

The Effect of Seed Maturity, Temperature and Storage Period on Vigor of *Picrasma javanica* Bl. Seedling

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ABSTRACT

A study on the effect of seed maturity, temperature and period of storage on vigor of *Picrasma javanica* Bl. seedling was conducted at the Macropropagation Laboratory, Research Centre of Biology, LIPI, Cibinong, from March to September 2008. The research was arranged using Randomized Block Design with 3 factors and 3 replications, in which each replication had 20 samples. The first factor was stage of seed maturation with 2 levels i.e. pre-mature and mature stage; the second factor was storage temperature with 3 levels i.e. ambient temperature ($28\pm 1^\circ\text{C}$), 20°C and 5°C ; and the third factor was storage period with 4 levels i.e. 0, 1, 2, 3 months. The result showed that the germination time of *Picrasma javanica* seed varies between 11-23 days. The mature seeds had better growth as compared to pre-mature seeds. Storage on the ambient temperature ($28\pm 1^\circ\text{C}$) caused decrease of seed vigor, so is not suggested to seeds store in the ambient temperature. The storage under lower temperature (5°C and 20°C) was able to maintain seed vigor until 3 months storage.

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Key words: seeds maturity, temperature, storage period, vigor, *Picrasma javanica* Bl. seed.

INTRODUCTION

The synonym of *Picrasma javanica* Bl. is *Picrasma nepalensis* A.W. Bennet, *Picrasma philippinensis* Elmer. The plant belongs to Simaroubaceae family. The local name of this plant is 'ki pahit', 'kaju paek' which can be grown in Java island at 150-1400 m altitude (Heyne, 1997), or even at 1500 m (Hidayat, 2003). The bark of this plant can be used as a febrifuge to substitute for quinine (Heyne, 1997; Hidayat, 2003). The bark has been isolated and tested of its anti malaria activity *in vitro* using hexane extraction method (Saiin et al., 2003). Extract of leaves, seeds, stem and root of *P. javanica* Bl. consist of anti bacteria compound (Arbain and Sargent, 1987). Laloo et al. (2006) reported the use of *P. javanica* as anti-malaria medicine. Moreover Praptiwi et al. (2007) said that chemically active compound of bark extract of 'ki pahit' consist of alkaloid, flavonoid, saponin, tannin and steroid.

Heyne (1997) and Laloo et al. (2006) stated that the distribution of 'kaju paek' in nature is rare recently. Uji (1995) and Bahktiar (2005) reported that *P. javanica* in Sumatra is accounted as endangered species. It is caused by the harvesting of material medicinal plant was done by logging, at the same

time the cultivation not yet intensive done. The utilization of 'ki pahit' bark as a material of medicine requires some adult trees or minimum has bark.

To fulfill the necessity, some effort needs to be carried out for large scale cultivation of this plant. In the effort of these cultivation, optimal number, best quality and good continuity of seed stokes are needed. Sutarno and Utami (2007) said that seed viability of *P. javanica* is low i.e. 10-15%, and in $30-32^\circ\text{C}$ temperate. Seed viability with $4.8-20.8^\circ\text{C}$ pre treatment during 51 days resulted higher viabilities and uniform seedlings i.e. 90-100%.

In the effort to support the supply of seeds stocks with best quality, continuous and optimal number of seeds stocks, study about the effect of seeds maturity, temperature and storage period on vigor of *P. javanica* seedling is needed. The objective of the study is to know vigor of *P. javanica* seed during storage period. From the result of the study, it is expected to obtain data about technique of seed selection and seed storage of *P. javanica*, so that the seeds with high quality will always available.

MATERIALS AND METHODS

This study was carried out at the Macropropagation Laboratory, Research Centre of Biology, LIPI, Cibinong-Bogor, West Java, Indonesia from March to September 2008. *P. javanica* obtained from plant collection of Bogor Botanical Gardens was used as raw material in this study.

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Seed was harvested and separated into two different stages of maturity based on morphology characteristic of fruit peels color; pre-mature represented by white orange, and mature by green toska. Seeds storage were done by wrapping with aluminum foil, and further germinated on plastic boxes upon each treatment. Prior germination, part of seeds were taken as sample to examine water content by cutting about 5 g the seeds, oven at 105°C until constant was obtained, the water content based on fresh weight (ISTA, 1985). Besides, ion leakage of the seeds also tested by soaking of 5 g seeds in 50 mL deionized water during 24 hours at 15°C, its water solution measured with conductivity meter in μScm^{-1} per g of seeds (Hanson, 1983).

The study was arranged by using Randomized Block Design in Factorial with 3 factors and 3 replications, in which each replication had 20 samples (for germination percentage), whereas for seeds vigor was taken 3 samples of seedling aged 2 months. The first factor was stage of seed maturity with 2 levels i.e. pre-mature and mature stage; the second factor was storage temperature with 3 levels i.e. ambient temperature ($28\pm 1^\circ\text{C}$), 20°C and 5°C ; and the third factor was storage period with 4 levels i.e. 0, 1, 2, 3 months.

The observation was started from germination seeds until 2 months ages. The parameters were observed i.e. germination percentage (to know the seeds viability), seedling height, number of leaves, leaves length, leaves width, stem diameter, number of roots and seedling fresh weight (for vigor parameters). Seedling in germination boxes was watered daily, to maintain the relative humidity.

RESULT AND DISCUSSIONS

The result showed that time of germination of *Picrasma javanica* seed was varies between 11-23 days. After 2 months cultivation to germination of seed can be observed. At ebony seeds, the variation of time germination between 10-30 days (Sumiasri and Setyowati, 2006). The result of this study also showed that time of storage *P. javanica* seeds at ambient temperature during 2-3 months could not maintain seeds viability (germination percentage = 0%), in the pre-mature or mature seeds. Even on pre-mature seeds storage during 1 month at the ambient temperature seeds viability decrease until 0% (Table 6). It can be said that seeds storage of *P. javanica* at the ambient temperature cause death of seeds.

Seeds maturity stage

Seeds maturity is significantly correlated to seeds viability of *P. javanica*. It can be shown from the result of statistical analysis with significant different in all parameter observed at 5% Duncans test (DMRT). The mature seeds gave a better growth response as compare to pre-mature seeds shown by germination percentage (59.86; 44.44 %), seedling height (10.63;

8.20 cm), number of leaves (2.18; 1.87 leave), leaves length (3.44; 2.98 cm), leaves width (1.72; 1.47 cm), stem diameter (0.09; 0.08 cm) and number of root (6.45; 5.32), respectively. Although seedling fresh weight statistically showed no significant difference, but the fresh weight of mature seeds were higher than that of pre-mature seeds (Table 1).

Similar condition was also reported by Hartutiningsih and Utami (1998) in which seeds maturity stage of '*palem kipas*' was highly significant on its germination. The best germination showed by mature seed. Study on the '*palem putri*' study also revealed that seeds mature affected on germination rate and viability of seeds (Utami and Hartutiningsih, 2000). Moreover, the study of *Brucea javanica* seeds showed that the best germination percentage was in mature seed (Setyowati and Utami, 2008). According to Sutopo (1985) said that seed which harvested before physiologically mature stage has no high viability, at this stage, seeds were estimated lack of nutrient and impaired of embryonic development. The reason is after fruiting, weight and measurement of the seeds is increasing, until physiologically mature. The increasing of nutrient namely carbohydrate, lipid and protein, depends on species of seeds (Byrd, 1983). The reserve nutrient is substances which is used for hydrolysis during germination and transferred to embryo axis for seedling growth. Thus resulting on seed germination or seedling derived from mature seed to grow better than that from pre-mature seeds.

Seed water content

According to seed water content condition, early water content of pre-mature seeds is higher than mature seeds (Table 2), and there were no significant difference during storage. The pre-mature seeds showed decreasing water content during storage in 3 months. The water content about 39.47-33.14% for pre-mature seeds and 26.55-30.28% for mature seeds. The degradation of seeds viability estimated is caused by water content which is higher in pre-mature seeds. Sutopo (1985) said that seeds viability with high water content will undergo quick degradation. High water content will increase enzymes activities to accelerate respiration process, so that nutrition degradation was higher. The heat energy and humid condition stimulate microorganism which could damage seeds.

In this study, it could not decrease of water content, because in the preliminary study the water content could decrease viability of *P. javanica*, and estimated that the seeds belong to semi recalcitrant (N.W. Utami, 2009, private communication). On the contrary with '*adas*' seeds which belong to orthodox species, which could maintain of viability upon storage with low water content and low temperature (Utami and Sutarno, 2008).

Storage temperature

The storage temperature significantly affected to viability of *P. javanica* seeds. The seeds storage under ambient temperature ($28\pm 1^\circ\text{C}$), which is 20°C and 5°C temperature, indeed able to maintain seeds viability and vigor, based on mean of germination and growth seedling parameters (Table 3). The storage temperature of 20°C and 5°C were no significantly different, except on leaves length and seedling fresh weight. The storage at 5°C better than 20°C (Table 3). According to Sutopo (1985), the high temperature on seeds storage causes seeds damage, due increased evaporation of liquid substances inside seeds, which lead to lose imbibition capacity and germination viability. The embryo protoplasm could be died because a part or all of seeds dryness. Sutopo (1985) also said that the optimum temperature for long term seed storage is between $(-18)^\circ\text{C}$ to 0°C .

Storage period

Generally, seeds without storage (0 month) posses better vigor compared with seeds which is stored (1, 2, and 3 months), these were demonstrated by all of observed parameters, except on germination percentage and seedling height, a control which is lower than 1 month seeds storage, but no significant difference based on Duncans Test 5% (Table 4). This phenomenon only showed variation of germination since no significant different was observed based on statistical analysis. Although 1 month storage showed highest mean (seedling high 10.75 cm; germination percentage 58.06 %) than 2, 3 months storage and control, but no significant different with control.

The result indicated from Table 4 known that 3 months period was range time where mature seeds of 'ki pahit' still endure seeds viability with high water content of fresh seeds, with storage temperature under 20°C .

Table 1. The single effect of seeds maturity stage to viability and vigor of *Picrasma javanica* seedlings.

Seed maturated stage	Germination percentage (%)	Seedling height (cm)	Number of leaves (leaf)	Leaves length (cm)	Leaves width (cm)	Stem diameter (cm)	Number of root	Seedling fresh weight (g)
Pra-mature	44.44 b	8.20 b	1.87 b	2.98 b	1.47 b	0.08 b	5.32 b	0.28a
Mature	59.86 a	10.63 a	2.18 a	3.44 a	1.72 a	0.09 a	6.45 a	0.30a

Note: Number followed by the same letters on the same column was not significantly different based on DMRT 5%

Table 2. Effect of seeds maturity, temperature and storage period on water content of *Picrasma javanica* seeds

Seed maturated stage	Control (pre-treatment)	Water content (%)								
		1 st month			2 nd month			3 rd month		
		Ambient temperature ($28\pm 1^\circ\text{C}$)	20°C	5°C	Ambient temperature ($28\pm 1^\circ\text{C}$)	20°C	5°C	Ambient temperature ($28\pm 1^\circ\text{C}$)	20°C	5°C
Pre-mature	37.37	38.92	39.47	37.41	38.16	35.69	34.29	36.27	35.81	33.14
Mature	29.57	29.28	29.98	30.28	29.06	26.99	26.55	28.71	27.11	26.67

Table 3. Effect of storage temperature to viability and vigor of *Picrasma javanica* seedling

Storage temperature ($^\circ\text{C}$)	Germination percentage (%)	Seedling height (cm)	Number of leaves (leaf)	Leaves length (cm)	Leaves width (cm)	Stem diameter (cm)	Number of root	Seedling fresh weight (g)
Ambient temperature (28 ± 1)	24.58 b	4.14 b	0.90 b	1.54 c	0.74 b	0.04 b	2.83 b	0.13 c
20	70.21 a	12.10 a	2.67 a	3.88 b	1.95 a	0.10 a	7.10 a	0.34 b
5	61.74 a	12.04 a	2.51 a	4.24 a	2.10 a	0.10 a	7.77 a	0.39 a

Note: Number followed by the same letters on the same column was not significantly different based on DMRT 5%

Table 4. Effect of storage period to viability and vigor of *Picrasma javanica* seedlings

Storage period	Germination percentage (%)	Seedling height (cm)	Number of leaves (leaf)	Leaves length (cm)	Leaves width (cm)	Stem diameter (cm)	Number of root	Seedling fresh weight (g)
0	50.83 ab	9.59 ab	2.61 a	4.32 a	1.94 a	0.10 a	7.67 a	0.40 a
1	58.06 a	10.75 a	2.18 b	3.02 b	1.64 b	0.08 b	5.69 b	0.26 b
2	56.39 a	8.53 b	1.67 c	2.71 b	1.37 c	0.07 c	6.35 b	0.22 b
3	42.35 b	8.65 b	1.61 c	2.75 b	1.39 c	0.07 d	3.67 c	0.26 b

Note: Number followed by the same letters on the same column was not significantly different based on DMRT 5%

Table 5. Effect of seeds maturity, temperature and storage period to ion leakage of *Picrasma javanica* seeds.

Seed maturated stage	Control (pre- treatment)	Ion leakage (μScm^{-1})								
		1 st month			2 nd month			3 rd month		
		Ambient temperature ($28\pm 1^\circ\text{C}$)	20°C	5°C	Ambient temperature ($28\pm 1^\circ\text{C}$)	20°C	5°C	Ambient temperature ($28\pm 1^\circ\text{C}$)		
Pre-mature	4440.00	7580.63	5803.98	4388.11	21802.58	8856.62	3637.05	476285.20	14295.43	4917.18
Mature	4780.61	6658.77	3669.43	4559.53	24311.33	4303.43	4141.13	374338.90	7918.09	4705.04

Table 6. Effect of treatment combination on seed maturity, temperature and period storage to viability and vigor of *Picrasma javanica* seedling.

Seed maturated stage	Storage temperature (°C)	Storage periode (month)	Germination percentage (%)	Seedling height (cm)	Number of leaves (leaf)	Leaves length (cm)	Leaves width (cm)	Stem diameter (cm)	Number of root	Seedling fresh weight (g)
Pre- mature	ambient 28±1	0	53.33	8.91	2.22	4.11	1.88	0.10	6.89	0.38
		1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
		2	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
		3	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
		0	53.33	8.91	2.22	4.11	1.88	0.10	6.89	0.38
		1	76.67	13.17	3.00	3.31	1.90	0.10	6.44	0.32
	20	2	86.67	13.11	2.56	4.20	2.04	0.11	9.33	0.34
		3	60.00	10.83	2.78	3.67	1.96	0.10	4.56	0.40
		0	53.33	8.91	2.22	4.11	1.88	0.10	6.89	0.38
		1	63.33	10.33	2.61	3.39	1.82	0.10	6.22	0.34
		2	83.33	12.28	2.55	4.50	2.04	0.10	10.89	0.41
		3	53.33	11.78	2.22	4.33	2.10	0.10	5.66	0.33
Mature	5	0	48.33	10.28	3.00	4.53	2.00	0.10	8.44	0.42
		1	85.00	13.95	2.00	3.67	2.013	0.10	7.33	0.26
		2	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	ambient 28±1	3	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
		0	48.33	10.28	3.00	4.53	2.00	0.10	8.44	0.42
		1	75.00	12.22	2.89	3.52	1.71	0.10	6.56	0.30
		2	85.00	13.39	2.56	3.36	1.86	0.11	8.78	0.22
		3	76.67	14.89	2.33	4.39	2.23	0.10	5.78	0.36
		0	48.33	10.28	3.00	4.53	2.00	0.10	8.44	0.42
20	1	88.33	14.83	2.55	4.22	2.36	0.10	7.56	0.36	
	2	83.33	12.39	2.33	4.22	2.29	0.12	9.113	0.36	
	3	81.67	16.61	2.67	4.78	2.34	0.10	7.33	0.51	

This could also be observed on ion leakage test, in which mature seeds storage at 5°C during 3 months shows no increasing of ion leakage concentration i.e. 4559.53; 4141.13 and 4705.04 μScm^{-1} , for storage 1, 2 and 3 months, respectively, there were no significantly different with control (4780.61 μScm^{-1}). However, seeds storage at 20°C showed a few ion leakages i.e. 7918.09 during 3 month storage. The higher enhancement of ion leakage was shown by storage at ambient temperature i.e. 374338.90 μScm^{-1} . Consistently, storage at ambient temperature causes a highly increase of ion leakage concentration. The ion leakage was a reflection on cell membrane degradation. Higher ion leakage concentration, more clear to know of seeds damage indication (Copeland, 1976). It causes decreasing of seeds viability.

Effect of treatment combination

The pre-mature seeds which is stored at the ambient temperature ($28\pm 1^\circ\text{C}$) were unable to maintain its viability, so the storage of seeds at 1

month could not germinated (0%) (Table 6). The mature seeds could be germinated at 1 month storage; however at 2 months storage the seeds could not be germinated. This phenomena was caused by ion leakage enhancement which become higher and higher during storage at the ambient temperature, i.e. the early of ion leakage of pre-mature seeds (4440.00 μScm^{-1}); increase to 7580.63; 21802.58; 476285.20 μScm^{-1} , for storage on 1, 2 and 3 months, respectively (Table 5). Decreasing of seeds viability during storage perhaps caused by plasma membrane damage so that ion leakage occurred. Transportation of substance, carbohydrate hydrolysis and transpiration enzyme activities were disturbed and causes on germination failed (Hanson, 1983). Increasing on ion leakage was indicated by increasing of ions and soluble substance in soaking solution. It shows positive correlation between increasing of ion leakage and seeds deterioration (Hartutiningsih and Utami, 1996/1997).

The seeds storage until 3 months at 5 and 20°C, still could maintain seeds viabilities and vigor, for

mature or pre-mature seeds (Table 6). However, seeds viability and seedlings growth of mature seeds are better than pre-mature seeds; therefore mature seeds were preferable recommended for seeds storage (Sutopo, 1985). That food supplement transferred for growth of seedling after germination, so generally viability and vigor of mature seeds are better than pre-mature seeds (Byrd, 1983).

The germination percentage on control of pre-mature seeds was 53.33% higher than mature seeds (48.33%) (Table 6), because ion leakage stage of mature seeds ($4780.61 \mu\text{Scm}^{-1}$) are higher than pre-mature seeds ($4440.00 \mu\text{Scm}^{-1}$) (Tabel 5). The phenomena perhaps caused by physical condition of seeds selected as sample in this study, where the seeds sample was selected randomly. The germination percentage increased during storage at 20°C and 5°C, it was supported by data of concentration ion leakage where it were not highly significant different with control. During storage at 20°C and 5°C, the seeds still undergo maturation process, so that food supplement in the embryo was optimum enough for germination process, this causes the increasing on seeds germination.

CONCLUSSIONS

The conclusions of this study were (i) the first germination of *Picrasma javanica* seed was varies between 11-23 days., (ii) The mature seeds had better growth as compared to pre-mature seeds, (iii) Storage on the ambient temperature ($28 \pm 1^\circ\text{C}$) caused decrease of seed vigor, (iv) The storage under lower temperature (5°C and 20°C) was able to maintain seed vigor until 3 months storage.

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