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BEBERAPA METODE PEMECAHAN DORMANSI BENIH Leucaena leucocephala (Lmk. de Witt.) DAN BEBERAPA FUNGI PATOGENIK YANG BERASOSIASI DENGAN BENIH

Some Methods for Breaking the Seed Dormancy of Leucaena leucocephala (Lmk. de Witt.) and Some Pathogenic Fungi Associated With the Seeds

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ABSTRAK

Penelitian ini bertujuan untuk menentukan (1) metode pemecahan dormansi benih dan (2) menentukan fungi patogenik yang berasosiasi dengan benih.

Pemecahan dormansi dengan perendaman benih dalam asam sulfat 65-75% selama 25-35 menit sangat efektif untuk benih no. 3, kurang efektif untuk benih no. 4, tidak efektif untuk benih no. 2 dan mematikan benih no. 1. Akan tetapi untuk semua tingkat kemasakan, makin tinggi konsentrasi asam sulfat atau makin lama perendaman benih dalam asam sulfat, makin tinggi daya berkecambah benih. Terdapat interaksi antara tingkat kemasakan benih dengan konsentrasi asam sulfat.

Metode pemecahan dormansi dengan skarifikasi memakai "sample seed huller" dengan tiga kali pelewatan cukup efektif untuk benih no. 2, no. 3 dan no. 4. Perlakuan yang sama mengakibatkan semua benih no. 1 menjadi rusak. Perendaman benih dalam air mendidih selama 24 jam juga efektif, kecuali untuk benih no. 1 yang menjadi rendah daya berkecambahnya sesudah perlakuan tersebut. Pemecahan dormansi dengan penempatan benih pada 40^0 C selama 24 jam kurang efektif.

Fungi patogenik yang berasosiasi dengan benih adalah : Aspergillus sp., Penicillium sp., Colletotrichum sp., Pythium sp. dan satu jenis yang tidak dikenal (species X), menyebabkan kerusakan pada benih.

INTRODUCTION

Leucaena leucocephala (Lmk. de Witt.) (kemlandingan) is a tree, which has an important role both as forest plant and cultivated crops. In Indonesia, L. leucocephala has been known for along time and is used for intercrops at teak plantation or as shade plants and green manure producers in plantation (Martadiredja, 1971). This species is also very

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[1)] Staf pengajar dan peneliti di Lab. Patologi Hutan, Fakultas Kehutanan IPB, Kampus IPB Darmaga P.O. Box. 168 Bogor useful for erosion prevention (Martadiredja, 1971; Benge, 1976), alang-alang eradication (Knoop, 1910; Mathews, 1974, Benge, 1976) and soil amendments (Martadiredja, 1971).

One problem in management of L. leucocephala crop was difficulty in procurement of high quality seed in sufficiently amount. Several things have not been known yet, for instance those concerning dormancy breaking method, and proper condition for storage. Various fungi can develop in the seeds during in field or storage, and this can result in rapid decline of seed viability.

For the purpose of solving these problems, several factors those affect seed viability of L. leucocephala have been studied, which were among other things as follows:

1. Method of breaking seed dormancy.
2. Fungal pathogen, which attack seed.

Hopefully the research results can be used for developing method of handling seeds of this species, so that good quality seeds are available for planting L. leucocephala.

MATERIALS AND METHODS

Seeds of L. leucocephala

Seeds of L. leucocephala used in this research were those produced by the seed trees, which grew in IPB Campus of Darmaga, Bogor. Seed lots were obtained from pods with four levels of maturity, which were usually harvested for planting purposes, and they were numbered indicating its level of maturity. Explanations on pod and seed development on each number are as follows:

Seed no. 1: Seeds were succulent, and the color ranged from yellow to dark brown. Their pods were yellow and having spots of black or brown color, still fresh and succulent.
Seed no. 2: Seeds were flat and black. Nearly the whole part of the pod was black. Only the stalk and base of pod were still greenish yellow.
Seed no. 3: Seeds were flat and black. Pods were black colored. Their stalks were still fresh and greenish yellows.
Seed no. 4: Seeds were flat and black colored. Pods were black colored, entirely dry, nearly open.

Method of Seed Germination Testing

Seed germination test was conducted by using between paper tests with rice straw paper as germination medium, and by using germination type IPB-2A/B. The observed data, seed germination capacity were the number of the seeds, which germinate normally at the fourth and sixth days after the seeds were placed in the germination. Each treatment comprised four replications of 100 seeds each. Seed germination test was conducted 100 seeds each.

Breaking of Seed Dormancy

Chemical method

Experiment was conducted by soaking seeds in 65%, 70%, and 75% sulfuric acid with duration of soaking for each level of sulfuric acid concentration was 25 minutes, 30 minutes, and 35 minutes, after which the seeds were washed with water. Data in the form of seed germination capacity were analyzed by using factorial design with 4 x 3 x 3 levels (seed lots, sulfuric acid concentration and duration of soaking respectively).

Physical method

Sample of seeds were treated with three treatments, namely (a) soaking in boiling water, which was put away from the heat source for 24 hours, (b) storage at 40^0 C (in incubator) for 24 hours, (c) scarification using sample seed huller with three time passing through. The treated seeds were afterwards tested for their germination capacity. As a control group, is that group of seeds, which did not receive treatment before testing of germination capacity. For this experiment, the design used was completely randomized block design.

Identification of Pathogenic Fungi

Pathogenic fungi, which commonly occurred in seeds of the four levels of seed maturity were studied. Identification was conducted on seeds right after extraction. Seeds were incubated inside Petri dishes in room conditions. After the fungi grow, isolation was conducted and the fungus was grown on PDA. Afterwards the fungus was identified under microscope.

RESULTS AND DISCUSSIONS

Seed Dormancy Breaking

Chemical method

Germination capacity of seeds from various levels of maturity soaked in sulfuric acid at various concentrations with various soaking duration can be seen in Table 1.

Effect of sulfuric acid concentration on seed germination capacity was highly significant. Relation between sulfuric acid concentration (X) and seed germination (Y) was linear with regression equation $Y = - 33.80 + 0.786X$. The higher the concentration of sulfuric acid, the greater was the seed germination capacity (Figure 1).

Table 1. Germination capacity of L. leucocephala seeds from various levels of pod maturity (A) whose soaked in sulfuric acid in various concentrations (B) and duration (C) (in arc sin $\sqrt{\%}$)

Treatment	Replication				Total
	1	2	3	4	
$a_1b_1c_1$	0.00	0.00	0.00	0.00	0.00
$a_1b_1c_2$	0.00	0.00	0.00	0.00	0.00
$a_1b_1c_3$	0.00	0.00	0.00	0.00	0.00
$a_2b_1c_1$	40.98	43.28	47.87	42.13	174.26
$a_2b_1c_2$	39.82	42.13	46.15	50.77	178.87
$a_2b_1c_3$	42.13	45.00	44.43	45.00	176.56
$a_3b_1c_1$	12.96	16.43	12.92	12.92	55.23
$a_3b_1c_2$	15.34	16.43	12.92	14.18	58.87
$a_3b_1c_3$	16.43	17.46	11.54	12.92	58.35
$a_4b_1c_1$	5.74	8.13	5.74	15.34	34.95
$a_4b_1c_2$	11.54	8.13	9.98	9.98	39.63
$a_4b_1c_3$	11.54	11.54	14.18	17.46	54.72
$a_1b_2c_1$	0.00	0.00	0.00	0.00	0.00
$a_1b_2c_2$	0.00	0.00	0.00	0.00	0.00
$a_1b_2c_3$	0.00	0.00	0.00	0.00	0.00
$a_2b_2c_1$	38.65	42.71	46.98	54.94	183.28
$a_2b_2c_2$	50.18	49.02	46.15	47.87	193.22
$a_2b_2c_3$	47.39	47.29	50.18	52.53	197.39
$a_3b_2c_1$	24.35	20.27	25.10	21.97	91.69
$a_3b_2c_2$	18.44	26.56	21.97	25.10	92.07
$a_3b_2c_3$	21.97	25.84	28.66	30.00	106.47
$a_4b_2c_1$	11.54	8.13	14.18	16.43	50.28
$a_4b_2c_2$	12.92	9.98	12.92	17.46	53.28
$a_4b_2c_3$	16.43	18.44	5.74	14.18	54.79
$a_1b_3c_1$	0.00	0.00	0.00	0.00	0.00
$a_1b_3c_2$	0.00	0.00	0.00	0.00	0.00
$a_1b_3c_3$	0.00	0.00	0.00	0.00	0.00
$a_2b_3c_1$	43.85	43.85	42.71	46.15	176.56
$a_2b_3c_2$	46.15	43.85	40.40	46.72	177.12
$a_2b_3c_3$	42.13	49.02	51.94	49.60	192.69
$a_3b_3c_1$	30.66	27.28	33.21	24.35	115.50
$a_3b_3c_2$	33.21	33.21	33.21	31.95	131.58
$a_3b_3c_3$	38.06	31.95	33.83	33.66	137.50
$a_4b_3c_1$	27.28	22.13	25.84	21.97	97.22
$a_4b_3c_2$	19.37	29.33	22.79	17.46	88.95
$a_4b_3c_3$	21.97	23.58	22.79	26.56	94.90

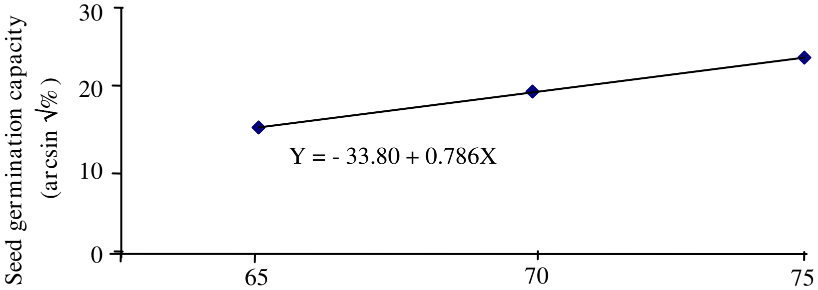

Figure 1. Relationship between concentration of sulfuric acid and seed germination capacity

Test of Honestly Significant Difference revealed that seed maturity level produced highly significant difference (Table 2). Seed germination capacity decreased with the increasing age of the seed.

Table 2. Difference in germination capacity between various levels of seed maturity (A)

Level of Factor A	Average	Value difference for			
		a_1	a_2	a_3	a_4
a_1 (seed lot no.1)	0	-	-	-	-
a_2 (seed lot no.2)	45.66	45.66^{**}	-	-	-
a_3 (seed lot no.3)	23.45	23.45^{**}	22.21^{**}	-	-
a_4 (seed lot no.4)	15.77	15.77^{**}	29.89^{**}	7.68^{**}	-

Remark: HSD (0.05) = 1.83; HSD (0.01) = 2.20; ** Highly significant

Results of test of Honestly Significant Difference showed that sulfuric acid concentration had highly significant effect on seed germination capacity (Table 3).

Table 3. Difference in germination capacity between seeds soaked in various concentration of sulfuric acid (B)

Levels of Factor B	Average	Value difference for		
		b_1	b_2	b_3
b_1 (65 %)	17.32	-	-	-
b_2 (70 %)	21.17	3.85^{**}	-	-
b_3 (75 %)	25.17	7.85^{**}	4.00^{**}	-

Remark : HSD (0.05) = 1.42; HSD (0.01) = 1.79; ** Highly significant

Effects of soaking duration in sulfuric acid solution were highly significant on seed germination capacity. Relation between soaking duration (x) and seed germination capacity (y) was linear with the following regression equation: y = 14.92 + 0.21x. The longer the soaking duration in sulfuric acid, the higher was the seed germination capacity (Figure 2).

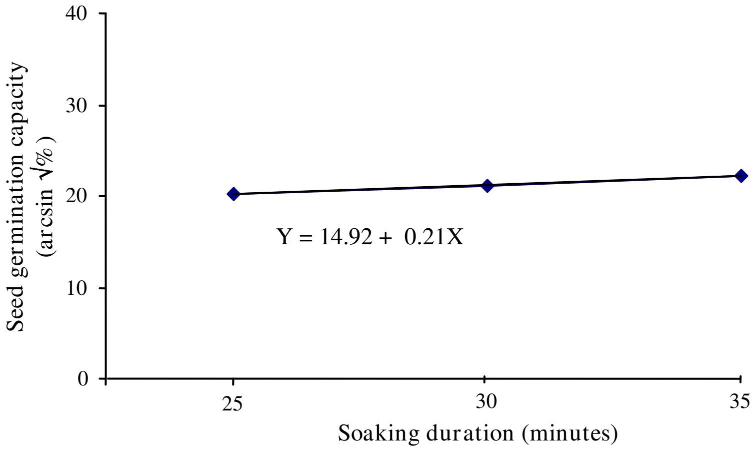

Figure 2. Relationship between soaking duration in sulfuric acid concentration and seed germination capacity.

Results of Honestly Significant Difference test showed that germination capacity of seeds soaked in sulfuric acid for 25 minutes differed in highly significant magnitude with those soaked for 35 minutes (Table 4).

Table 4. Difference in germination capacity between seeds soaked for various duration in sulfuric acid solution (C)

Levels of Factor C	Average	Value difference for		
		c_1	c_2	c_3
c_1 (25 minutes)	20.25	-	-	-
c_2 (30 minutes)	21.12	0.87	-	-
c_3 (35 minutes)	22.30	2.05^{**}	1.18	-

Remark : HSD (0.05) = 1.42; HSD (0.01) = 1.79; ** Highly significant

For first and second level, only interaction between seed maturity level (A) and sulfuric acid concentration (B), which had highly significant effect. After further examination, it turned out that interaction between sulfuric acid concentration and seed lot no.3 and 4 had highly significant effect (Table 5).

Table 5. Analysis of variance for the effect of interaction between seed maturity level (A) and sulfuric acid concentration (B) on seed germination capacity

Source of Variation	Degree of Freedom	Sum of Square	Mean Square	F computed
Between B within a_1	2	0	0	-
Between B within a_2	2	30.40	15.20	1.78
Between B within a_3	2	916.37	458.19	53.78^{**}
Between B within a_4	2	533.21	266.61	31.29^{**}

Remark: ** Highly significant; P (0.01) = 4.82

Observation results showed that soaking in various concentration of sulfuric acid concentration, produced better germination capacity for seed lot no.3 and no.4, as compared to seed lot no.1 and no.2. Soaking in sulfuric acid damaged all seeds from seed lot no. 1.

Effects of sulfuric acid concentration on germination capacity of seed lot no. 3 and 4 were linear (Figure 3). The increase in sulfuric acid concentration, enhanced seed germination capacity.

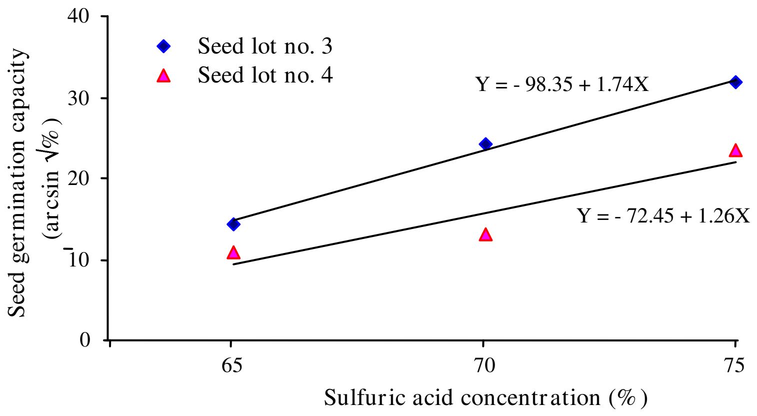

Figure 3. Relationship between sulfuric acid concentration and seed germination capacity

Results of Honestly Significant Difference test showed that for seed lot no. 3, concentration of sulfuric acid produced highly significant difference for germination capacity. For seed lot no. 4, there were no significant difference between seeds soaked in sulfuric acid 65% and those of 75%. Difference in germination capacity between seeds soaked in sulfuric acid 65% and those of 75% appeared significant (Table 6).

Table 6. Difference in germination capacity of seeds for maturity level no. 3 (a_3) and those of no. 4 (a_4) soaked in sulfuric acid 65% (b_1), 70% (b_2), and 75 % (b_3)

Factor Level	Average	Difference		
a_3b_1	14.37	-	-	-
a_3b_2	24.19	9.82^{**}	-	-
a_3b_3	31.80	17.43^{**}	7.61^{**}	-
a_4b_1	10.78	-	-	-
a_4b_2	13.20	2.42	-	-
a_4b_3	23.34	12.56	10.14^{**}	-

Remark: HSD (0.05) = 4.02; HSD (0.01) = 4.68; ** Highly significant

Physical method

Germination capacity of seeds which had received physical treatments, namely soaking in boiling water for 24 hours, storage at 40°C for 24 hours, scarification with "sample seed huller" with three times of passing through, and control treatment, were presented in Table 1. Results of variance analysis showed that treatments did not have any significant effect toward seed germination capacity (Table 7).

Table 7. Analysis of variance for the effect physical treatments on seed germination capacity

Source of Treatment	Degree of Freedom	Sum of Square	Mean Square	F computed
Block	3	718.55	239.52	0.34 (2.94)[1)]
Treatment	3	1,243.56	414.52	0.58 (1.72)
Treatment error	9	6,408.82	712.09	
Total	15	8,370.93		

[1)] ( ) : 1/F computed

For getting information from each maturity level concerning the effect of various treatments on seed germination capacity, variance analysis was conducted and that the treatments had significant effect.

Results of Honestly Significant Difference test for seed germination capacity from each maturity level treated with various physical means were shown in Table 8.

Table 8. Difference in germination capacity between treatments (T) at various level of seed maturity (A)

Treatment		Average	Value difference with			Remarks
			t_0	t_1	t_2	
a_1 (seed lot no. 1)	t_0	73.58	-	-	-	HSD (0.05) = 9.97
	t_1	40.16	33.42^{**}	-	-	
	t_2	58.86	14.72^{**}	17.30^{**}	-	
	t_3	0.00	73.58^{**}	40.16^{**}	58.86^{**}	
a_2 (seed lot no. 2)	t_0	40.25	-	-	-	HSD (0.05) = 9.70 HSD (0.01) = 12.71
	t_1	47.01	6.06	-	-	
	t_2	16.00	24.75^{**}	31.01^{**}	-	
	t_3	71.96	31.01^{**}	24.95^{**}	55.96^{**}	
a_3 (seed lot no. 3)	t_0	17.06	-	-	-	HSD (0.05) = 6.93 HSD (0.01) = 9.08
	t_1	48.74	31.68^{**}	-	-	
	t_2	9.84	7.22^{**}	38.90^{**}	-	
	t_3	59.38	42.32^{**}	10.64^{**}	49.54^{**}	
a_4 (seed lot no. 4)	t_0	11.45	-	-	-	HSD (0.05) = 5.71 HSD (0.01) = 7.48
	t_1	45.58	34.13^{**}	-	-	
	t_2	8.73	2.72	36.85^{**}	-	
	t_3	45.86	34.41^{**}	0.28	37.13^{**}	

Remark: * Significant at HSD (0.05); ** Highly significant at HSD (0.01);
t_0: Control (without treatment); t_1: Soaking in boling water for 24 hours;
t_2: Storage at 40^0C for 24 hours; t_3: Scarification with sample seed huller

Relation between seed maturity level and seed germination capacity for various physical treatments showed that soaking in boiling water for 24 hours could increase germination capacity of seed lot no. 2, no. 3, and no. 4. However, germination capacity of seed lot no. 1, could not be increased, even it was lower than that of control. Also, storage at 40^OC for 24 hours caused a decrease in germination capacity for each maturity level. The older the seed, the lower was the germination capacity after storage at 40^OC for 24 hours. Seed scarification with sample seed huller with three times of passing through, increased the germination capacity of seed lot no. 2, no. 3, and no. 4. Germination capacity of seed lot no. 2 was higher than that of no. 3, and that of no. 3 was higher than that of no. 4. On the other hand, seed scarification with sample seed huller made the seed lot no. 4 unable to germinate (Figure 4).

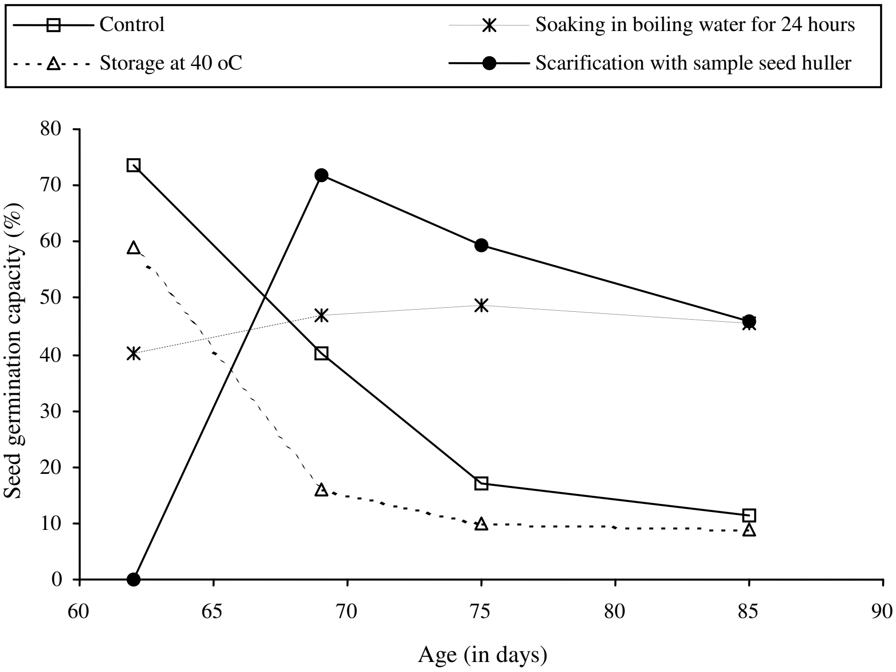

Figure 4. Relation between maturation level and germination capacity of seed treated with various physical means

In this study, all physical means to break dormancy, namely seed soaking in boiling water, seed storage at 40°C and seed scarification, had no effect on seed germination capacity. This was because the effect of dormancy breaking technique varied with various levels of seed maturity (Table 9).

Variation in the effect of treatments at various levels of maturity on the seed germination capacity was due to difference in response from the seed coat of varying levels of maturity, toward a certain treatment (Figure 4). The phenomenon could be described as follows (Figure 4):

1. Soaking in boiling water; many seeds of seed lot no. 1 were damaged and destroyed, due to their seed coat, which were still soft. In such condition, pathogenic fungi developed well and caused the germination capacity of treated seeds to be lower than that of control. For older seed, boiling water was able to soften the seed coat so that germination capacity could exceed that of control, although it did not reach maximum. Maximum results from seed soaking in boiling water could be achieved by adjusting the volume and temperature of water, and the soaking duration (Goor and Barney, 1968, and Woody Plant Seed Manual, 1948). Probably, the maximum germination capacity of seed lot no. 2, 3, and 4 could be achieved by longer soaking duration, or by