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Effect of Seed Maturity and Storage Duration on Germination of Sambiloto (*Andrographis paniculata*)

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History Article

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Keywords

Andrographis paniculata; germination; maturity; seed; storage

Abstract

Seed maturity and its storage are one of problems on propagation and developing of medicinal plants such as sambiloto (*Andrographis paniculata* (Burm.f.) ex Nees). The research aimed to determine effects of seed maturity and storage duration on seed germination of sambiloto was conducted in a green house of Purwodadi Botanical Garden from November 2014 to November 2015. The experiment was done by completely randomised design with two treatments namely seed maturity and storage duration. The seed was classified into immature seed (0.061 g/100 seeds), semimature seed (0.113 g/100 seeds) and mature seed (0.166 g/100 seeds). The seed's storage duration was classified into seed was not stored, seed was stored for six months and seed was stored for twelve months. Each treatment combination was replicated five times. The results showed that there were significant interactions between the treatments on percentage and rate of seed germination. The highest percentage of seed germination was the treatment of mature seed and stored twelve months (98.50%). On the contrast, the lowest seed germination percentage was the treatment of immature seed and stored twelve months (4.25 %). The fastest seed germination rate was the treatment of mature seed and stored six months (3.88 days), whereas the slowest seed germination rate was the treatment of immature seed and without stored (28.58 days). This study is expected to be applied to improve genetic and cultivation of medicinal plant as well as increasing plant growth and yield.

How to Cite

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INTRODUCTION

Plant propagation and cultivation play important roles to develop, conserve and produce medicinal plants including sambiloto (*Andrographis paniculata* (Burm.f.) Nees). It is an urge to fulfil the need of medicinal plant materials for public consumption and industrial sustainably. The need of herbal medicine tends to increase at this time. The trade value of world herbal medicine about 12-15 billion USD in 2001 then increased to 60 billion USD in 2011, including export of herbal medicine from Indonesia about IDR 10-11 trillion (Kadarusman, 2011).

Plant propagation by seed is an important stage of medicinal plant cultivation to produce high quality of plant seeds for increasing plant growth and yield. It is also important to genetic improvement of plant species which is achieved by selection, first from population in fields that continued unique genotype fixed by vegetative propagation (Surya et al.,2016). Maturity and storage of seed need to be attended correlating to seed quality for seed propagation. This is often to become a problem for developing and cultivation of the medicinal plant.

Seed quality can be obtained by propagation with high seed quality, which is characterised by high viability, uniform, pure, free of pests and diseases. A good quality seed can increase plant yield by 15-20% (Ambika et al., 2014). The viability of seed was influenced by seed maturity and weight at harvested (Takač et al., 2015; Negasu, 2015). Seed maturity could be seen visually by the seed and fruit colour. The mature seed of sambiloto was produced by ripe fruit which has purple or brown fruit skin and brown seed. Whereas the immature seed was produced by unripe fruit which has green peel and cream or light yellow seed (Solikin, 2008; Solikin, 2016). Zhang et al (2013) also reported that the colour of over mature and immature seed of *Brassica napus* was black and light brown respectively. The dry weight of mature seed was higher than those of immature seed, as reported Negasu (2015) that the mature seed weight of castor bean (*Jatropha curcas* L.) was about 537.57 to 592.10 g / 1000 seeds; whereas the immature seed about 416.16 g / 1000 seeds. The mature seed has full mature embryo with maximum weight and size also greater germination percentage and faster germination rate than immature seed which not fully mature embrio and low seed viability, even not capable to germinate (Solikin, 2016).

Seed storage was commonly conducted for the plant cultivation, germplasm conservation, delivery and economy. Storage associated with the management of seed moisture content, temperature and humidity of room to maintain seed viability for long time. Longevity of seed storage is influenced by the characteristics of each type of seed and environmental condition. Recalcitrant seed cannot be stored longer because of its rapid declining viability. In contrast, orthodox seed can be stored or saved until a few years duration as reported by Van Treuren et al., (2013) that the seed of *Spinacea oleracea* L. was able to germinate well (about 90.3%) after being stored for four years. Storage of orthodox seeds was also important to solve the problem of physiological dormancy after harvesting so that the seeds can germinate optimally. Solikin (2014) reported that the seed germination of *Stachytarpheta jamaicensis* was increased from 56.8% when the seeds directly planted into 96.8% after stored for eight months after harvesting.

This study aimed to know the effect of seed maturity, storage duration and its interaction of these treatments on the seed germination of sambiloto (*Andrographis paniculata* (Burm.f.) Nees) to develop and to improve cultivation of medicinal plant in nursery and field.

METHODS

Seeds were harvested simultaneously from fruits of sambiloto grown in Purwodadi Botanical Garden in October 2014. The fruits (different stages of maturity with green to purple or brown fruit skin) were inserted into a plastic bag for sun drying and to split the seeds for a week. This was conducted to prevent the seeds scattering around and facilitating to collect seeds. Seeds had been sorted from fruit skin and dried under direct sunlight for two days, then they were selected into three categories (treatments) as shown in Table 1 (Solikin, 2016).

The experiment was conducted in a green house of Purwodadi Botanical Garden from November 2014 to November 2015 at altitude of 300 m above sea level. Minimum and maximum temperatures in the garden were 20.5 ^{o}C and 30.3^{o}C respectively with average temperature 27.6^{o}C and relative air humidity 72.9% during the experiment. The experiment used completely randomised design with two treatments (factorial) namely seed maturity and storage duration. The seed maturity was classified into the immature seed (0.061 g/100 seeds), semimature (0.113 g/100 seeds) and mature seed (0.166 g/100 seeds). The seed storage duration was classified by seed was not stored, stored for six months and stored for

Table 1. The seeds description of sambiloto for experiments (Solikin, 2016)

Maturity	Colour	Seed colour (RGB Color Codes Chart)	Weight/100 seeds (g)
Immature	Cream	*Pale golden rod* (EEE8AA); *light golden rod yellow*(FAFAD2); *light yellow*(FFFE0)	0.061
Semimature	Light brown	*Peru* (CD853F); *golden rod*(DAA520)	0.113
Mature	Brown	*Saddle brown* (8B4513; *seinna* (A0522D	0.166

Note: Seeding media used in this experiment was river sand which had been screened by 2mm sieve

twelve months. Each treatment combination was replicated five times with 100 seeds for each replication.

Seeds were sowed into furrow on media in polybags 13x7 cm as depth as 0.5 cm under media surface. Smooth river sand was used as media in the experiment. The polybags (15 polybags) had been put into a seeding box 38 x 28 x 15 cm before sowing the seeds. The seeding box was covered by transparent plastic and black paranet with 9.14% light penetration on the media surface. Watering had been done by hand sprayer after sowing the seeds and twice a week after that.

The observation was conducted on variables of seed germination percentage, seed germination rate, beginning and end of seed germination. The seed germination percentage was calculated by (germinated seed number/total tested seed) X 100 %; and the seed germination rate was calculated by $(N_1T_1+N_2T_2 + ... N_XT_X)$/ total number of seed germinating, where N is the number of seed germinating between beginning of the test and the end of the particular interval of measurement (Sutopo, 1988).

Data was analysed by analysis of variance (ANOVA) using MINITAB 16 software ($p<0.05$). Data was transformed in arching before analysed. Average values of the variables from all the treatments were compared using Duncan's Multiple Range Test (DMRT) at $\alpha= 0.05$.

RESULTS AND DISCUSSION

The results showed that there was significant interaction between treatment of seed maturity and storage duration on percentage and rate of seed germination of sambiloto (Table 2). The interaction between the treatments was caused by mean values of seed germination on various seed maturity to the storage duration treatment was different. This was also showed by Figure 1 that the seed germination of immature seed declined steadily by increasing storage duration from 9.75%, (without stored) to 4.25% (stored for twelve months) with trend line $Y = -5.20\ln(x) + 9442$; $R^2 = 0.94$ (Figure 1). Table 2 showed that the seed germination of this treatment were 9.75%, 5.00 % and 4.25% (without stored, stored for six and stored for twelve months respectively). This was caused by the immature embryo and least food reserve in the immature seed among the other treatments (mature and semimature). On the contrast, the seed germination on semimature and mature seeds continued to rise from 35.80% and 71.00% (without stored) up to 64. 5% and 98.50% (stored for twelve months) respectively (Table 2, Figure 1). The trendline response of seed germination on the semimature and mature seed which were stored for twelve months were $Y = 27.50\ln(x) + 37.82$, $R^2 = 0.906$ and $Y = 25.87\ln(x) + 72.29$, $R^2 = 0.956$ respectively (Figure 1). The seed germination percentage of the mature seed was 71.00%, 93.8% and 98.5% (without stored, stored for six and twelve months respectively) (Table 2). Increasing seed germination on the mature seed during the storage was also reported by Solikin (2014) on the seed of *Stachytarpheta jamaicensis* (L.) Vahl that its germination continued rising up to the storage for eight months.

Increasing seed germination during storage could be caused by the increasing physiological seed maturity (after ripening) during storage to mitigate dormancy and improve the seed germination such as showed at Figure 2 for the mature and semimature seeds. It was commonly happened on orthodox seeds such as seed of sambiloto. The continuing seed maturity after harvesting (after ripening) was also reported by Hartman et al., (2002) that some types of seed required storage time in dry condition for several days, months or years to be able to germinate optimally as a genetic trait of seed to adapt to the environment. This is a natural mechanism for the seeds of certain plant species to adapt their life cycle with the environment in order to remain viable and prevent extinction, particularly for wild plants. Ekpong (2009) proved that the seeds germination of *Cleome gynandra* which was directly sown the lowest among 1,2,3,4 and five months of storage.

Declining seed germination on immature seed after being stored was caused by the immature seed embrio and fewer food reserves used as germination energy than those in the mature or

Table 2. Percentage and rate of seed germination of sambiloto (*Andrographis paniculata* (Burm.f.) Nees) on the treatment of seed maturity and storage duration

Treatment	Germination percentage (%) *)	Germination rate (day)*)
Immature seed and without stored	9.75[a]	21.66[c]
Immature seed and stored six months	5.00[a]	8.22[a]
Immature seed and seeds stored twelve months	4.25[a]	9.34[b]
Semimature seed and without stored	35.80[b]	28.58[d]
Semimature seed and stored six months	62.50[c]	5.57[a]
Semimature seed and stored twelve months	64.50[c]	7.09[a]
Mature seed and without stored	71.00[c]	10.82[b]
Mature seed and stored six months	93.80[d]	3.88[a]
Mature seed and stored twelve months	98.50[d]	5.60[a]

Note : Numbers followed by the same letters in the same columns were no significantly different by *Duncan's Multiple Range Test* (DMRT) at α= 0.05.
*) : There was significant interaction among the treatments

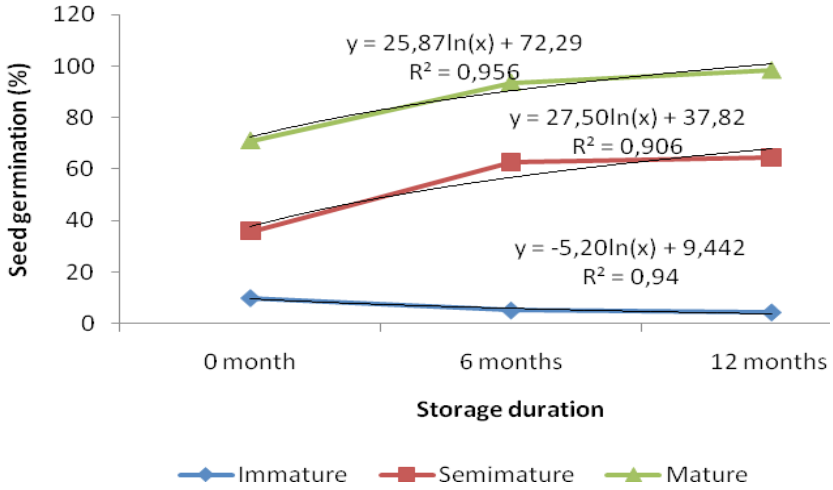

Figure 1. Seed germination of sambiloto (*Andrographis paniculata* (Burm.f.) Nees) on the seed maturity and storage duration

the semimature seed, so most of the immature seeds failed to germinate such as germination on immature seed of groundnut (*Arachis hypogaea*) that its germination lower than those on mature seed (Nautiyal et al., 2010).

Negasu (2015) reported that there was positive correlation between the content of the food reserve (weight) in the seed and the seed germination of castor bean (*Jatropha curcas* L.). Negasu (2015) also proved that germination of the mature seed of castor beans was 90%, whereas the immature seed was 65.67%. Seed coat of mature seed also contained higher calsium than immature seed (Nautiyal et al., 2010) which can increase preventing seed from diseases attacking. Nevertheless the mature seed still required storage after the seeds harvested to mitigate physiological dormancy and increase seed germination as shown in Table 2.

The seed germination rate at treatment of mature seed (the heaviest seed) and stored for six months was the fastest among the other treatments (3.88 days). Figure 2B showed that the seed number which germinated at the mature seed reached 90% in 3-6 days. This might be caused by the optimal seed physiological maturity after being stored for six months. The slowest seed germination rate was the treatment of semimature seed and without stored i.e. 28.58 days (Figure 2A) which showed that the seed germination continue to rise until 55 days. It might be caused by most of the seed maturity at harvesting still requiring a longer time (ripening) to embryo within the seed to germinate. Nevertheless, the seed germination percentage of the semimature seed was higher than those of immature seed. (Table 2).

The beginning time of seed to germinate

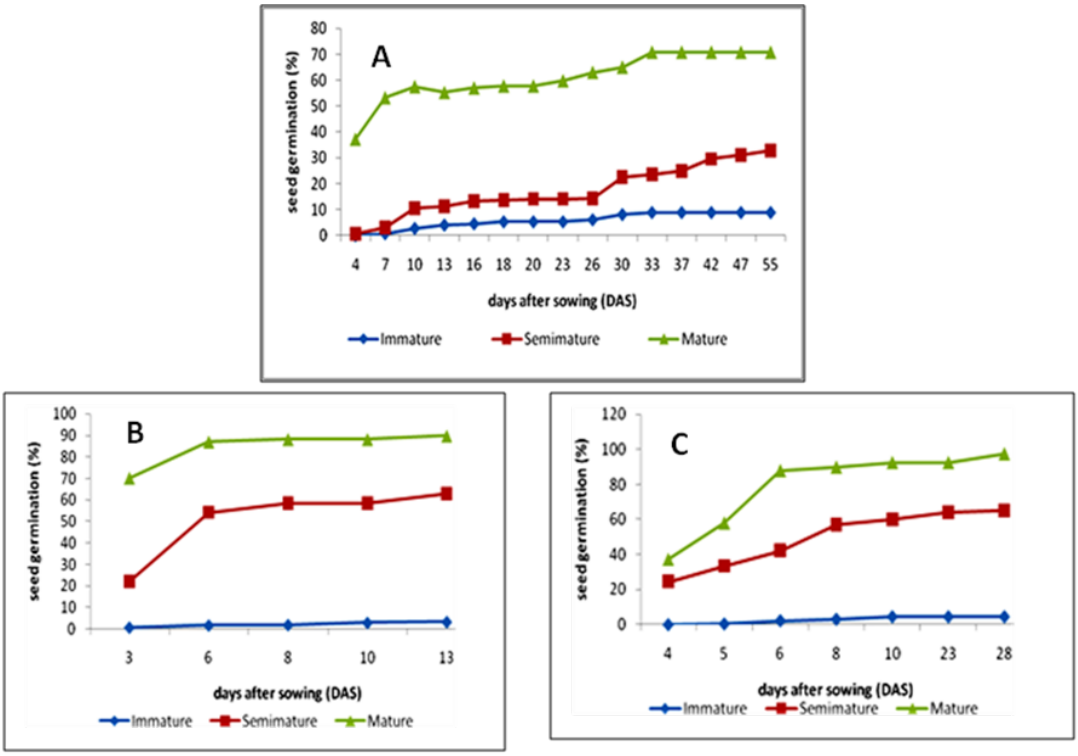

Figure 2. Seed germanination of sambiloto (*Andrographis paniculata* (Burm f.) Nees) on the treatment of seed maturity and without stored (A); stored for six months (B); store for twelve months.

was faster when the seed was stored than the seed without stored such as showed at Table 3 and Figure 2A that the beginning time of seed to germinate on the seed stored for twelve months was the quicker (3.67 days) whereas on the seed without stored was the slower (6.17 days). It was indicated that the time needed of the seed to start germinating on orthodox seed such as seed of *Andrographis paniculata* decrease when the seed stored until twelve months. This was consistent with the results of research by Solikin (2014) that the seed germination rate of *Stachytarpheta jamaicensis* without stored was slower (15.20) days than those of the seed stored two months (5.53) days. Gardner et al., (2008) also stated that some orthodox seeds required a certain period of time in order to germinate optimally.

Seed maturity of plant species has correlated to the change of seed coat (Atis et al., 2011) such as the seed coat of *Brassica napus* change from light colour to blackish colour following seed maturity from immature to over mature (Zhang et al., 2013). Seed colour of sambiloto also changed from cream or light yellow to brown or blackish brown from immature to mature seed (Figure 3). The seed maturity also correlated to the chemical compound(sugar, starch, oil and protein) contained in the seed. Zhang et al., (2013) reported that sugar and protein contain in seed of *Brassica napus* declining by increasing seed maturity, however the oil contain increase by increasing seed maturity; (Vera et al., 2007) also reported that the seed oil contain of canola was increase by increasing seed maturity. Increasing organic compounds which were accumulated in seed during maturity process will be followed by increasing weight as reported by Negasu (2015) at castor bean. Table 1 and Figure 3 showed that the weight of immature seed was 0.036 g/100 seeds whereas mature seed was 0.116 g/100 seeds.

Lipids containing in seed was very important to seed germination as source of germination energy (Hu et al., 2009) so the mature seed of sambiloto which may contain high lipids compound has greater germination energy and faster to begin germinate than immature seed (Table 4). Table 4 shows that the beginning time of seed germination of the mature and semi mature seed was faster (4.55 and 3.25 days respectively) than immature seed (6.25 days). This might be caused by lipids content in mature seed was greater than those of immature seed so its germination was faster than immature seed. This was also showed in Figure 1 that the seed number germinating at mature and semimature in 3-4 th days after sowing was more than immature seed, whereas immature seed starting germinated in 6-7 the days after sowing. Zhang et al., (2013) also proved that

Table 3. The beginning and end time of seed germination of sambiloto (*Andrographis paniculata* (Burm.f.) Nees) on the storage duration treatment

Treatment	The beginning of germination (day)	The end of germination (day)
Without stored	6.17[b]	41.67[b]
Stored six months	4.41[ab]	9.58[a]
Stored twelve months	3.67[a]	17.75[a]

Note: Numbers followed by the same letters in the same columns were no different significantly by *Duncan's Multiple Range Test* (DMRT) at α=0.05

Table 4. The beginning and end time of seed germination of sambiloto (*Andrographis paniculata* (Burm.f.) Nees) on the seed maturity treatments

Treatment	The beginning of germination (day)	The end of germination (day)
Immature seed	6.25[b]	21.58[a]
Semimature seed	3.25[a]	20.00[a]
Mature seed	4.75[a]	20.42[a]

Note: Numbers followed by the same letters in the same coloums were no different ignificanly by *Duncan's Multiple Range Test* (DMRT) at α=0.05

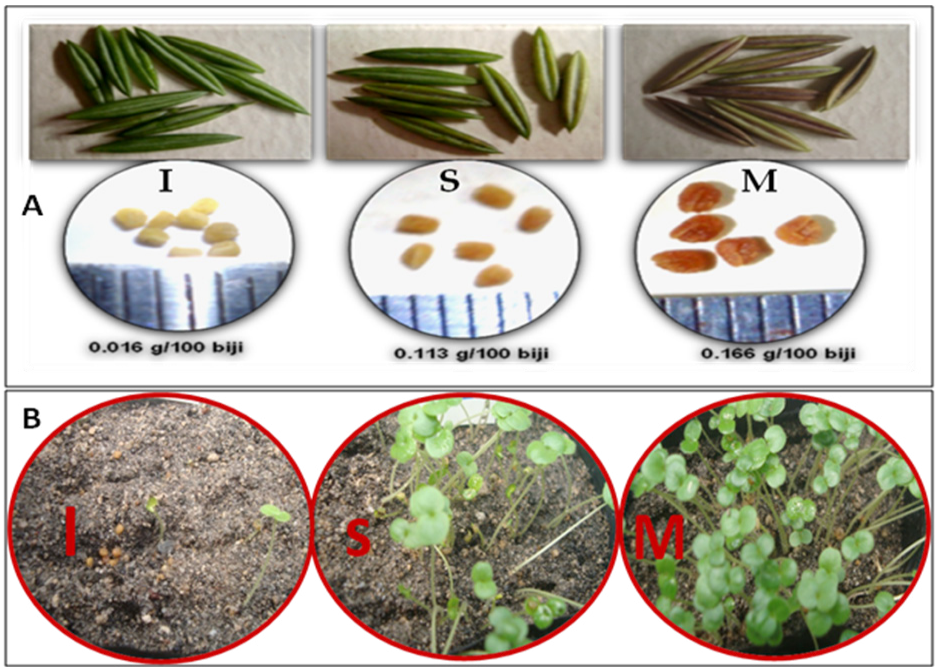

Figure 3. Sources of seeds **(A)** and their germination **(B)**: Immature(I),Semimature (S), Mature (M)

mean germinating time of mature seed of *Brassica napus* was lower (2.4 days) than immature seed (4.2 days). Statistically, the beginning time seed germination of the semimature and mature seed on sambiloto no significant different.

The end of seed germination of all maturity seed stages was no different statistically (Table 4) i.e 20.00 – 21.58 days. It was indicated that effect of seed maturity on the initial germination was determined in the first week, especially at the 1st - 3rd days. Starting of seed germination in this time was influenced by the lipids as or germination energy in seed and seed embrio maturity. After this stage, seed germination occured or continued normally as reported by Zhang et al., (2013) that the seed germination rate of *Brassica napus* at or after 7 th days was no significantly different. It was also showed in this experiment that the end of seed germination was no significantly different i.e. 20.00 -21.58 days.

This experiment indicate that seed maturity at harvesting is important to get high seed quality for sambiloto cultivation and development to increase plant production. Fruits of sambiloto was splitted and seeds scattered when the fruits over rippen so it is a problem for seed collection

in the field. By this research, mature seed can be collected from pinkish green – brown fruits coat to ensure and guarantee that the fruit coat no broken when harvesting or picking. Mature seed can also collected by sorting seeds if fruits harvesting conducted totally.

CONCLUSIONS

The highest percentage of seed germination of sambiloto (*Andrographis paniculata* (Burm.f.) Nees) was obtained from mature seed which was stored for twelve months (98.50%). The immature seed was not appropriate for propagation of sambiloto (*Andrographis paniculata* (Burm.f.) Nees) and indicated by the lowest seed germination percentage (4.25-9.75%). The fastest seed germination rate (3.88 days) was obtained from the mature seed which was stored for six months. This study is expected to be applied to improve plant genetic by seed propagation as well as increasing plant growth and yield of medicinal plant.

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