

A Preliminary Study on the Germination of *Eurycoma longifolia* Jack (Tongkat Ali) Seeds

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Keywords: *In vitro* germination, jiffy, endocarpe, MS culture medium, seedlings

ABSTRAK

Eurycoma longifolia (Tongkat Ali) menghasilkan buah dalam satu tangkai terdiri daripada 200 - 300 biji. Pokok Tongkat Ali didapati berbuah dengan banyak pada bulan September tetapi bilangan anak benih yang tumbuh di atas lantai hutan hanya sedikit sahaja. Kami mendapati bahawa biji benih yang mempunyai endokarpa keras hanya mula bercambah 43 hari selepas disemai dalam 1:1 campuran pasir dan tanah dan percambahan biji berlaku sehingga 99 hari. Campuran tanah ini adalah setanding dengan kandungan tanah hutan yang paling optimum untuk percambahan biji benih Tongkat Ali. Biji benih yang tidak menanggalkan lapisan endokarpa bercambah dalam pelet jiffy di antara 35 hingga 85 hari. Tetapi dengan menanggalkan lapisan endokarpa, biji benih Tongkat Ali didapati bercambah dalam tempoh masa dua minggu dengan mengkulturkan biji benih secara *in vitro* dalam medium kultur asas Murashige dan Skoog (MS) (Murashige & Skoog, 1962). Biji yang matang didapati bercambah dengan baik dalam campuran tanah dan pasir (1:1) dan juga dalam pelet jiffy. Tetapi biji benih muda dengan menanggalkan lapisan endokarpa bercambah lebih cepat (CVG = 0.2053) secara *in vitro*. Kesemua anak benih didapati mempunyai corak pertumbuhan yang sama dalam jangka masa 120 hari dari segi ketinggian anak benih, bilangan daun yang dihasilkan dan garis pusat batangnya walaupun biji benih dicambah dalam medium percambahan yang berlainan.

ABSTRACT

Eurycoma longifolia fruits are borne in large bunches consisting of 200-300 fruits in each bunch. Even though trees produce abundant fruits during the peak fruiting season in September, yet the number of seedlings found growing in the forest floor is very low. Our results indicated that the seeds with hard endocarp sown in a 1:1 soil and sand mixture only start to germinate 43 days after sowing and continue to germinate over a period of 99 days. The soil and sand mixture (1:1) is equivalent to that of the forest sandy soil combination that was optimum for germination of *Eurycoma longifolia* seeds. The seeds with endocarp intact that are sown in jiffy germinated within 35-85 days. However, when the endocarp of seeds were removed, the seeds germinated within two weeks via *in vitro* culture using basic MS medium. The ripe seeds germinated better when sown in the 1:1 soil and sand mixture than in jiffy pellets. But the unripe seeds with the endocarp removed seemed to germinate faster (CVG = 0.2053) when cultured *in vitro* in basic MS medium. All the seedlings were found to have the same growth pattern in terms of seedling height, number of leaves produced, and the stem diameter irrespective of germination methods over a period of 120 days.

INTRODUCTION

Eurycoma longifolia Jack belongs to the family Simaroubaceae, which is commonly known as Tongkat Ali in Malaysia and Singapore. It is also native to Indochina, Borneo and Sumatra. This tree can grow to about 12 meters and is usually unbranched or with a few upright branches. Each branch is crowned by an umbrella-like rosette of pinnate compound leaves of 20-30 cm in length. Each leaf consists of 20-30 pairs of

narrowly oblong, leathery, dark green entire type of leaflets with shining dark brown leaf stalk (Corner 1988). In Malaysia, *E. longifolia* Jack commonly grow at low altitude, up to 700 meters in beach forests on sandy soil as understorey treelets (Nooteboom 1962).

E. longifolia is dioecious, producing hairy, purplish-crimson bell-like flowers in long and branched panicles. The female flowers consist of five petals, an ovary, one style with a 5-lobed

stigma and always with large but sterile stamens. The male flowers produce five stamens with a sterile pistil. The ovoid shape fruits are borne in a large dangling axillary bunch. Its peak flowering season is from June to July and with peak fruiting in September (Corner 1988).

Even though trees produce abundant fruits and seeds during each fruiting season, the number of seedlings found growing around the adult trees is low. Until now, the germination behavior of the seeds has not been studied. Therefore we wished to determine the general morphology of *E. longifolia* fruits and seeds, and how its structures influenced seed germination behavior. Their capacity to germinate under laboratory conditions and the growth pattern of its seedlings were studied. The possibility of using *in vitro* seed germination as an alternative method for enhancing the seed germination of *E. longifolia* Jack was also investigated.

MATERIALS AND METHODS

Fruit and Seed Morphology

E. longifolia fruits were collected from a secondary forest in Penang, Malaysia at three different sites namely Bayan Lepas, Teluk Bahang and Teluk Kumbar. A study was done on the external morphology and cross-section of the fruit and seed.

Germination Test

a. Effects of Germination Methods and Seed Maturity on Seed Germination

The fruits were removed from each bunch which consisted of approximately 200 to 300 fruits and grouped as young, unripe, green seeds and matured, ripe, red or dark-red seeds. Twenty seeds were taken randomly from each bunch and from each grouping to study the effect of each of the germination methods on seed germination.

The three germination methods were:-

1. The seeds were sown approximately one cm deep in a 1:1 soil and sand mixture.
2. The seeds were sown in jiffy pellets (Jiffy Products Ltd., Norway). These jiffy pellets were made up of peat soil and each seed was placed in each pellet.
3. The seeds were germinated via the *in vitro* technique. For this technique, the epicarp and mesocarp of the fruits were removed. The seeds were washed with detergent, then rinsed in running tap water for 30 minutes.

The seeds were then immersed in a 250 ml conical flask containing 20% (v/v) Clorox® solution which contained 5.25% sodium hypochlorite and three drops of tween-20 for 20 minutes, with continuous agitation. This was followed by rinsing three times with sterile distilled water. Surface sterilization of these seeds was repeated with 15% Clorox® solution for 15 minutes and again rinsed three times with sterile distilled water. The sterilized seeds were then placed on the surface of 15 ml Murashige and Skoog basic medium (MS) (Murashige & Skoog 1962) contained in 25x150mm culture tubes capped with autoclavable plastic caps (Jenaerglas, Rasotherm, Germany).

Twenty seeds were used for each germination method and the study was repeated three times. Percentage of germination for each method was recorded over a 120-day period. Germination was determined by the emergence of the radical and epicotyl on the germination medium surface. The effects of germination methods and maturity of seeds and their interactions on percentage of germination were analyzed using analysis of variance (ANOVA).

b. Influence of Endocarp (testa) on Seed Germination

The endocarp was removed after the seeds were surface sterilized twice as mentioned above. The seeds with the endocarp removed were again surface sterilized with 5% Clorox® solution for 10 minutes, rinsed three times with sterile distilled water and placed in 25 x 150 mm culture tubes containing 15 ml MS basic medium. Twenty seeds were used for each trial and the experiment was repeated three times. Percentage of germination was recorded over a period of 120 days.

c. Determination of the Coefficient of Velocity of Germination

The coefficient of velocity of germination (CVG) was computed based on Hartman and Kaster (1968):

$$CVG = \frac{\text{Total number of germination}}{A_1 T_1 + A_2 T_2 + \dots + A_n T_n}$$

where A = number of fresh germination recorded at each day interval
T = number of days from sowing.

The effects of germination methods and the maturity of seeds, and their interactions on the coefficient of velocity of germination were computed using ANOVA.

The Growth Pattern of Seedlings

Two weeks after germination, the seedlings were transferred to 15 x 23 cm polybags containing a 1:1:2 mixture of organic manure: top soil: sand. These seedlings in polybags were placed in a plant house at a temperature of between 28-30°C. The height of the seedlings was recorded every week starting from the emergence of the epicotyl, while the stem diameter was recorded every month. Plant height was taken as the distance from the tip of the shoot apex to the first node on the plant. Stem diameter was measured with a pair of calipers (Kern, Germany) at the fifth node of the stem. The number of leaves produced over a fortnight period was estimated by counting the last tagged leaf of the previous recording to the most recently produced leaf.

RESULTS AND DISCUSSION

The fruits of *E. longifolia* were borne in a large dangling axillary bunch. The bunches of fruits that were collected consisted of 200-300 seeds per bunch. The fruits were produced in groups of 1-5 on the bunches (Fig. 1).

The fruits were yellow to light green when young, and became red to blackish-red when ripe. The ripe and unripe fruits were distributed randomly in the same bunch. The variation in fruit maturity within the bunch serves to minimize the competition for substrate for successful seed germination at the forest floor which is often overcrowded with secondary growth. As stated by Villier (1972), some seeds appear to be

involved in controlling germination by restricting it to periods and conditions most favorable for seedling growth.

The fleshy drupe fruits were ovoid in shape and about 10-20 mm long and 5-12 mm broad. It consisted of a thin shining epicarp, fleshy mesocarp, hard and stony endocarp. The seed consisted of two large expanded cotyledons and a chlorophyllous capitate embryo. With the endocarp removed, the seed could be seen to be covered with a thin papery covering, which could be easily removed from the inner surface of the endocarp (Fig. 2).

Seeds with the endocarp intact sown in the 1:1 soil and sand mixture started to germinate from the 43 days and continued to germinate until 99 days after sowing (Table 1). The inhibition and delay in germination could be due to a high degree of impermeability of the endocarp to water or oxygen or to both. This phenomenon was similar to that of winged bean seeds (*Psophocarpus tetragonolobus* L.) which showed very low percentage of germination due to impermeability of the seed coat to water (Rudrapal *et al.* 1992). Rolston (1978) also reported that impermeability of hard seed coats was typical of legume seeds.

Seeds sown in jiffy pellets germinated earlier and within a shorter period of time (35-85 days) as compared to those sown in 1:1 soil and sand mixture (43-99 days). This was because jiffy pellets consisted mainly of peat soil and were able to retain higher moisture content compared to the 1:1 soil and sand mixture, hence allowing more water absorption by the seeds. None of the seeds with endocarp intact germinated when cultured *in vitro* using the MS culture medium (Table 1). Blackening occurred on the non-germinated seeds with the endocarp

TABLE 1
Effect of germination methods and seed maturity on the duration of *E. longifolia* seeds germination (days) within a 120-day period

Germination methods for <i>E. longifolia</i> seeds a	Duration of germination (days)	
	Ripe seeds	Unripe seeds
sown in soil and sand mixture	45-99	43-93
sown in jiffy pellets	37-85	35-70
<i>In vitro</i> culture	No germination	No germination

a seeds used for germination are with endocarp intact

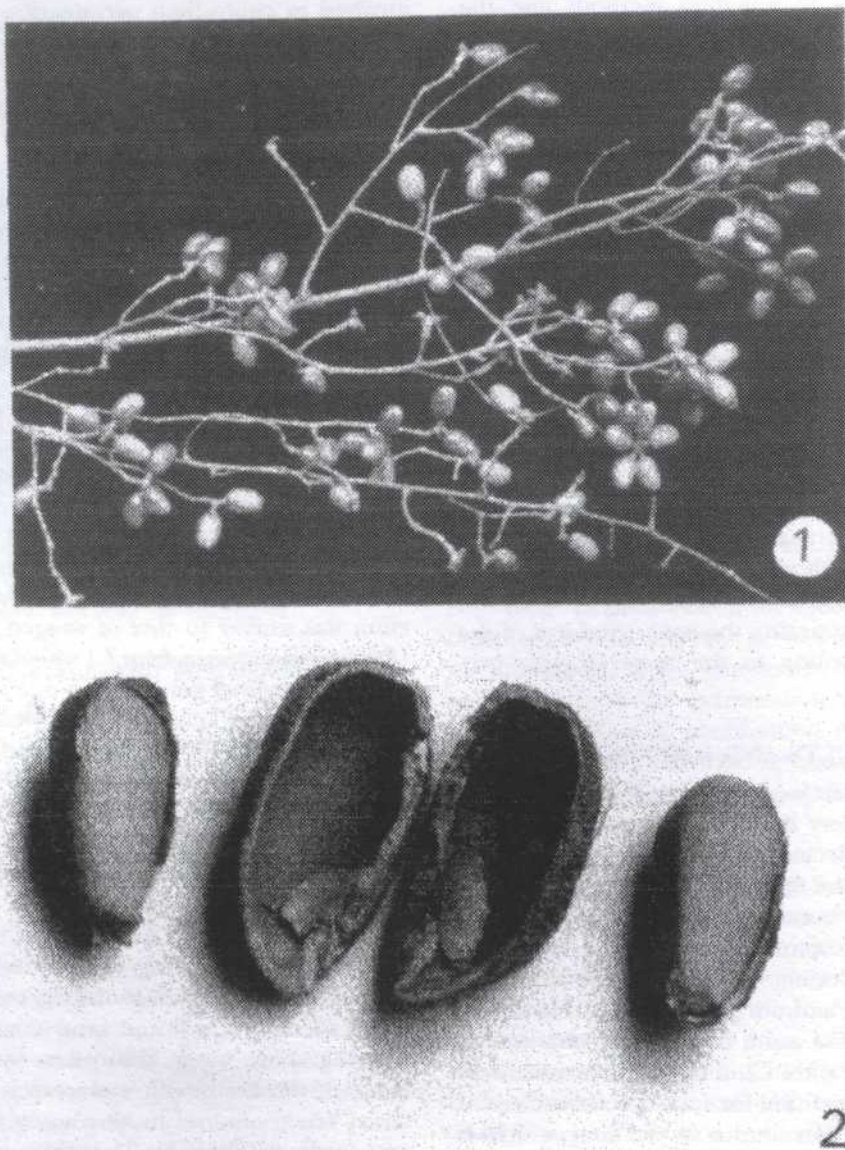


Fig. 1-2. *E. longifolia* fruits and seeds

1. *E. longifolia* fruits and groups of 1-5 in part of the bunches. 2. The seed with two large cotyledons and a chlorophyllous capitate embryo covered with a thin papery covering which was easily removed from the stony endocarp

intact and also in the MS culture medium (Fig. 3). However, the seeds without endocarp did not release any black exudates (Fig. 4). Marbach and Mayer (1974) reported that black exudates released were mainly phenolic compounds and could contribute to the impermeability of seed coats to water, hence preventing germination of seeds.

Ripe *E. longifolia* seeds sown in a 1:1 soil and sand mixture or jiffy pellets germinated better than the unripe seeds. Ripe seeds sown in the

soil and sand mixture (1:1) reached 58% germination over a 120-day period while only 46% of the unripe seeds sown in the same medium germinated at the same duration. Forty six percent of the ripe seeds sown in jiffy germinated over a 120-day period. Only 29% of the unripe seeds sown in jiffy germinated over the same duration. All the ripe and unripe seeds cultured *in vitro* did not germinate (Fig. 5). Rudrapal *et al.* (1992) proposed that the delayed germination of immature seeds was due to lower free

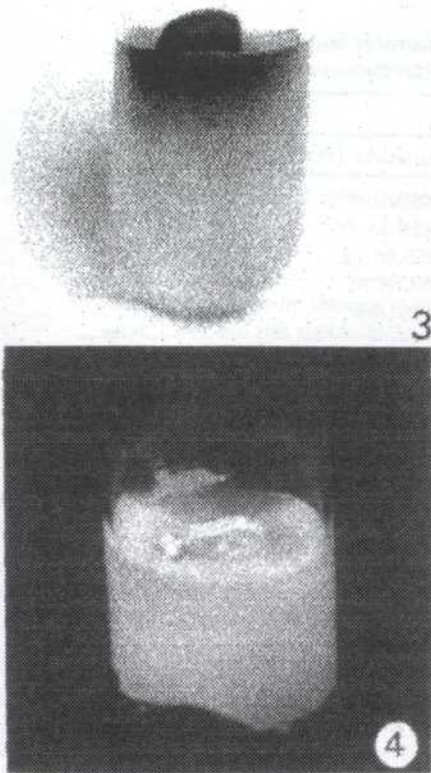


Fig. 3-4. Germination of *E. longifolia* seeds on MS culture medium.

3. Seed with endocarp intact released black exudates into the MS culture medium.
4. Seed without endocarp on MS culture medium clear of black exudates.

gibberellin content in embryo-cotyledon at the immature stage resulting in slower rate of water imbibition. Analysis of variance indicated that the different germination methods significantly affected ($p=0.01$) the percentage of germination of *E. longifolia* seeds. However, there were no significant differences in percentage of germination between the ripe and unripe seeds, and there was also no interaction between the ripeness of seeds and the different types of germination methods (Table 2).

The experimental results thus indicated that the stony endocarp did contribute to the germination process of these seeds. This was further supported by our findings on seeds with the endocarp removed starting to germinate 14 days after *in-vitro* cultured on MS medium. They continued to germinate until 64 days and none of the seeds with endocarp intact germinated on the same MS medium (Table 3). Edwards (1968) reported that most of the inhibition compounds that inhibited seed germination were usually located in the fruit wall or seed coat. Hence, this explained that *E. longifolia* seeds without endocarp would be able to germinate earlier.

With *in vitro* germination, the unripe seeds without endocarp germinated faster than the ripe seeds. The unripe seeds without endocarp showed maximum 53% germination while only 30% of the ripe seeds without endocarp germinated in the MS culture medium (Fig 6). This was further supported by the CVG results (Table 4) which indicated that unripe seeds with the endocarp removed, sown via the *in-vitro* method was the fastest to germinate, (CVG = 0.2053)

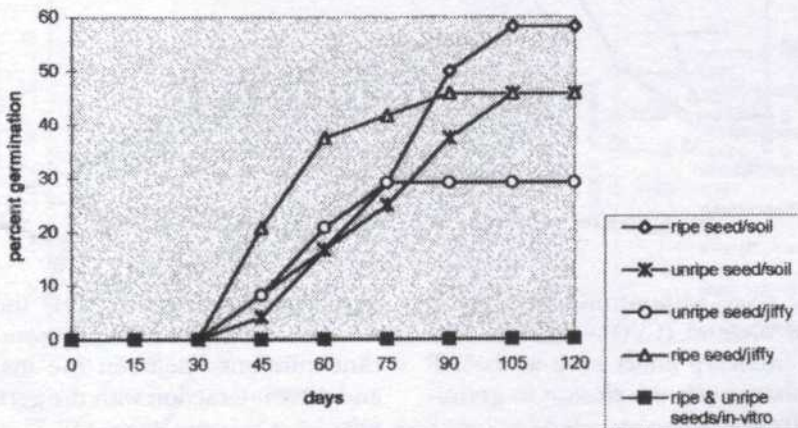


Fig. 5. Germination response of the ripe and unripe seeds of *E. longifolia* to different germination methods

TABLE 2
Analysis of variance for percentage of germination and coefficient of velocity of germination (CVG)

Source of variation	df	MS	
		Germination (%) ^y	CVG ^x
Treatment	5	1480.68 **	0.0159 **
Factor A (method)	2	3489.78 **	0.0363 *
Factor B (seed type)	1	255.08 ns	0.0015 ns
A x B	2	84.38 ns	0.0028 ns
Error	12	151.64	0.0013

^y analysis based on arc sine value.

^x analysis considers CVG data for in-vitro method using seeds without endocarp.

** significant at p=0.01.

ns not significant.

TABLE 3
Effect of seed endocarp on the duration of *E. longifolia* seed germination using in vitro technique

Condition of seeds	Duration of germination (days)	
	Ripe seeds	Unripe seeds
Seeds with endocarp intact	No germination	No germination
Seeds with endocarp removed	18-50	14-64

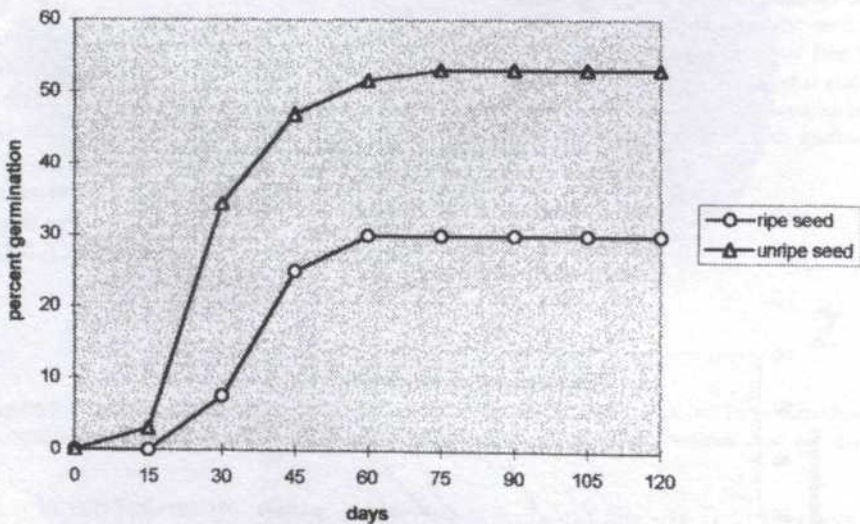


Fig. 6. In-vitro germination of *E. longifolia* seeds without endocarp

followed by ripe seeds without endocarp using the same *in vitro* method (CVG = 0.1377). The ripe seeds with endocarp intact sown in the 1:1 soil and sand mixture was the slowest to germinate (CVG = 0.0252).

The ANOVA presented in Table 2 also showed that the different germination methods

significantly affected (p=0.01) the coefficient of velocity of germination but there was no significant difference between the maturity of seeds and their interaction with the germination methods.

All the seeds sown in the 1:1 soil and sand mixture and in jiffy pellets showed the same

TABLE 4
Mean coefficient of velocity of germination (CVG) for *E. longifolia* seeds germinated with different germination methods

Germination methods	Mean CVG w	
	Ripe seeds	unripe seeds
sown in soil and sand mixture (1:1)	0.0252 a	0.0362 a
sown in jiffy pellets	0.0424 a	0.0456 a
In vitro v	0.1377 b	0.2053 c

* Means separation by Duncan's multiple range test, $p=0.05$. Values followed by the same letter are not significantly different.

v Seeds germinated via *in-vitro* method are with endocarp removed

growth pattern in terms of height, number of leaves produced and the stem diameter of the seedlings. The seedlings showed a rapid growth in height only in the first two weeks after germination followed by a gradual increase in height until 22 weeks. Then the seedlings grew slightly faster from 22 to 28 weeks after germination (Fig. 7). However, the stem diameter increase very slowly from sowing until the 5th month, followed by a slightly faster increase in stem girth after that.

The seedlings derived from seeds germinated without the endocarp via the *in-vitro* method also showed a rapid growth in height during the first two weeks after culturing, followed by reduction in growth rates. These seedlings showed the same growth pattern as seedlings arising from seeds germinated in the 1:1 soil and sand mixture and in jiffy (Fig. 8).

The number of leaves produced by the seedlings seemed to follow the same growth pattern as that of seedling height irrespective of germination methods. When seedling growth was slower, the number of leaves produced was reduced (Fig. 7 & Fig. 8). Since all the seedlings were planted in polybags and placed in the plant house, the seedlings were exposed to similar environmental condition, hence they have the same growth pattern irrespective of type of germination medium. Chan (1984) also noted that different varieties of *Carica papaya* L. grown in Malaysia had the same growth pattern because the constant environmental conditions encouraged continuous growth and development.

Our study indicated that *E. longifolia* seeds had a low germination rate. This was due to the impermeability of hard stony endocarps of its

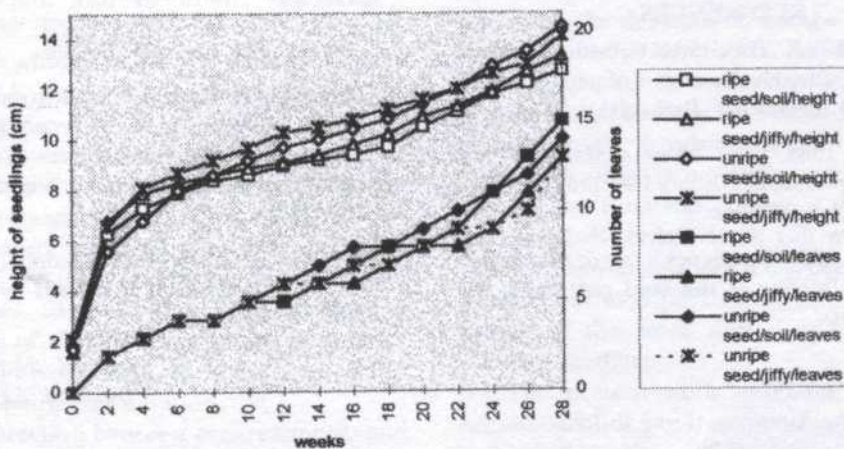


Fig. 7. The growth pattern of *E. longifolia* seedlings, derived from seeds germinated in soil and sand mixture and in jiffy pellets, in term of height and number of leaves produced

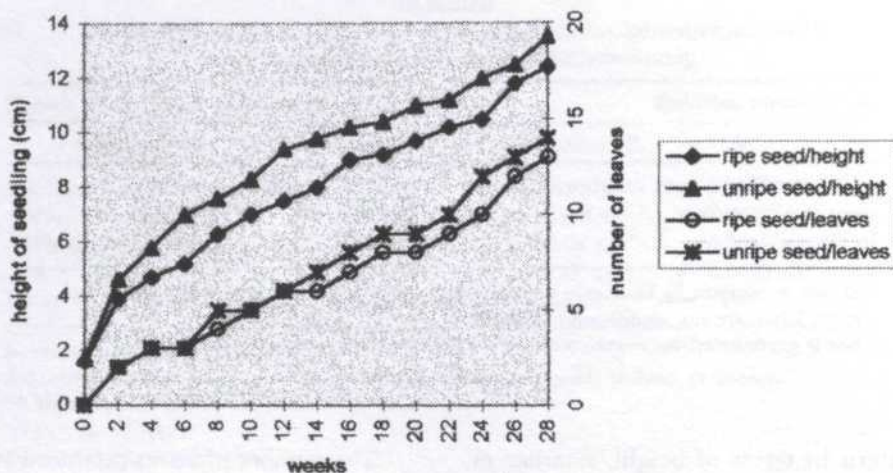


Fig. 8. The growth pattern of *E. longifolia* in-vitro seedling in term of height and number of leaves produced

seeds to water. The low germination rate of *E. longifolia* seeds could also be the reason why there is poor distribution of its seedlings in the forest floor. Since the seeds germinated faster when cultured in-vitro and there were no differences in the growth pattern of the seedlings, the in-vitro method of germination could be an alternative method for producing faster and more *E. longifolia* seedlings.

ACKNOWLEDGEMENT

We thank Universiti Sains Malaysia for providing research facilities and funding under IRPA Short Term R & D Grant No. 191-9632-0006.

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(Received: 24 February 1999)
 (Accepted: 17 August 2001)