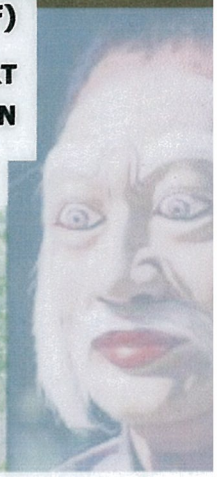
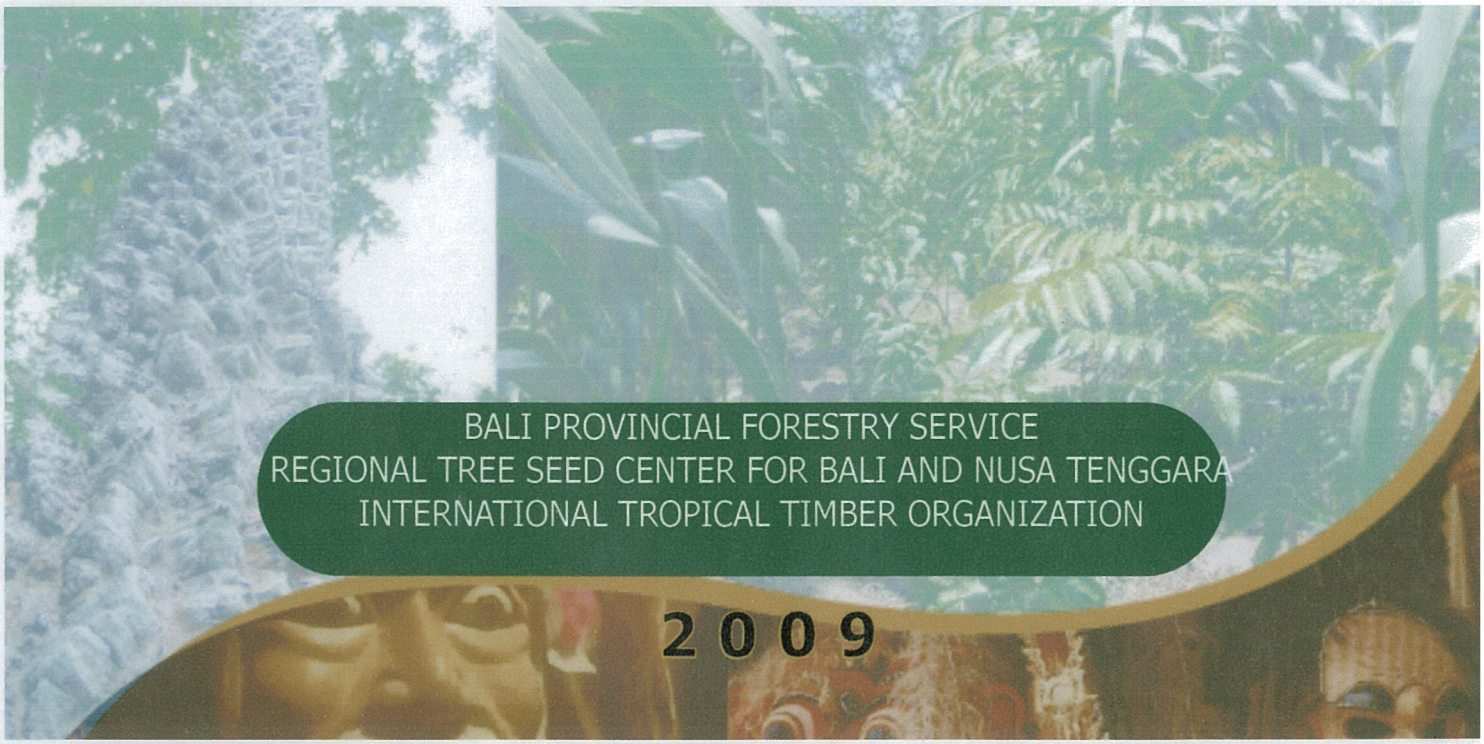
ITTO PD 386/05 Rev.1 (F)

TECHNOLOGICAL DEVELOPMENT FOR THE PRODUCTION OF PLANTING MATERIALS TO SUPPORT SUSTAINABLE PLANTATION OF BALI INDIGENOUS SPECIES THROUGH COMMUNITY PARTICIPATION



REPORTING ACTIVITY 1.2.3 DEVELOPMENT OF TISSUE CULTURE TECHNIQUES OF 6 SPECIES

PREPARED BY:
PROJECT EXECUTING TEAM



BALI PROVINCIAL FORESTRY SERVICE
REGIONAL TREE SEED CENTER FOR BALI AND NUSA TENGGARA
INTERNATIONAL TROPICAL TIMBER ORGANIZATION

2009

Reporting

Activity 1.2.3. Development of tissue culture techniques of 6 species (*Fagara rhetsa, Manilkara kauki, Alstonia scholaris, Wrightia pubescens, Planchonia valida*, and *Dysoxylum densiflorum*)

Project Executing Team ITTO PD 386/05 Rev.1(F)

Bali Provincial Forestry Service and
Regional Tree Seed Center for Bali and Nusa Tenggara and
International Tropical Timber Organization
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SUMMARY

Propagation method by tissue culture was developed for 6 species of interest namely *Fagara rhetsa, Manilkara kauki, Alstonia scholaris, Wrigtia pubescens, Planchonia valida* and *Dysoxylum densiflorum*. Explants for initiation of tissue culture were obtained from young seedlings. The ease of obtaining young explants from seedlings was variable.

In general, MS media with IBA growth hormon used for the initiation and multiplication stages provided ideal condition for the growth of the six species in culture, with the exception of M. Kauki which grew much slower.

In the rooting stage, IBA hormon was used at low dosage (0.01 mg/l) for bentawas and (20 mg/l) for panggal buaya and majegau gave the best results.

The acclimatization stage was successfully achieved for the species (*A. scholaris, W. pubescens, F. rhetsa* and *D. densiflorum*). Media composition of top soil, compost, and sand at 3 : 1 : 1 respectively is an ideal growing media.

1. INTRODUCTION

The activity 1.2.3 is development of tissue culture techniques of 6 species. Pulai (*Alstonia scholaris*), Sawo kecik (*Manilkara kauki*), Panggal buaya (*Fagara rhetsa*), Majegau (*Dysoxylum densiflorum*), Putat (*Planchonia valida*), and Bentawas (*Wrightia pubescens*) are selected because of their importance in providing timber for the local handicraft industry in Bali. Whilst other activities of the project deal with plantation issues and community participation, the tissue culture propagation activity is focused on developing techniques as well as producing plants for genetic test.

Tissue culture is a vegetative propagation technique that is widely used in agriculture and horticulture for plant production. The use in forestry, however, is not common due to either technical or practical matters. Technically, forest tree species are quite diverse and in general are not easy to be propagated by vegetative means. The phenomenon of juvenility and aging is critical for the success of tissue culture of tree species.

The development of tissue culture techniques for Bali indigenous species reported here is to provide alternative for propagation of planting materials. In case when seeds are not available, vegetative propagation is the only alternative for production of planting materials. Conventional propagation by cuttings, marcoting, or grafting is often as problematic as tissue culture propagation. The potential advantage of tissue culture over cuttings is seen as an attractive option.

The techniques being developed by this project is expected to be used for operational application in the future. One of the hurdles of tissue culture propagation is the high cost due to investment on infrastructure and high production cost. If conventional propagation is a success, it would be much less expensive and technically simpler.

2. MAIN TEXT

The study was carried out at the Tissue Culture Laboratory of the Centre for Forest Biotechnology and Tree Improvement in Yogyakarta.

2.1. Procedure

Source of explants

Explants were collected from newly grown seedlings, leaf and seed to ensure success and ease of sterilization. Seedlings were grown in the glass house to minimize source of contaminants. Juvenile explants will have more chance of success than older one. In order to initiate juvenile materials pruning can be performed and shoots of 1 – 3 cm can be collected 2 -3 three weeks later.

Sterilization

Sterilization is an important step to obtain explants free of any contamination

Sterilization process includes :

Immerse in 1% fungicide (10 g/l) for 15 minutes.

Flush with running water and immerse in detergent for 15 minutes.

Flush with destilled water.

Working under the laminar cabinet, soaked the explants in 70 % alcohol for 1 minute.

Flush with sterile distilled water then immerse in 2% NaOCl for 10 minutes.

Flush with sterile distilled water several times, and explants are ready for initiation.

Initiation

Tissue culture initiation aims at obtaining sterile materials for multiplication stage. Explants for initiation may come from axillary shoots of seedlings or newly germinated seedlings. Initiation may takes several weeks and sub-culturing often is required before explants are ready to be transferred to multiplication stage.

Multiplication

In the multiplication stage explants are grown in media supplemented with growth regulator (giberellic acid) to stimulate multiplication. The aim is to obtain maximum multiplication. Explants will be subcultured over several cycles before being transferred to rooting media.

Rooting

Vigorous explants from multiplication are transferred to rooting media supplemented with auxin.

2.2. Result

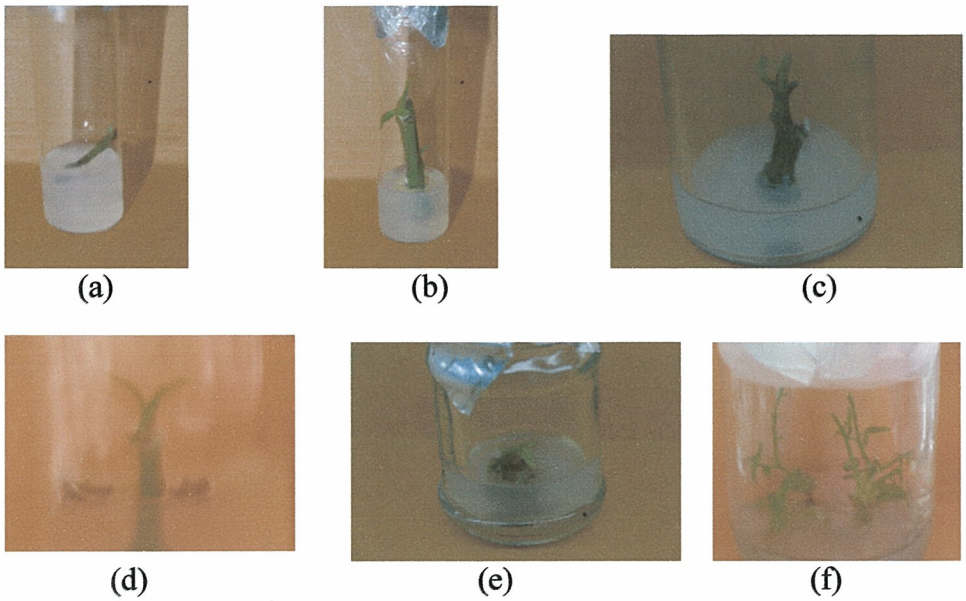
Initiation

The explants were cultured onto media MS (Murashige dan Skoog, 1962) supplemented with growth regulator of cytokinin and auxin.

Initiation of cultures has been carried out to all species at different time depending upon the availability of explants. Only two species, i.e. *A. scholaris* and *W. pubescens* have shown positive response and attained growth of axillary branching. *F. rhetsa* and *M. kauki* have not shown any axillary branching.

Table 1. Initiation stage of the 6 species

Species	Media composition	Growth regulators
A. scholaris	MS (Murashige and Skoog)	BAP 0,5 mg/l + Kinetin 0,15 g/l
M. kauki	½ MS (Murashige and Skoog)	BAP 1 mg/l + Kinetin 0,5 mg/l + NAA 0,01 mg/l
F. rhetsa	GD (Greshof and Doys)	BAP 0,5 mg/l + NAA 0,01 mg/l
D. densiflorum	MS (Murashige and Skoog)	BAP 1 mg/l + NAA 0,01 mg/l
P. valida	MS (Murashige and Skoog)	BAP 1 mg/l + NAA 0,01 mg/l.
W. pubescens	MS (Murashige and Skoog)	BAP 0,5 mg/l + NAA 0,01 mg/l



Picture 1. Induction result of (a) *Manilkara kauki*; (b) *Planchonia valida*; (c) *Fagara rhetsa*; (d) *Alstonia scholaris*; (e) *Dysoxylum densiflorum*; (f) *Wrightia pubescens*

2.2.1. Multiplication

In general media composition for multiplication is the same as media for initiation. Some modifications, however was applied to certain species, in this case *A. scholaris* additional growth hormone namely GA/gibberelic acid of 0,1 mg/l was added to the initiation media. The other species were cultured on the same media as in the initiation.

Repeated sub-cultured over 4 weeks interval is the standard procedure in most tissue culture techniques. Of the 6 species tested, three species namely *D. densiflorum, M. kauki* and *F. rhetsa* did not multiply. The other species have succesfully produced new shoots from axillary branching.

2.2.2. Rooting

Overall, *A. scholaris* and *W. pubescens* have been successfully cultured in multiplication and rooting stages. Plants have been acclimatized and were growing in the nursery. However only *W.pubescens* has sufficient number for the trial (99 plants were successfully raised in time for the trial), and have been transferred to the ITTO nursery in west Bali.

(a) (b) (c)

Picture 2. Rooting of *Alstonia scholaris* (a); *Fagara rhetsa* (b); and *Dysoxylum densiflorum* (c)

(a) (b)

Picture 3. Acclimatization in green house (a) *Alstonia scholaris*; (b) *Wrightia pubescens*

Table 2. Stages of development of the cultures are described in the following table

Species	**Tissue Culture Stage**				
	Initiation	**Multiplication**	**Rooting**	**Acclimatization**	**Nursery**
A. scholaris	√	√	√	√	√
M. kauki	√				
F. rhetsa	√	√			
W. pubescens	√	√	√	√	√
P.valida	√				
D.densiflorum	√				

3. CLOSING

Conclusion of development of tissue culture techniques are:

- Explants can be successfully obtained from the seedlings, an indication of the juvenile nature of the vegetative shoots.
- Generally, the basic media of MS supplemented with BAP is suitable for both initiation and multiplication.
- In case where axillary branching is difficult, callus culture can be an alternative. The media needs to be supplemented with 2,4 D hormone. *M kauki* and *D. densiflorum* are two species that can be propagated by callus culture.
- In the rooting stage, IBA hormon was used at low dosage (0.01 mg/l) for bentawas and (20 mg/l) for panggal buaya and majegau gave the best results.
- Four species have been successfully acclimatized (*A. scholaris, W. pubescens, F. rhetsa* and *D. densiflorum*) using media composition of top soil compost and sand (3 : 1 : 1). However only *W. pubescens* has sufficient number for field experiment.