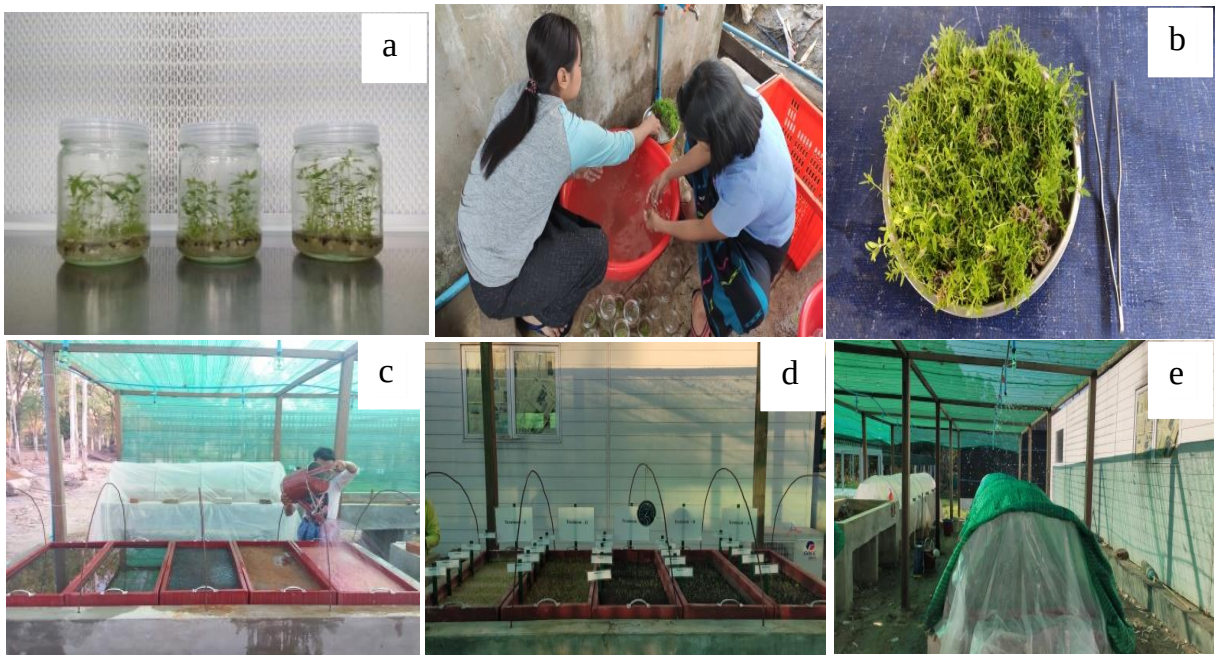

Figure 2. Preparation of the Hardening Medium

a. 90% rooted plantlets in vitro
b. Washing off artificial media
c. Preparation of the Hardening Medium and Sterilization of soil
d. Planted transplants are ready to be covered
e. Plantlets in polytents under high relative humidity

5. Results and Discussions

5.1 Comparison on survival rate (%) among five treatments

The data were analyzed in terms of survival rate on different treatments. In the first 3 days of transfer the plantlets were weak but after 1 week of transfer these plantlets started acclimatized in the mist chamber and started growing in all treatments. After two weeks of transfer, the nursery net cover was removed from the plastic container. After five weeks, further acclimatization had done by exposing the plantlets to the green house, shade house and open light.

According to the statistical analysis, after 1month of transfer the mean survival rate of treatment observed that treatment (A) 47.8%, (B) 53.2%, (C) 48.4%, (D) 96.8% and (E) 92.4% respectively. The hardenings of plants are then carried out to increase in height, and it was observed the effect of various treatments. Among the different treatments of the maximum survival rate was observed in treatment D, and the minimum was Treatment A (Table-1).

Table 1. Effect of soil mixtures on plant survival rate %

Descriptives

Survivalrate

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
A	5	47.80	7.694	3.441	38.25	57.35	41	60
B	5	53.20	6.907	3.089	44.62	61.78	46	63
C	5	48.40	15.868	7.096	28.70	68.10	29	69
D	5	96.80	3.962	1.772	91.88	101.72	90	100
E	5	92.40	5.273	2.358	85.85	98.95	85	97
Total	25	67.72	23.968	4.794	57.83	77.61	29	100

According to ANOVA test, it was found a significant difference in mean survival rate of tissue eucalyptus plantlet among the five treatments $F_{(4, 20)} = 37.849$, $P<0.05$. Tukey post hoc test revealed that treatment D and treatment E are not significant in survival rate. However, these two treatments had produced a significantly difference than other three treatment A, B & C at the significant level ($P<0.05$). Therefore, tukey test showed that treatment D (96.80±3.962) had produced a significantly higher survival rate than among five

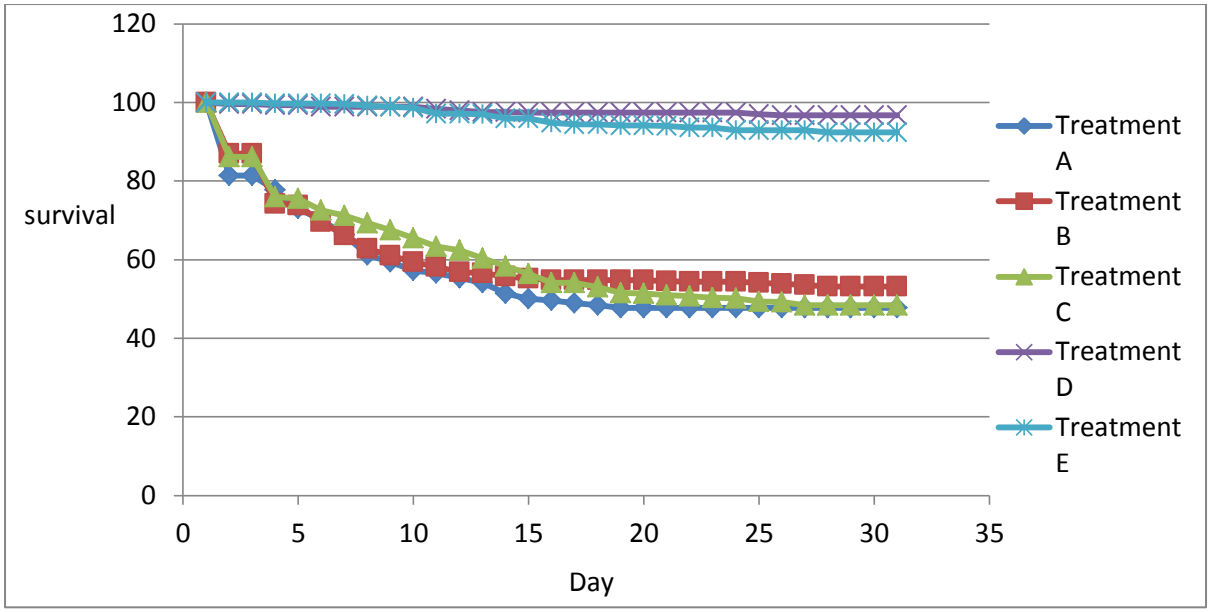

Figure 3. Comparison on survival rate (%) among five treatments

Table 2. Pairwise comparison among five treatment
Dependent Variable: Survival rate
Tukey HSD

(I) fertilizer	(J) fertilizer	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
A	B	-5.400	5.672	.873	-22.37	11.57
	C	-.600	5.672	1.000	-17.57	16.37
	D	-49.000*	5.672	.000	-65.97	-32.03
	E	-44.600*	5.672	.000	-61.57	-27.63
B	A	5.400	5.672	.873	-11.57	22.37
	C	4.800	5.672	.913	-12.17	21.77
	D	-43.600*	5.672	.000	-60.57	-26.63
	E	-39.200*	5.672	.000	-56.17	-22.23
C	A	.600	5.672	1.000	-16.37	17.57
	B	-4.800	5.672	.913	-21.77	12.17
	D	-48.400*	5.672	.000	-65.37	-31.43
	E	-44.000*	5.672	.000	-60.97	-27.03
D	A	49.000*	5.672	.000	32.03	65.97
	B	43.600*	5.672	.000	26.63	60.57
	C	48.400*	5.672	.000	31.43	65.37
	E	4.400	5.672	.935	-12.57	21.37
E	A	44.600*	5.672	.000	27.63	61.57
	B	39.200*	5.672	.000	22.23	56.17
	C	44.000*	5.672	.000	27.03	60.97
	D	-4.400	5.672	.935	-21.37	12.57

*. The mean difference is significant at the 0.05 level.

5.2 Effect the soil properties of different planting media on survival rate

Some scientists studied the effects of media in acclimatization stage and the mixture of soil: sand: manure. They found the mixture of soil: sand: manure (1:1:1) was observed 85 - 95% success in field survival rate in the eucalyptus hybrids. And Eucalyptus hybrid made satisfactory growth in soils with pH less than 9.0 (Arya, Sharma, & Arya, 2009). Kengar & Paratkar(2015) was observed the hardening media soil: sand: compost 1: 1: 1 showed 90% survival rate with 12-13 cm shoot length having erect stem in *Ruta graveolens*. Plant grown in cocopeat showed 76% of survival rate (Meera M Chabukswar and Mamjushri A Deodhar, 2004) followed by cocopeat and sand (1:1) with 75%of survival in *Garcinia indica* Chois (Chabukswar & Deodhar, 2005). For most species, the mixture of compost and sand media is better improvement for hardening. Soil medium or potting mixers are important to be porous enough to prevent water logging conditions and bad aeration after transplantation.

Table 3. Results of soil chemical analysis of potting soil mixtures

No	**Soil Mixture (Treatment)**	**pH**	**Total N %**	**Available P%**	**Extractable**		**OM**	**Particle size distribution%**			**Texture**
					K. %	**Ca %**		**Sand**	**Silt**	**Clay**	**Soil test**
1	Treatment- A	7.39	0.055	0.00647	0.018	0.164	2.0	85	4	6	Sand
2	Treatment- B	7.37	0.019	0.00894	0.050	0.171	2.0	83	5	7	Sand
3	Treatment -C	7.34	0.019	0.00337	0.022	0.221	4.0	79	10	7	Loamy Sand
4	Treatment -D	6.42	0.015	0.00011	0.011	0.067	2.0	53	16	30	Sandy-Clay-Loam
5	Treatment- E	4.98	0.011	0.00035	0.003	0.027	3.0	55	17	25	Sandy - Loam

Survival rate of eucalyptus tissue plant are affected by the different soil mixtures used as shown in table-3. Survival rate of eucalyptus tissue plant in Treatment D & E are generally better than that of Treatment A, B & C.

From the soil analysis data, the potting mixture of Treatment -D (Sand Clay Loam) and Treatment -E (Sand: clay ratio, 8: 2) are to be closest to idea for eucalyptus tissue culture. During the initial period, the potting mixtures in this study are suitable for transplanting. However, it is different from some results because they used compost containing optimal nutrients content. In treatment A, B & C, soil distribution pattern are not statistically different among the particular soil mixtures. They are sand and loamy sand and statistically different Treatment D & E. Small size, poorly developed cuticles and soft, immature tissues is not tolerant cores texture in our results.

In general, the pH of the soil mixture is important for tissue culture plant in the few weeks. Soil acidity of the potting mixtures in our study was pH 5- 7.3. The significantly different survival rates of euclayptus in Treatment D &E were found in treatments A, B & C. It may be a low survival rate in sand with high pH value as compared to other soil mixture with low pH value. Plantlets of euclayptus spp transplanted into soil mixtures (treatments) do best at a pH between 5 and 6.5.

In nitrogen few differences among the treatments are noted because probably due to Treatment A. It is evident that the percentages of available phosphorous and calcium in Treatment A, B & C were significantly higher than Treatment D &E. However, it contains a lower percentage of potassium. The percentage of organic matter in all soil mixtures was same condition, except treatment C. Treatment D & E (potting mixture) providing for the best growth of tissue-cultured euclyptus plantlets have a good balance between its pH and amount of nutrient contents.

To assist tissue cultured plants in acclimatizing to soil mixes, treatments D & E have allowed successful transplantation of tissue cultured. This could be due to the low percentage of nutrients and slightly acid soil with medium texture in the soil mixture.

6. Conclusion and Recommendation

Acclimatization stage is the very important the whole process in tissue culture method. The aim of this study was to test the effect of different hardening media by Eucalyptus tissue culture. Hardening media can have impact on the success of survival rate in acclimatization.

Sandy clay loam and sandy loam are generally found to be suitable transplanting soil media for eucalyptus tissue culture. However, soil nutrients are observed to be an appropriate soil media. In order to higher survival percentage of eucalyptus tissue plant, the soil mixture should be slightly acidic. A pH range of is recommended 5 to 6.5.

The study thus revealed vital information related to rapid hardening of *in vitro* raised plantlets of Eucalyptus. This can be very useful for the mass production of Eucalyptus by various commercial entrepreneurs.

7. Acknowledgements

I would like to express my sincere thanks to Forest Department and Forest Research Institute for giving me a chance to do this paper. I am greatly indebted to Dr. Thant Shin (Assistant Director) and Dr Phyu Phyu Lwin (Staff officer) for their valuable suggestions and encouragement for our research paper. I highly appreciate to soil laboratory of Forest Research Institute, Yezin for testing soil samples. Thanks to the staff of Mobile tissue culture lab for their help data collection.

8.References

Arya, I. D., Sharma, S., & Arya, S. (2009, November). Micropropagation of superior eucalyptus hybrids FRI-5 (Eucalyptus camaldulensis Dehn x E. tereticornis Sm) and FRI-14(Eucalyptus torelliana F.V. Muell x E. citriodora Hook): A commercial multiplication and field evaluation.

Chabukswar, M. M., & Deodhar, M. A. (2005, July). Rooting and hardening of in vitro plantlets of Garcinia indica Chois.

Jones, N., & Staden, J. (2016, July 20). Micropropagation and establishment of Eucalyptus grandis hybrids.

Kengar, A. A., & Paratkar, G. T. (2015, August 01). Efficient Rooting and Biological Hardening of In Vitro Plantlets of Ruta Graveolens L.

Khaing, T T., Mu, T. T., & Thent, Y. Y. (2015, December). Preliminary Study on Influence of Different Media and Rooting Hormone of Shoot Cutting of Eucalyptus.

Kirdmanee, C., Kitaya, Y., & Kozai, T. (1995, January). Acclimatization of Eucalyptus Plantlets by Controlling Photosynthetic Photon Flux Density and Relative Humidity.

KOZAI, T. (1991). 1.8 Acclimatization of Micropropagated Plants.

Ma, S., Cai, C., & Liu, J. (2016, January). A Study on the Tissue Culture of Eucalyptus Urophylla x Eucalyptus Grandis.

POSPÍŠILOVÁ, J., TICHÁ, I., KADLEČEK, P., & HAISEL, D. (1999, May). Acclimatization of micropropagated plants to ex vitro conditionsatization of micropropagated plants to ex vitro

Preece, J., & Sutter, E. (1991, January 01). Acclimatization of micropropagated plants to the greenhouse and field.

Yonghuai, H., Ningnan, Z., Pulin, H., Shaobin, H., Huanhua, H., & Ruiping, C. (2014). Resistance of Different Eucalyptus Strains to Leptocybe invasa Fisher & La Salle.