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The effects of physical and hormonal treatments on dormancy breaking and the changes in seed coat ultrastructure of *Delonix regia*

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Abstract. *Solichatun, Santosa, Dewi K, Pratiwi R. 2016. The effects of physical and hormonal treatments on dormancy breaking and the changes in seed coat ultrastructure of* Delonix regia. *Nusantara Bioscience 8*: *94-102.* The seed dormancy is described as the inability of an intact viable seed to complete germination under favorable conditions. *Delonix regia* (Hook) Raf. also known as flamboyant is a member of Fabaceae that has seed dormancy. Every seed has a different structure in their seed coat that responsible for initiating imbibition of water due to dormancy breakdown and germination. The aims of this research were to investigate the effect of physical and hormonal treatment in dormancy break of flamboyant seed and to investigate the structure of seed coat on the dormancy and germination. The treatments were applied to break the dormancy of *D. regia* seeds were physical treatments and hormonal treatments (ABA, BAP, GA, and IBA). Morphological and anatomical changes during dormancy breakdown were investigated using scanning electron microscope. The imbibition pathway tracked using blocking experiment. The results indicated that physical treatment that seeds dipped in hot water (98ºC) for 5 minutes was the most effective treatment to dormancy breakdown of flamboyant seeds (95%). The application of GA_3 100 ppm results in the highest of germination percentage (22%) among other hormones. The result of blocking experiment showed that hilum and lens play an important role during imbibition processes; and the type of flamboyant seed water gap was the lens gap type.

Keywords: *Delonix regia*, dormancy, germination, seed

INTRODUCTION

Seed, the dispersal unit of the plant, plays an important role in the higher plant life cycle. Many seed plants evolve some mechanism to rises successful next generation by setting the timing of germination. Therefore, many mature seeds committed to entering a dormant state. Seed dormancy, the term devoted regarding the inability of viable seed to germinate under the environmental conditions favorable for germination (Kermode 2005; Finch-Savage and Leubner-Metzger 2006).

Seed structure plays a critical role in the dormancy establishment. In typical angiosperm seeds, the embryo is surrounded by two covering layers, i.e. the endosperm and testa (seed coat). These components may contribute both in single or combination in the dormant state of the seed. Morphological formed dormancy represents by seeds that have an immature embryo and need extended time to grow and germinate. Physiological dormancy, generally related to abscisic acid (ABA) and gibberellins (GA) as well as other hormones metabolism inside the seed. Another type of dormancy imposed by water-impermeable seed coat is known as physical dormancy. Endosperm breakdown followed by seed coat rupture is the two important events that initiate germination in several seeds, including tomato and tobacco seeds (Gong et al. 2005). Germination commences with water uptake, imbibitions by quiescent dry seed and terminates with the elongation of the embryonic axis (Bentsink and Koornneef 2008).

Seed dormancy and germination were the least understood phenomena in the field of seed biology considering the biological complexity processes occur in the seed, and many influent factors are not yet clearly understood (Nonogaki 2006; Nonogaki et al. 2010; Nambara and Nonogaki 2012). These include, first, the control of water entry into the seed by imbibition as the onset of germination. Second, the involvement of a special structure on the seed coat that varies for each type of seed. Third, the setting of the balance of seeds endogenous hormones as well as a physiological and biochemical state inside the seed that importance to successful germination.

Flamboyant, *Delonix regia* (Hook) Raf. is one of the members of Fabaceae that has committed seed dormancy (Vozzo 2001). In 2010, the flamboyant included on the IUCN Red List of Threatened Species Version 2011. 2 with vulnerable status. In 2013, flamboyant Red List status was near threatened, and the last status in 2014 was the least concern (Rivers 2014). In some countries, flamboyant were exploited as firewood. The high exploitation is generally not counterbalanced by plant propagation efforts, so that decrease the population. Flamboyant has many uses as well as medicine, food sources due to their high protein content, and natural dyes (Adje et al. 2008; Kale et al. 2009; Chitra et al. 2010). Flamboyant is a legume plant widely grown in tropical and sub-tropical regions. The tree is cultivated as an ornamental or a boulevard tree due to its beautiful red flowers and its dense foliage, providing shade. Flamboyant

seeds have a hard, thick, and impermeable coat. Dormancy of flamboyant seeds is an interesting area of research.

Physical dormancy usually could be broken using physical treatments and/or hormonal treatments. There are five groups of hormones are known to play a major role in dormancy and germination, i.e., of ABA (ABA), gibberellin (GA), ethylene, brassinosteroids (BRs), and jasmonic acid (JAS) (Finkelstein et al. 2008; Linkies and Leubner-Metzger 2012). Abscisic acid (ABA) and jasmonic acid are known to control the status of seed dormancy and inhibiting germination. GA, ethylene, and brassinosteroids are known to promote germination (Brady and McCourt 2003; Penfield and King 2009). Changes in the nature of dormant into non-dormant seeds are influenced by the balance of endogenous hormones in the seed (Brady and McCourt 2003; Kucera et al. 2005; Penfield and King 2009). The aims of this research were to investigate the effect of physical and hormonal treatment in dormancy breaking of flamboyant seed and to investigate the structure of seed coat on the dormancy and germination.

MATERIALS AND METHODS

Seed collection

Seeds of *D. regia* were collected from the East Java region in July-August 2012 by Barokah Jaya collector of seeds Inc. (Bogor, Indonesia).

Seed sterilization method

Seeds were washed under running tap water for six hours. These seeds were soaked and shook in 2% sodium hypochlorite solution for 5 min. The sodium hypochlorite was removed with three times shaking in sterile distilled water at intervals of 10 min.

Experimental procedure

Effects of physical treatments on breaking of seed dormancy

In this study, the influence of physical treatments on breaking of seed dormancy has been studied. Seeds were sterilized and applied with physical treatments as follows:

Scarification: seeds cut edges using an electric grinder.

Dry heat: seed transferred into an aluminum tray and stored in an oven at 60^{o}C for 5 minutes.

Dry chilling: seeds transferred into a plastic bag and stored in a refrigerator at 4^{o}C for 30 days.

Hot water: seed soaked in hot water at 98^{o}C for 5 minutes

Each treatment consists of 100 seeds. The seeds were transferred onto separate Petri dishes with moist sterile Whatman No. 1 paper filter and incubated at 25°C temperature in the growth chamber with constant light. In addition, the same amount of seeds with no treatments was used as controls. The percentage of seed germination was calculated with the following formula:

$$\text{Percentage of germination} = \frac{\text{No. of germinated seeds}}{\text{No. of total seeds}} \times 100$$

Effects of plant growth regulators on breaking of seed dormancy

Four plants growth regulators which were ABA (Sigma-Aldrich), BAP (Sigma-Aldrich), IBA (Sigma-Aldrich), and GA_3 (Merck) applied on seeds of *D. regia.* Each hormone divided into two level hormones which are 10 and 100 ppm. Seeds were sterilized and transferred to petri-dish and moistened with hormone solution (10 ml). Seeds were incubated at room temperature (25°C) in constant light. The complete randomized design with ten replicates was used. The seeds were observed every day, and the number of germinated seeds was compared after 30 d. Data were subjected to ANOVA and LSD was calculated ($p>0,05$) by DMRT.

Morphological changes during dormancy breaking

Taking by a dissecting microscope equipped with a digital camera, a micrograph of seeds were taken before and after germination to compare morphological changes that occur during the breaking of dormancy. Light microscopy and scanning electron microscopy (SEM) imaging to investigate the water structure and seed structure was carried out according to methods of Serrato-Valenti et al. (2000), Van Dongen (2003), Ghosh et al. (2009), and Turner et al. (2009). Three *D. regia* seeds were selected for SEM analysis. The seed treated with hot water, GA_3 100 ppm, and non-treated dissected transversally near the micropyle region. Samples were sputter coated with platinum to a depth of 30 nm. Coated samples were scanned with an FEI-Quanta scanning electron microscope using an accelerating voltage of 10,00 kV and micrographs compared to identify the changes around the area where the proposed water gap is located. Light microscopy for anatomical sections was made from non-treated seed using paraffin method.

Blocking experiment (Gama-Arachchige et al. 2010)

The hilar and non-hilar region of flamboyant seeds was blocked with Castol-glue (methyl-2-cyanoacrylate) in 100 seed with a sharpened toothpick. One set of 100 seeds was blocked at the hilum, micropyle, lens, and extra-hilar region. Ten replicates of ten seeds for each treatment were placed on wet filter paper in Petri dishes and incubated for 30 d under the same conditions used in germination experiments. The number of germinated seeds was counted at the end of the experiment.

Statistical analysis

All data of germination and imbibition percentage were analyzed using one-way ANOVA, and Duncan's multiple range test was performed to determine significant differences between each treatment ($P<0.05$). All analyzes were carried out using SPSS ver 15 software.

RESULTS AND DISCUSSION

Effects of physical treatments on breaking of seed dormancy

The dry seeds of flamboyant did not germinate under room temperature condition and therefore were dormant. Analysis of variances showed that all physical treatments except with low temperature (cold storage) had a highly significant effect on breaking of seed dormancy (Figure 1). Dry heat, scarification and soaking seed in hot water raised the germination percentage of flamboyant seeds. The seeds under hot water treatment reached the maximum germination rate (95%) at six days after germination. The un-treated seed had a very low germination percentage due to their hard seed coat dormancy, and similar with Hassanein (2010) result.

Figures 1-2 showed that physical treatments can break seed dormancy of *D. regia* (flamboyant), except the treatments with low temperature (cold storage). The graph demonstrated that hot water treatment significantly influenced breaking of seed dormancy with highest seed germination of 95%. This experimental proof strongly indicated that breaking of seed dormancy was related to temperature and moisture. The treatment of soaking seeds in hot water causes changes in the permeability of the seed coat which promote seed imbibition that ultimately stimulate the germination of seeds. High temperatures would cause damage to the seed coat which encourages the entry of gas into the water and seeds. The influx of water and gas into the seed are important factors that outbreak seed dormancy and germination process begins (Varier et al. 2010).

Scarification treatment succeeded in raising the percentage of seed germination of *D. regia* (Figure 1). However, the resulting germination rate is still lower compared to the treatment of soaking the seeds in hot water. The study found that the seeds that have been scarified exposing most of their endosperm and they will quickly absorb water on germination media. Absorption of water will spur the endosperm softening, but unfortunately, the exposed endosperm promoted the growth of fungus massively. The growing fungus may interfere with the germination.

The rate of entry of water into the scarified seeds is also believed to be a factor that led to the failure of germination. The entry of excessive amounts of water in a relatively short period is not favorable for the success of seed germination flamboyant. From experience, it is known that the seeds failed to germinate in this treatment because of seed decay. The water that goes into the seed will encourage changes in the structure of macromolecules in cells, such as proteins that are hydrophilic which will absorb water and expand. This change will increase the turgor pressure in the cell, and if the pressure is too intense due to the high rate of water influx into the cell, it will cause damage to the cell membrane. Varier et al. (2010) suggest that the excessive entry of water into the seeds will lead to a decrease in germination success.

Dry heat treatment could stimulate seed germination of flamboyant, but it was not the maximum levels. Dry heat stimulated the damage of epidermal waxy cuticle, and induced crack on the testa; but the treatment did not produce the cracking through the macrosclereid layer (Bazin et al. 2011), so the impermeability of seed coat persist. Morrison et al. (1998) suggested that the seeds germination of *Cassia* (Caesalpinioideae) could not be stimulated by dry heat methods because cracks that occur in the region of the seed coat was not at the point of water entry. The different result of dry heat effect on seed dormancy was observed in other seeds, which is *Swartzia madagascariensis* (Leguminoceae) and *Tamarindus indica* (Ajiboye 2010; Amri 2010). Dry heat caused a dormancy-breaking on both seeds, and it was suggested that the structural difference among the seeds caused different respond due to their capability to germination.

Chilling storage did not influence the germination of flamboyant. After being stored at 4^{o}C for 30 days, there is no physical change observed in seeds. It indicates that there no alteration of seed permeability that allows imbibition process. The effect of chilling storage on dormancy breaking relies on the water content of seeds itself. Seeds with seed moisture content less than 15%, would not change the capability to germinate after chilling storage treatment. That treatment was only effective to induce dormancy breakdown, if the seed moisture content was higher than 20% (Probert 2000; O'Reilly and De Atrip 2007). The high moisture content of the seeds indicates the high water content in seeds. At low temperature, water molecules formed larger molecules that would induce the damage of cell membrane. This condition would induce the alteration of permeability. The flamboyant dried beans have a moisture content of the seeds is very low, perhaps only at 10-15% (Arora et al. 2010), so that the effect of chilling storage could not afford to encourage germination.

Soaking seeds in hot water increase the germination percentage and fresh weight. Soaking seed in hot water causes damage to the cuticle layer, leaching of secondary metabolites deposited in testa, also stimulate the occurrence of cracks through the macrosclereid layer. The combination of these results increases seed coat permeability to water and O_2. Water is a basic requirement for germination. It is essential for the activation of the enzyme, as well as the breakdown and translocation of food reserves. Early induction of optimal germination will affect physiological processes in the seed, and will result in the optimal growth of seedling (Varier et al. 2010).

Effect of exogenous PGRs in dormancy break

Evidence showed that exogenously applied plant growth hormones (PGRs) had increased the breaking of seed dormancy in many species. Gibberellic acid is known as important PGR and commonly involved in the breaking of seed dormancy. Another plant hormones that give the same effect of inducing germination are auxin (IBA, NAA) and cytokinin (BAP) (Kucera et al. 2005). In addition, gibberellins, especially GA_3 play a critical role in seed germination and are an important factor in the classification of seed dormancy.

Analysis of variance showed that plant growth hormones (PGRs) had a highly significant influence on

breaking of dormancy of flamboyant seeds (Figures 3-4). The results revealed that cytokinins (BAP 10 ppm), auxin (IBA 10 and 100 ppm), and GA_3 (10 and 100 ppm) increased germination percentage of flamboyant. The application of BAP at 100 ppm has different effects on seed germination, due to no significant effect on promoting germination. The results indicated that the seeds treated with ABA 100 ppm showed the increase of dormancy level, and ABA 10 ppm has no effect on seed germination. The maximum seed germination under GA_3 100 ppm treatments was 22%, and it could conclude that GA_3 showed the most effective exogenous growth regulator in breaking of seed dormancy and germination of flamboyant.

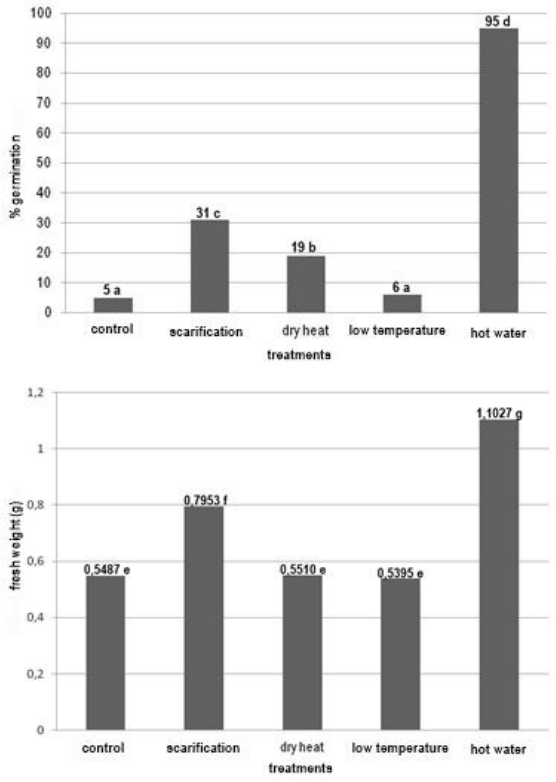

Figure 1. The effect of physical treatment on dormancy breakdown of flamboyant seeds. Different letters above bars indicate significant differences ($P \leq 0.05$) on DMRT.

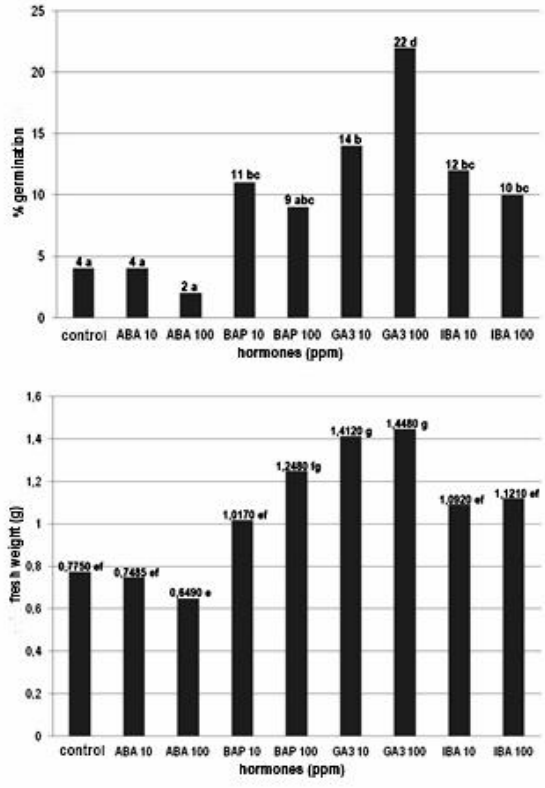

Figure 3. Effect of exogenous plant growth hormones on seed germination of flamboyant. Different letters above bars indicate significant differences ($P \leq 0.05$) on DMRT.

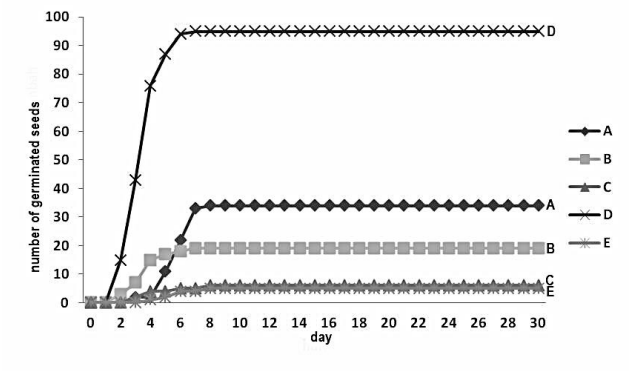

Figure 2. The rate of flamboyant seed germination by physical treatment of dormancy breakdown. A: scarification; B: dry heat; C: Low temperature; D: soaking in hot water temperature of 98°C; E: control

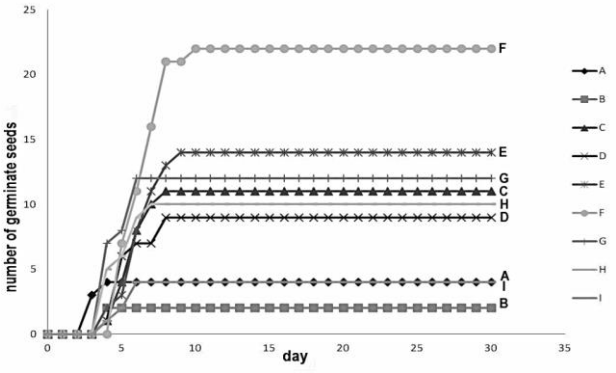

Figure 4. The rate of seed germination of flamboyant in exogenous plant growth hormones treatments. A: ABA10 ppm; B: ABA100 ppm; C: BAP10 ppm; D: BAP100 ppm; E: $GA_3$10 ppm; F: $GA_3$100 ppm; G: IBA 10 ppm; H: IBA100 ppm; I: control (non-treated)

Gibberellin influences the most extensive and effective in inducing germination than other hormones such as cytokinin and auxin (Kucera et al. 2005). Some hormones are successfully used for dormancy breaking of Leguminoceae seeds including gibberellin (GA), benzyl aminopurine (BAP), kinetin, indole butyric acid (IBA) and indole acetic acid (IAA) (Hassani et al. 2009; Otroshy et al. 2009). Gibberellic acid, ethylene, and BRs increase germination success through his influence in softening the endosperm and embryo growth (Brady and McCourt 2003; Kucera et al. 2005). Softening the endosperm making possible for radicle to grow and penetrate the seed coat through the micropyle. Auxin (IBA) and cytokinin (BAP) have a role in increasing the potential growth of the embryo and generate a greater percentage of germination than the control. These findings suggest that certain hormone in the specific amount necessary to induce germination of flamboyant seed. Treatments by various hormones that stimulate germination, only 100 ppm BAP treatment were not significantly different from controls. In affecting growth and development, auxin and cytokinin interact with each other, and the resulting response depends on the dose or concentration of each hormone (Bessler 1997)

Finkelstein et al. (2002), reported that cyp707a2 mutant *Arabidopsis* seeds contain higher ABA compared with wild type and mutant increased levels of dormancy. At flamboyant seed, application of exogenous hormones ABA maintains the level of seed dormancy flamboyant. Provision of 100 ppm ABA tended to raise the degree of dormancy. In controlling the state of dormancy and seed germination, all hormones in seeds interact and crosstalk each other. Research on *Albizia julibrissin* seeds Durazz., It is known that the hormone GA_3 and benzyladenine application for dormancy breaking will increase the activity of enzymes in glyoxylate cycle, enzyme activity isocitrate lyase, and malate synthase. Allegedly glyoxylate cycle activities affect sugar production of lipids (gluconeogenesis) so as to spur growth and germination (Sedghi et al. 2011).

The flamboyant hard seed coat is thought to be a major factor that inhibits water imbibition included in the solution of hormones. If physical barriers such as a hard seed coat have not been eliminated, so the effect of hormones to stimulate germination could not be achieved.

Anatomical structure of the flamboyant seed

Figures 5-7 demonstrated light microscope and SEM images of seed coat structure of the flamboyant seed. The outermost layer, superimpose lining the surface of the epidermis, is the waxy cuticle with variable thickness, which represents the first hydrophobic barrier for water imbibed into the seed. The epidermis is a layer of thick-walled and compact-arranged of elongated palisade cells, called macrosclereids, with the long axis oriented perpendicularly to the surface. In some species, in each single palisade cell, a light refractive, apparently denser region can be distinguished using optical microscopes, called "*linea lucida*" or "light line". A light line is a region of palisade wall material that appears brighter than the surrounding wall areas when observed with a compound microscope; this line is located near the outer surface of the palisade layer. Proximal from the epidermis, a single layer of sub-epidermal cells form the hypodermis, which is also, called hourglass cells, pillar cells, osteosclereids or lagenosclereids, depending on their pattern of cell wall thickness and shape. They are usually larger than adjacent cell layers and are separated by wide intercellular spaces, excluding in region proximal of the hilum cleft where they are absent. The innermost layer of the seed coat, the interior parenchyma is formed by six to eight layers of thin-walled, protoplast-free, tangentially elongated parenchyma cells, uniformly distributed throughout the whole testa, except in the area of the hilum, where a smaller number of layers can be distinguished.

The impermeability feature of legume seed is determined by the macrosclereid and osteosclereid tissue. The control of the influx of water for the first time into a seed can consist of several structures. Some of this structure form a regulatory system the entry of water into the seed and is called a water gap system (Ma et al. 2004; Shao et al. 2007; Turner et al. 2009; Mavi 2010). Observation of the structure of the seed surface controlling water entry into the seeds is done by scanning electron microscope (SEM) and anatomical observations flamboyant seed (Figures 3-4). Here, we show the structure of the water gap in the form of hilum, micropyle, and a lens on the flamboyant seed.

The permeability property of a hard seed coat should be facilitated by the special structure. A typical legume seed coat contains many specialized areas, i.e., hilum, micropyle and lens, and the rest of the seed coat commonly known as the extra-hilar region. The hilum is a scar formed in point of the funiculus separates from the seed at maturity. The hilum is a particularly important structure controlling embryo-external environment relationships. Adjacent to the hilum, a micropyle is a small opening remnant of the embryo sac and it is sometimes naturally closed by a waxy "lid" which may function to regulate susceptible response to pathogen infection. The micropyle, through which the radicle protrudes during seed germination, is the pore that formed where the margins of the integuments meet in the early development of the ovule. In the opposite of the hilum, there is the lens (also called strophiole). Lens is a weak area of the seed coat that under certain environmental conditions, it ruptures.

Different letters above bars indicate significant differences ($P \leq 0.05$) on DMRT.

The results on blocking experiment (Table 3) showed that both hilum and lens (or strophiole) play an important role in imbibition process leading to seed germination of flamboyant. Hilum and lens may create a water gap system that controls water entry into the seed cells. Morrison et al. (1998) suggested that on the seed coat there are certain parts of the weakest obstacle for general mechanical layers that compose macrosclereid not as thick as in the other layers. In Leguminoceae these areas are referred to strophiol or lens. Lens will physically damage when the

critical temperature is reached. The critical temperature is different for each type of seed. For *Acacia elongata, A. longissima, Glycine clandestina* critical temperature 80°C, while for *Acacia suaveolens, Daviesa alata, Platylobium formosum* critical temperature 60ºC (Faboideae, Mimosoideae) (Morrison et al. 1998).

Changes in seed coat structure due to the physical and hormonal treatments

The results of physical treatments showed that the application of soaking seed on hot water at different temperature cause seed dormancy breakdown of flamboyant seed. Every level of temperature causes different germination rate of flamboyant seed. The temperature of 98ºC gives the highest germination percentage of flamboyant seed. In general, the legume seed has a special interface for the point of water entry. Such interface may consist of single or multiple structures in the seed coat. Collectively, the structures formed a regulatory system the entry of water into the seed and are called a water gap system (Turner et al. 2009). Observation of the structure of the seed surface to determine the control system of flamboyant water entry into the seeds is done by scanning electron microscope (SEM) (Figures 8-10).

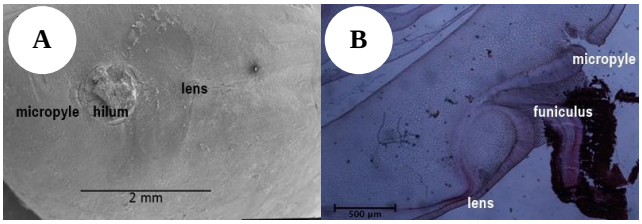

Figure 5. The morphology and anatomy of the water gap of flamboyant seeds using SEM and light microscope imaging. A.: micropyle, hilum, and lens in seeds flamboyant under SEM. B.: the anatomy of micropyle, hilum, and lens of flamboyant seed under a light microscope. The three structures (micropyle, hilum, and lens) created the water gap system, a special structure in the seed coat that controls the entry of water into the seed.

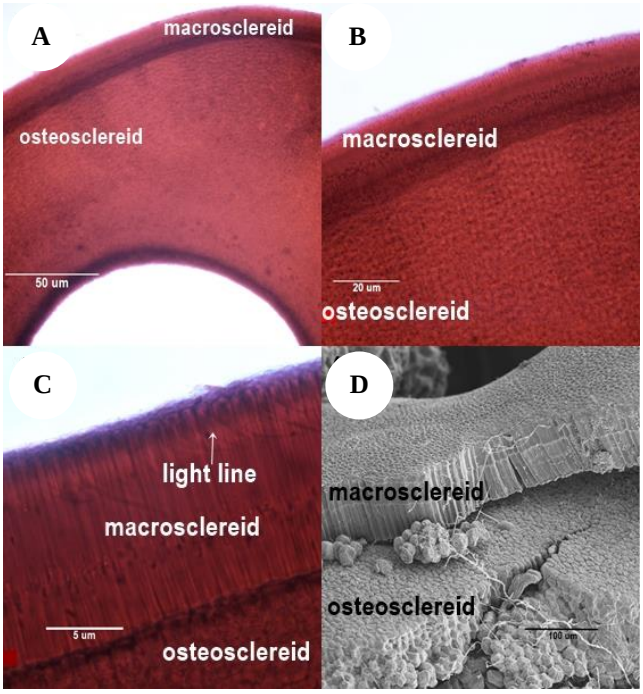

Figure 6. The longitudinal section of a flamboyant seed showed the structure of seed coat. A-C. Anatomy of seed coat consists of macrosclereid and osteosclereid tissues. D. The ultrastructure of seed coat of flamboyant under scanning electron microscope showed the macrosclereid cell shape (palisade cell) and osteosclereid cell shape (hourglass cell).

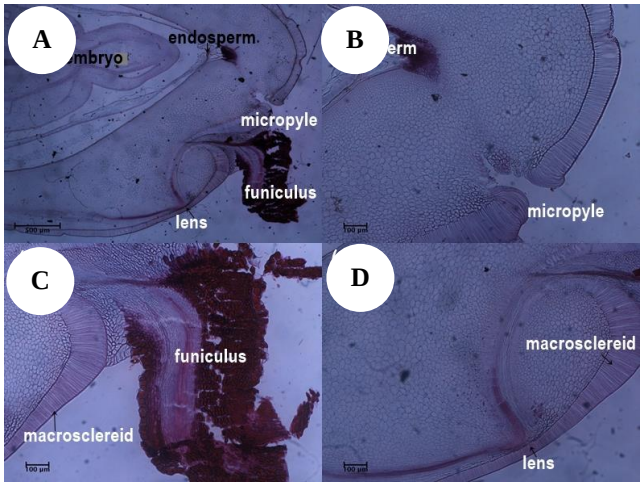

Figure 7. The longitudinal section of flamboyant seed was observed with a light microscope. A. The three main parts of the seed can be observed, which are the seed coat, endosperm, and embryo. B. The structure of micropyle, a small slit where radicle protruded. C. The structure of funiculus and macrosclereid of the seed coat. D. The structure of lens, the weakest part of seed coat which has macrosclereid thinner than the other part of the seed coat.

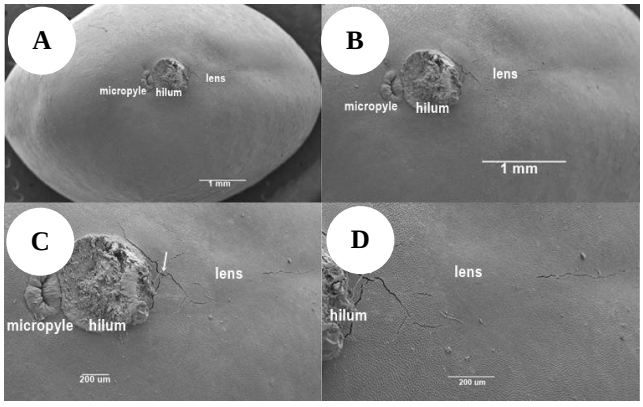

Figure 8. Ultrastructure water gap (micropyle-hilum-lens) of non-treated flamboyant seed. There are cracks around the hilum (arrows) were not to penetrate the macrosclereid layers. The is no alternation of hilum, micropyle, and lens condition (A-D).

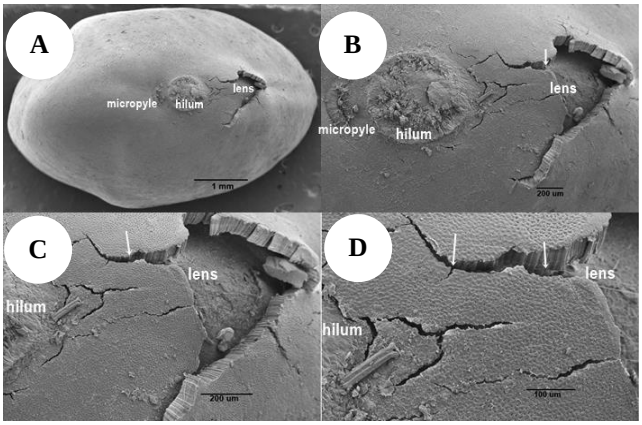

Figure 9. Ultrastructure water gap (micropyle-hilum-lens) on a flamboyant seed treated by hot water treatment. There is rupture of micropyle and cracks around the lens due to alternation of seed permeability (A-D).

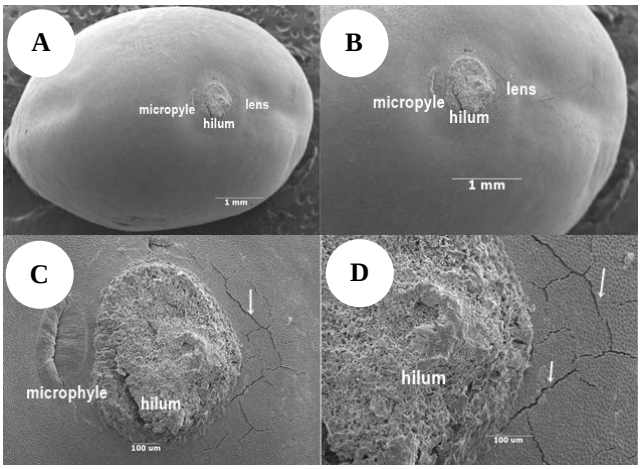

Figure 10. Ultrastructure water gap (micropyle-hilum-lens) on a flamboyant seed treated by GA_3 100 ppm. There are cracks (arrow) around the hilum. There is no alteration of micropyle, hilum and lens do not condition (A-D).

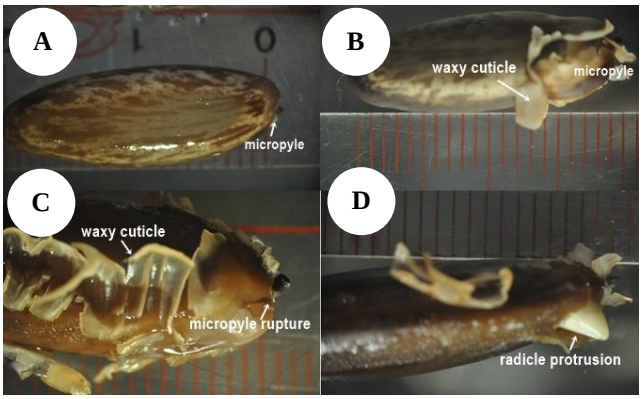

Figure 11. Changes in the morphology of the flamboyant seeds during germination process. A. Dry seed; B. Early imbibition process; C. Micropyle rupture; D. Radicle protrusion.

Table 3. Blocking experiment of flamboyant seed germination

Treatments	% germination
Non-treated (control)	9[b]
Close of entire surface of the seed	0[a]
Close of hilum, lens and micropyle (hilar region)	0[a]
Close of extra-hilar region	9[b]
Close of hilum and micropyle	6[b]
Close of hilum and lens	0[a]
Close of hilum	6[b]
Close of lens	6[b]
Close of micropyle	7[b]

Figures 8-10 showed that physical and hormonal treatments in order to break seed dormancy in flamboyant had different effect on seed coat structure. The hot water treatment caused cracking on water gap area (hilum and lens) that improve germination percentage. The hot water caused ultrastructure damage which is formed by deep cracking through the macrosclereid layer and lens rupture. The weakest part of the testa is responsible for the process of imbibition. Usually, the weakest part of the testa is the lens or strophiole region. Lens structure, hilum, and micropyle together form a structure called a water gap. According to Turner et al. (2009), water gap at the seed serves as a signal detector environment, and water this gap will open when the environment provides a high probability of the seeds to germinate and grow better. The favorable environmental conditions include high humidity indicating a high availability of water in the environment (Baskin 2003).

There are several types of water gap which has been proposed by Gama-Arachchige et al. (2013) which is type 1 is hilar slit (example in *Rhus glabra*-Anacardiaceae), type 2 is lens gap (lid water gap example at *Albizia lophanta*, *Leucaena leucocephala*-Fabaceae) and type 3 is chalazal gap (plug water gap example on *Geranium carolinianum*-Geraniaceae). From the data, it can be concluded that the water gap of flamboyant seeds is lens gap type (lid water gap).

Seeds with physical dormancy (PY) cannot imbibe water even under favorable environmental conditions due to a water-impermeable layer(s) of cells. Specialized structure is involved in occlusion of the water gaps (Baskin et al. 2000). The breaking of PY involves disruption or dislodgment of "water-gap" structures, which act as environmental signal detectors for germination. Once the closed water gap opens, a seed can imbibe water rapidly and germinate under a wide range of conditions (Baskin et al. 2000). Cell elongation is necessary and is generally accepted to be sufficient for the completion of radicle protrusion (Kucera et al. 2005).

Temperature would affect the structure of macromolecules including proteins inside the cells. Soaking the seeds in hot water flamboyant promoted leaching of secondary metabolites deposited on the seed coat. Generally, natural compounds of legume seed coat are phenolic compounds such as tannins that can act as

inhibitors of germination (Moise et al. 2005). Soaking the seeds in hot water causes the leaching of inhibitor and this would reduce the inhibition of germination.

The hormonal treatment did not change the seed coat permeability because there is no ultrastructure damage to the seed coat. Unlike the hot water treatment, there is no crack formed near the lens, so the germination percentage of the flamboyant seed treated using hormonal application still low. The ultrastructure of seed coat of GA_3 treatments and untreated seed was similar.

Analysis of morphological changes during imbibition phase until germination

Figure 11 is a series image of the morphological changes of the seed coat in the onset of germination of flamboyant seed. In the beginning, through the hilum and/or lens water entered into the seed by turgor-driven process. The increasing of turgor caused the release of waxy cuticle layer. Water entered into palisade and hypodermis through apoplastic movement and then entered into parenchymatous tissue and embryo via symplast transport. The loss of the cuticle causes the seed becomes easier imbibed by water. At this stage, seed initiated to synthesis enzymes and hormones, as well as other important metabolic processes to be used in germination. GA will promote the endosperm breakdown and the growth of the embryo. The next stage is the elongation of radicle cells that cause a rupture in the micropyle and the seed dormancy will be terminated by radicle protrusion from the seed coat.

Other seeds show various mechanisms in the onset of germination. In the Solanaceae and Brassicaceae seeds, they need two successive events i.e. dislodging of the seed coat and followed by breaking of the endosperm (Muller et al. 2006; Liu et al. 2013). In raspberry (*Rubus coreanus*) seeds, at the time of germination, seed coats will be torn apart in the middle, and all parts of the cotyledons and radicle will sprout out the seed coat (Rahman et al. 2011). Seed *Lactuca sativa*, *Nicotiana tabacum*, and *Lycopersicon esculentum* also showed a similar thing. In such seeds, the endosperm is the layer that surrounds the embryo as a whole so that the outbreak of the endosperm to be one of the critical success factors of seed germination (Muller et al. 2006).

At flamboyant seed, endosperm not completely cover the embryo, it just laid on the top and bottom of the embryo and does not cover the area of the tip of the radicle. The tip of the radicle which are not fully covered with endosperm facilitate the process of germination. This is similar to carob bean (*Ceratonia siliqua* L.), fenugreek (*Trigonella foenum-graecum* L.) and senna (*Cassia tora* L.) (Fabaceae) in which seed germination is controlled by the cell wall structure around the micropylar endosperm and not merely because softening the endosperm. The cell walls in the micropylar endosperm have thin cell walls so they more easily penetrated by the radicle. According to Gong et al. (2005) in the event of dormancy break for the hard seed coat, radicle emergence of the seed coat can occur by two mechanisms. First, endosperm and other tissues that cover the embryo undergo weakening as a result of the activity of the enzyme that breaks endosperm. Therefore, the weakened endosperm is more accessible for radicle to penetrate the seed coat. Second, alternatively, radicle able to penetrate of the seed coat is not due to the activity of enzyme breaker endosperm but because the micropylar region around radicle has no enough mechanical resistance. Such modification in the form of thinner cell walls in this area led to the micropylar endosperm easily penetrated by radicle. Based on the above, then for seed germination occurs flamboyant mechanism is a combination of several mechanisms that have been proposed by Gong et al. (2005) and Rahman et al. (2011). At flamboyant seed, radicle tip is not covered by the endosperm so that it can be said the flamboyant seeds have endosperm modifications in the micropyle region; besides the softening endosperm will also encourage germination.

In conclusion, the results indicated that physical treatment that seeds dipped in hot water (98ºC) for 5 minutes was the most effective treatment to dormancy breakdown of flamboyant seeds (95%). The application of GA_3 100 ppm results in the highest of germination percentage (22%) among other hormones. The result of blocking experiment showed that hilum and lens play an important role during imbibition processes; and the type of flamboyant seed water gap was the lens gap type.

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