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PHYSICAL AND CHEMICAL TREATMENTS TO BREAK SEED DORMANCY ON LERAK (*Sapindus rarak* DC.)

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ABSTRACT

Sapindus rarak, which has local name as lerak, is a saponin producing plant belongs to the family Sapindaceae. Lerak has not been cultivated and grows wild in the forest. Utilization of lerak fruit pericarp includes traditional detergent, biopesticides, and for health purposes. *Sapindus rarak* and its related species in the genus are known for their delayed, uneven and low germination that in turn inhibit the regeneration. *Sapindus* genus usually undergo physical or physiological dormancy. Seed germination can be increased after the treatment of sand paper scarification, hot water, hydrochloric acid or gibberellin treatments. This study aimed to evaluate the effect of sand paper scarification, hot water, *hydrochloric* acid or gibberellin treatments on seed dormancy breaking of lerak. Germination experimental unit is a tray filled with a mixture of garden soil and sand in the ratio of 1 : 1. Each tray contains 30 seeds of 3 replications arranged in a randomized block design. The data on seed germination was collected daily and continued until complete germination (maximum up to 90 days). Parameters recorded were germination percentage and median length of germination time (MLG). The study showed that the highest percentage of germination (81.11%) and the shortest MLG (38.67 days) was shown by the treatment of hot water soaking with the temperature of 50°C for 20 minutes. The lowest percentage of germination (31,11%) and the longest MLG (67.20 days) was shown by the control (untreated) seeds.

Keywords: *Sapindus rarak*, seed scarification, GA_3 soaking, seed germination

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INTRODUCTION

Sapindus rarak DC. has local names lerak (Indonesia), rerek (Sunda), klerek, werak (Java) or lamuran (Palembang) (Kasahara & Hemmi, 1986). Lerak belongs to the family Sapindaceae, other species of similar genus with *Sapindus rarak* is *S. mukorossi*, *S. emarginatus*, and *S. saponaria*. Lerak plants which has the shaped trees grow well in almost all types of soil and climatic conditions, from the lowlands to the mountains with an altitude of 450-1500 m above sea level (Udarno, 2009). The average tall of lerak tree is 10 m, although it can reach 42 meters with diameter 1 m. Lerak commonly grows wild in the forest and shade grown.

Lerak fruit pericarp contains saponin, while the seeds contain oil (Sunaryadi 1999 & Stoffels, 2008). Lerak pericarp is used for washing batik cloth in Java and maintaining the color of the fabric to become more durable and does not fade (Herman, 2007; Stoffels, 2008), and also commonly used to wash precious metals made jewelry (Fatmawati, 2014), natural washers, biopesticides, and utilized for health purposes (Piputri & Lutfiati, 2014; Mediana & Prijono, 2014; Silviani & Puspitaningrum, 2015).

The usage of lerak based soap is one example of an environmentally friendly soap. The advantages of using lerak soap compared to artificial chemical soap more in terms of environmental protection. Lerak can be classified as a biopesticide crop because the saponin content of lerak pericarp can be used as insect (mosquitoes and cockroaches) killers. Saponins content of lerak may also have anthelmintic and moluscisidal activities (Nunik, 1998; Hamburger et al., 2007).

Based on it is multifunctional benefits, lerak plants need to be conserved. Lerak can be propagated by seed, so lerak seedling can be obtained in large quantities without the need of sophisticated equipment and does not require high costs. However, there is a problem in lerak propagation. Lerak has a low propagation success rate and have a low germination rate (Sunlayanuban, 1991). Seed germination of similar genus with lerak namely *Sapindus mukorossi*, *Sapindus emarginatus* and other species, were known to be low, long and uneven (Thapa & Gautam, 2005; Dobhal et al., 2012; Swaminathan & Revathy, 2013).

Mature and ripe seed which is ready to germinate requires appropriate climatic and growing conditions to be able to break dormancy and begin the process of germination. Seeds can undergo physical and physiological dormancy (Sutopo, 2002). Physical dormancy is caused by structural barriers to germination, such as a hard seed coat and waterproof and mechanical

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resistance of the seed coat to the growth of the embryo. Physiological dormancy caused by a number of physiological factors including plant growth regulators or immature embryos.

Sapindus genus has the type of physical or physiological dormancy (Cook et al., 2008). Physical dormancy in *Sapindus* is caused by the existence of the hard seed coats (testa) which interfere the absorption of water. Lerak seeds also has structure of the hard testa as well as kepel (*Stelechocarpus burahol*) that can lead to the difficulty of germinate (Isnaeni & Habibah, 2014).

Physiological dormancy is caused by physiological processes in seeds such as embryo immaturity. Embryonic development stages include pre-embryo quadrant and octane, globular, heart, torpedo stages in the seed. Embryos that not fully developed or immature, which is still in *the* torpedo stage, requires a certain period of time in order to germinate (Cook et al., 2008). Sautu (2004) states that seeds of *Sapindus saponaria* have physiological dormancy type with median length of germination time (MLG) normally of 74 days.

Gibberellin has been reported to be able to overcome seed dormancy in many species, so that *seeds* can germinate and the mobilization of endosperm reserves during the early stages of seed germination (Hopkins & Huner, 2008). One of the effects of gibberellins in seeds is pushing radicle cell elongation so that it can penetrate the endosperm, seed coat or rind that limits growth (Salisbury & Ross, 1992). Gibberellin can be produced by the plants, but the amount is not enough to stimulate seed germination mainly on hard-testa seed (Asra, 2014). Soaking the hard-testa seed with GA_3 usually been done to speed up the germination process.

Plant *growth* regulators Sakawa (cytokinin, Gandasil D, *Chlorella pyrenoidosa*) has been reported effective on increasing seed germination and seedling growth of lerak in compost media (Sumiasri et al. , 2010). Sakawa with the concentration of 2 mL/L gave the best effect on the growth parameters observed (percent germination, plant tall, leaf number, root length, and number of roots).

Seed dormancy on lerak also likely caused by the barrier of seed coat (testa). Woods (2004) states that physical scarification on lerak seeds can accelerate seed germination. Soaking the seeds in hot water is able to soften and open the pores of the hard seed testa (Baskin et al., 2004). Soft seed testa will allow water to be easily absorbed by the seed so that the physiological processes in seed germination can take place and happen.

The germination percentage and germination rate in lerak seed treated with sandpaper scarification and soaking the seeds in hot water of 65°C for 20 minutes resulted in better germination and seedling growth than soaking the seeds with KNO_3 solution or untreated seeds

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and soaking in auxin (Wardhani & Elik, 2007). Seed germination of *S. mukorossi* increased (32%) after been given hot water treatments than untreated seeds (Dobhal et al., 2012). The germination of *S. mukorossi* treated with saturated hydrochloric acid scarification for 75 and 110 minutes effective to accelerate and increase germination of *S. mukorossi* (Thapa & Gautam, 2005).

This study aimed to evaluate the effect of sand paper scarification, hot water, hydrochloric acid or gibberellin treatments on seed dormancy breaking of lerak for developing an extensive and well-planned lerak cultivation.

MATERIALS AND METHODS

Lerak seeds material

The physiologically matured fruit of lerak were obtained from a one plants grown in the Malang city. The collection was made in the month of February until April, 2015. The fruit dried at under shade room temperature and stored in plastic sacks until October 2015.

Treatments for dormancy breaking

Factors for dormancy breaking applied in this study includes sand paper scarification, hot water, hydrochloric acid or gibberellin treatments. ***Sand paper scarification*** consist of two variation as to scarify testa at 1) hilum, 2) hilum and back seed position. ***Hot water treatment:*** The seeds were soaked separately in three level of hot water (50°C, 65°C, 80°C) for 20, 30, 40 min. ***Hydrochloric acid treatment:*** The seeds were presoaked separately in three level concentration of HCl (80%, 90%, and concentrated) for 5, 10, 15 min. After treatment the seeds were washed 5 times with distilled water to remove the traced of acid and transferred to trays for germination. ***Gibberelin treatment***: The seeds were soaked in three level of GA_3 at 80, 90, and 100 ppm for 30, 60, 90 min separately.

Germination experimental unit is a tray filled with a mixture of garden soil and sand to 1 : 1. Each tray contains 30 seeds 3 replications arranged in a randomized block design.

Data collection

The seed germination data was collected daily and continued until all the seeds germinated (maximum up to 90 days). Parameters recorded were germination percentage (g) and median length of germination time (MLG).

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$g = N_g/N_s \times 100$
where: N_g = number of seeds germinated,
N_s = total number seeds,
$MLG = (N_1 \times H_1) + (N_2 \times H_2) + \ldots + N_k \times H_k) / N_1 + N_2 + \ldots + Nk$,
where MLG = median length of germination time,
N = number of seeds germinated on particular day (i),
H = day of emergence for germination
(Sautu, 2004; Kamble et al., 2013; Sandi et al., 2014).

Statistical analysis

The data were analyzed statistically using Anova (non-Factorial) in Randomized Block Design at the 5% probability level (Gomez and Gomez, 1986). Means differing significantly were compared using Scott-Knott test at the 5% probability level. Variability in the data has been expressed otherwise as mean ± SE (standard error).

RESULTS AND DISCUSSION

Germination percentage

All the treatment apply in this study enhanced the percentage of germination. The highest percentage of germination (81.11%) was shown by the treatment of hot water soaking with the temperature of 50°C for 20 minutes and the lowest percentage of germination (31,11%) was shown by the control (untreated) seeds (Figure 1 A). The results of analysis of variance showed that the kind of treatment affect on germination percentage of lerak. Scott-Knott test results show that soaking hot water with the temperature of 50°C for 20 minutes resulted in germination percentage (81.11 %) were not significantly different from the treatment of GA_3 100 ppm for 30 minutes (73.33 %), and hot water soaking with the temperature of 50°C for 30 minutes (72.22 %) (Figure 1A). The soaking hot water with the temperature of 50°C for 20 minutes was significantly different from other kinds of treatments.

Among the treatment of the seed coat sandpaper scarification, the highest percentage of germination (60.00%) was shown by treatment of the seed coat sanding on the side of the hilum, followed by the sanding on the two sides (34.44%), and by the untreated (31.11%) respectively. Whereas the treatment of hot water showed that hot water soaking with the temperature of 50°C for 20 minutes gave the highest percentage of germination (81.11%), followed the temperature of 50°C for 30 minutes (72.22%). The lowest percentage (37.78%) was shown the temperature of 80°C for 40 minutes. For the hydrochloric acid treatment, HCl 80% soaking for 5 minutes gave

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the highest percentage of germination (64.44%) on the other hand treatment of HCl 100% for 15 minutes gave the lowest percentage of germination (44.45%). For the gibberelin soaking treatment, the highest percentage of germination (73.33%) was shown by the treatment of GA_3 100 ppm for 30 minutes and the lowest percentage (46.67%) was shown treatment of GA_3 90 ppm for 30 minutes.

Median length of germination time (MLG)

The median length of germination time (MLG) was also accelerated by all the treatment apply in this study. The fastest MLG (38.67 days) was shown by the treatment of hot water soaking with the temperature of 50°C for 20 minutes and the longest MLG (67.20 days) was shown by the control (untreated) seeds (Figure 1B). Results of analysis of variance showed that the kind of treatment affect the MLG of lerak. Scott-Knott test results show that soaking hot water with the temperature of 50°C for 20 minutes resulted germinate the fastest time (38.67 days), but not significantly different with hot water soaking with the temperature of 50°C for 30 minutes (40.65 days), and treatment of HCl 80% soaking for 5 minutes (42.23 days) and significantly different from other kinds of treatments.

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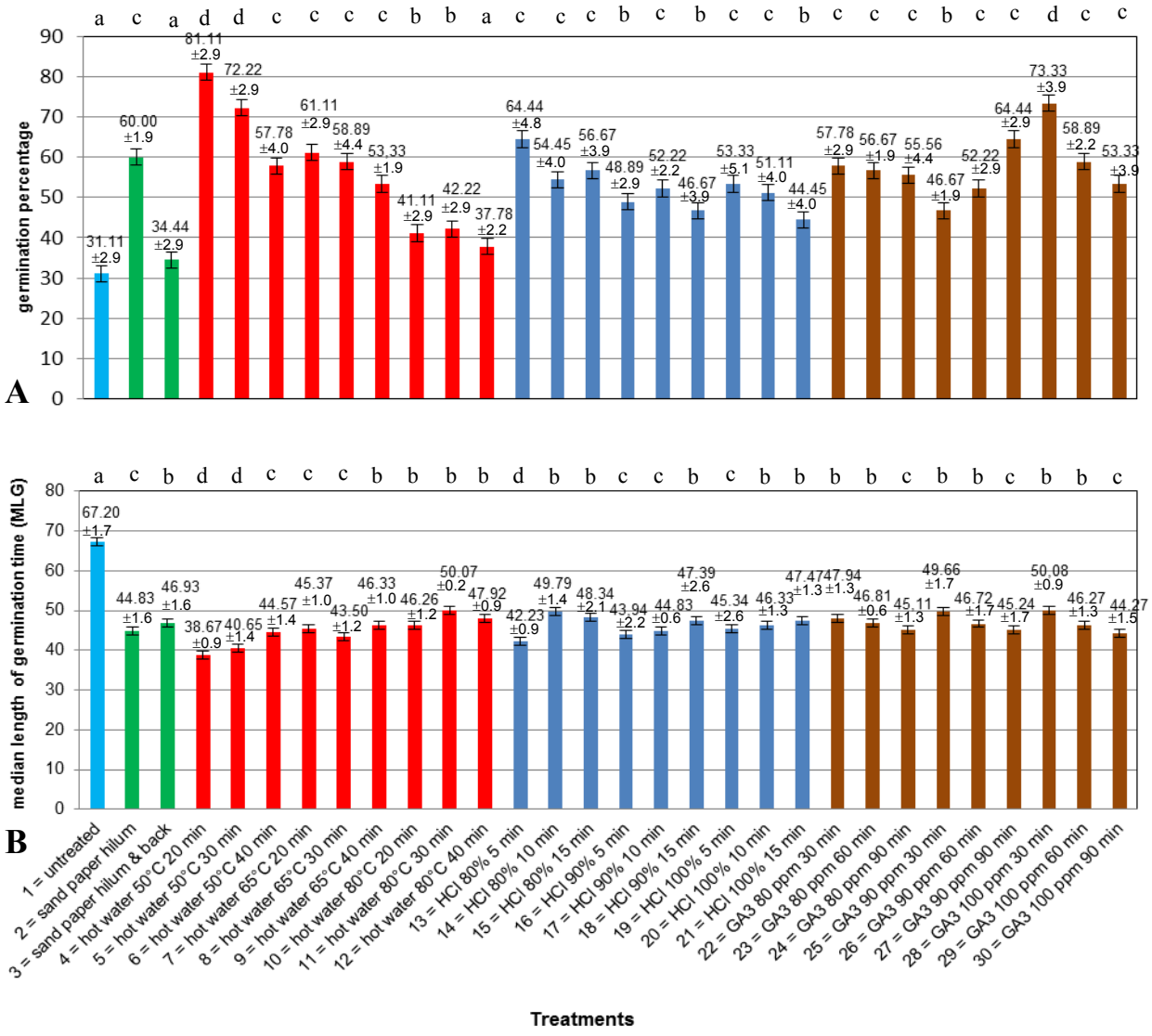

Figure 1. Effect of of physicall and chemicall dormancy breaking treatment germination percentage and median length of germination time (MLG) of lerak seed .

A. Germination percentage; B. median length of germination time (MLG).

untreated; the treatment of sandpaper scarification; the treatment of hot water; the treatment of hydrochloric acid; the treatment of gibberelin.

Numbers above column showed the mean ± SE (standard error). Notation by same letter above graph are show not significantly different at the 5% level with Scott-Knott test.

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For sandpaper scarification treatment, the fastest MLG (44.83 days) was shown by sanding one the side of the hilum, followed by the sanding on the two sides (46.93 days), and by the untreated (67.20 days) respectively. Whereas hot water soaking with the temperature of 50°C for 20 minutes gave the fastest percentage of MLG (38.67 days), followed the temperature of 50°C for 30 minutes (40.65 days). The longest MLG (50.07 days) was shown the temperature of 80°C for 30 minutes. For the hydrochloric acid treatment, HCl 80% soaking for 5 minutes gave the fastest of MLG (44.23 days) on the other hand treatment of HCl 80% soaking for 10 minutes gave the lowest of MLG (49.79 days). For the gibberelin soaking treatment, the fastest of MLG (44.27 days) was shown by treatment of GA_3 100 ppm for 90 minutes and the longest (50.08 days) was shown by treatment of GA_3 100 ppm for 30 minutes.

Lerak seed testa is impermeable for water (physical dormancy). In lerak seeds, hard testa structure as the main obstacle that resulted dormancy (Sautu et al., 2007). The treatment of the seed testa by sandpaper scarification facilitated water into the seeds for imbibition and facilitated germination. Whereas the seeds that were not sanded cannot absorb water well. Sanding of testa on two sides of the hilum and seed back, causing too much water entered the seed, so the seeds were oversaturated causing lower germination percentage and took longer time to germinate than the sanding on one side of the seed testa.

Soaking the seeds in hot water resulting in better seed germination and growth than the control (no treatment). The seed coat is made permeable to water through the hot water imbibition. Soaking seeds with hot water is able to soften and open “gap water” in the hard seed coat (Baskin et al., 2004). The softer seed coat caused water easily absorbed but slow enough to give a chance to the seed to facilitate physiological processes in seed germination to take place and happen.

Seed scarification can also be done with hydrochloric acid treatment. Soaking with hydrochloric acid also allows water entering the seeds for imbibition and facilitate germination. Chloride acid hydrolyze and increase the permeability of the seed coat. Acid scarification leads to partial or complete removal of inhibitors substances and weakening of the hard and impermeable seed coat (Naikawadi et al., 2012).

Seed growth can be stimulated by endogenous hormones seeds to germinate to produce normal seedling in unfavorable circumstances (Salisbury & Ross, 1992). Exogenous plant growth regulator that is given will interact with endogenous hormones. Growth hormone levels capable of causing a bit of a reaction either biochemical, physiological and morphological, which serves to influence the growth and development of plants. Gibberellin was able to overcome seed

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dormancy in many species. One of the effects gibberellin on seed is to stimulate elongation of radicle cell so that it can penetrate endopserma and seed coat endosperm that limited growth (Srivastava, 2002). Physiological effects of gibberellin among others are encouraging the activity of hydrolytic enzymes and the formation of amylase and enzymes that modify lipid into sucrose in the process of germination. Nurshanti (2009) stated that the provision of growth regulators (GA_3 75 ppm) influence on seed germination of *Roystonea regia* (king palm) 32% higher compared to controls. Lerak seed soaking treatment in GA_3 100 ppm for 30 minutes to produce 73.33 % germination percentage, 42 % higher than the control.

External gibberellin provided will change the level of internal gibberellin contained in the seed, that this level is a trigger factor for the germination process. Hopkin & Huner (2008) suggested that internal gibberellins responsible for the formation of the enzyme alpha-amylase occurred at the begining of germination. If the internal gibberellins are in limited quantities or not active then germination will be slow. The addition of an external gibberellins resulted in increasing the amount of gibberellin in the seed, thereby increasing the availability and activity of the alpha-amylase enzyme.

CONCLUSION

Soaking lerak seeds with hot water of 50°C for 20 minutes gave the highest percentage of seed germination and median length of germination time (MLG) compared with seed soaking treatment with HCl solution, GA_3 solution, and sandpaper scarification. Soaking treatment of hot water of 50°C for 20 minutes is recommended to break seed dormancy lerak, because it is cheaper and simpler equipment.

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