



CHARACTERISTICS OF MORPHOLOGY, ANATOMY AND DORMANCY BREAKS OF *CASTANOPSIS BURUANA* MIQ. SEEDS AS AN ENDEMIC PLANT OF SULAWESI, INDONESIA

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Abstract

Castanopsis buruana Miq. is one of the endemic plants that grows and spreads in the lowland forest of Sulawesi. This research aimed to determine the characteristics of morphology, anatomy and dormancy breaks of *C. buruana* Miq. seeds. The anatomical structure of the seed coat was studied according to Sass method. The lignin content was analyzed by using Klason method and the cellulose, as well as the hemicellulose content were analyzed by using Cross and Bevan methods. The dormancy break of the seeds was analyzed by using complete randomized design in the factorial pattern. The results of this research showed that *C. buruana* Miq. seeds have a very high variation in both physical forms and sizes. The length, diameter, weight and thick of the seeds were in ranges of 17.46 ± 0.40 - 19.34 ± 0.28 mm, 12.84 ± 0.31 - 15.03 ± 0.13 mm, 0.87 ± 0.015 - 1.68 ± 0.015 g and 169.0 ± 24.92 - 231.9 ± 34.45 μ m, respectively. The large size of seeds with IBA 50 ppm treatment was the best treatment due to it was able to increase the germination percentage, potential maximum growth and seedling germination rate of *C. buruana* Miq. in 83.33%, 88.89%, and 1.33% KN/etmal.

Key words: anatomy, *Castanopsis buruana*, germination, growth, morphology.

Introduction

Castanopsis is one of the important plants in Indonesian rainforests and belonging to Fagaceae family. This genus consists of 120 species and grows to spread from northeast of India to west of China, Korea, Japan, and all Malaysia (Prosea, 1995). Its growing and distribution are found starting from west to east Indonesia such as Borneo, Sumatera, Java, Sulawesi, Maluku (Soepadmo, 1968, Heyne, 1987). *Castanopsis buruana* Miq. is an endemic plant growing in lowland forest and distributing in Sulawesi and Maluku (Whitmore *et al.*, 1989). In the Southeast Sulawesi, this species is discovered abundantly in Buton Island, Muna, Konawe, South Konawe, and Kolaka (BKSDA, 2002). Ecologically, this

species inhabits in the primary and secondary forests from low hills until high mountainous areas with 1.000 metres above sea level (Prosea, 1995) and lives in evergreen forests with Latosol and Oxisol soils, as well as rainfall of 2.000-3.000 mm/year and dry season less than 6 months (Heriyanto *et al.*, 2007). Becoming an endemic species, *C. buruana* Miq. Plays an important role in mountainous ecosystem due to it possess a wide canopy that functions as life buffering, especially hydrological system and climate for surrounding areas (Heriyanto *et al.*, 2007).

In addition, *C. buruana* Miq. possesses high economic value. It becomes timber and non-timber producer. In terms of timber producer, it is classified into a strong wood grade, namely II-III class and a durable

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III class (Hildebrant, 1950), that is suitable for medium and high construction materials. Its fruit generates edible beans, high nutritious content and free gluten compound (Purwaningsih and Polosakan, 2016). However, currently, the overexploitation on this plant species due to illegal logging activities, mining activities and crops and agricultural land extension, threatens the existence of this species in the natural forests. While in the natural forest, this species regeneration is very difficult because of its *C. buruana* Miq. is easy to be trodden and possessing a tough and thick seed coat which takes a longer time to be germinated. Therefore, one of the efforts to accelerate the germinating process of *C. buruana* Miq. seeds is by a dormancy breaking through physical, chemical and hot water treatments. For instance, physical treatment is taken by scarification or rasping its spermatofit (Hartman *et al.*, 2002) and physical treatment is taken by immersing its seed into a growing regulator solution like an *indolebutyric acid* (IBA).

The research results indicate that soaking the seed of cape yellow wood in the 95% concentration of sulphide acid as long as 30 minutes and following with water in room temperature during a full day is able to break its dormancy which implicate to have potential growth, germination and growth rate are 40.7%, 39.7% and 0.69% KN/etmal, respectively (Puspitarini, 2003). A first treatment with cutting the seed and followed by soaking it into 0.525% concentration of sodium hypochlorite and water (80°C) left to be cool as long as 24 hours generate the highest germination of merbau seeds namely 78.68% (Nugroho, 2010). Increasing of viability and vigor of mindi seed can be conducted by soaking the seed into the solution of H₂SO₄ (95%) as long as 45 minutes with a delignification in the endocarp edge is able to increase the seed's germination as high as 57% (Yulianti, 2011).

Research on breaking seed dormancy through a chemical compound or soaking into the hot water to accelerate dormancy process of seeds has been frequently used, however, there is still lacking of the information regarding the method of breaking seed dormancy by using IBA, especially for *C. buruana* Miq. as an endemic plant of Sulawesi, Indonesia. IBA is able to push ahead sitokinin works in the process of cell division and elongation and to induce enzymes functioning in the cell division particularly apical meristem (Kumari *et al.*, 2010). Auksin also affects roots morphology through obstructing their elongation and escalates the lateral root production (Overvoorde *et al.*, 2010, Da-Costa *et al.*, 2013, Kazan, 2013). The research result indicates that IBA treatment with 1.5 mg/l can scale up the percentage of jatropa seed germination significantly

and shorten the germination time more than 350% than the control treatment (Kumari *et al.*, 2010). Hence, aims to investigate the characteristics of morphology, anatomy and chemical content of *C. buruana* Miq. seeds and investigate the methods for dormancy break of *C. buruana* Miq. seed.

Materials and Methods

Study sites

This research was carried out in February to May 2018. The analyzing of anatomical seed coat, morphological seed coat and seed chemical content of *C. buruana* were taken in three different laboratories namely the Physiology Laboratory of Indonesia Institute of Sciences (LIPI) Cibinong, the Entomology Laboratory of Silviculture Department at Forestry Faculty-Bogor Agricultural University (IPB) and the Chemical Wood Laboratory of Forest Product Department at Forestry Faculty-IPB, respectively. While the seed germination observation was conducted in the greenhouse of Silviculture Department at Forestry Faculty-IPB.

Procedures

The Seed Collection: The seeds of *C. buruana* Miq. were collected directly under natural tree stands at Nipa-Nipa Forest Park of Kendari City and South Konawe Regency. They were collected during a fruiting season from December 2017 until February 2018. The gathered seeds were weeded out and then cleaned up by washing the seeds. Further, they were dried up in the sunlight directly. The dried seeds were kept in plastic bags at room temperature until use.

The Seed Morphological and Anatomical Analysis

The used methods in the morphological analysis of seeds were observation and direct measurement for each seeds consisting of weight, length and diameter of seeds. While the testa thickness was checked by making longitudinal slices on the seed, then it was observed under a stereo microscope with a 10 times of magnification. Measurement of this testa thickness used a μm unit. The seed used in the morphological analysis was carried on sampling from some tree stands in one population of *C. buruana* Miq. to be a lot seeds. Furthermore, seeds were taken out randomly in each lot seed to become a bulk sample. From three bulk samples, five seeds were taken from each of them to be analyzed.

The anatomical structure of seed's skin was analyzed using the method of Sass (1961) which had been modified by conducting longitudinal slices of the seed's skin applying a freezing microtome of Yamato RV-240. The seed's skin was cut in ± 0.5 cm long. This cut was putted

into a gelation block, then it was frozen in a freezer. This gelatin block was then cut utilizing a frozen microtome with a range of 8-10 μm thickness and the cut slices further were observed under a microscope.

The Seed Chemical Content Analysis

A chemical content analysis of the seed was taken on entire parts of the seeds (nut and seed's skin). Analyzing seed chemical content was consisted of measuring lignin, cellulose, hemicellulose and fat levels using a 100g of seeds. The lignin, cellulose and hemicellulose was analyzed according to Klosan, Cross, and Bevan Methods, respectively, while fat level was analyzed by using a distillation method.

The Breaking of Seed Dormancy

The breaking of seed dormancy techniques were carried out by soaking seeds into hot water (80°C) and chemical solution of IBA. This method have been many applied and referred to ISTA (2006). Before germinating, the surfaces of seeds were sterilized utilizing NaOCl 0,5%.

Research Design

The breaking of seed dormancy used a completely random design with two factorial patterns. The first factor (treatment), the seeds were soaked into the IBA solutions composing of four level concentration namely 0 ppm, 50 ppm, 100 ppm and 150 ppm (method modified from Kumari *et al.*, 2010). The second factor (treatment), the seeds were classified into two size levels namely big size with a weight of 1.65-1.70 g, and small size with a weight of 0.84-0.89 g (method modified from Nugroho, 2010). Every treatment was duplicated three times generating 24 treatment units.

The Observed Variable

1. Germination percentage. Observation of germination percentage was conducted in five times namely 14, 21, 28, 35 and 42 days after planting.

2. The potential maximum growth. The potential maximum growth is a percentage of germinating percentage until the end of observation towards total germinated percentage.

3. Germination rate. The germination rate was counted based on the daily growing percentage accumulation in a percentage benchmark of a daily normal sprout growth during 42 days.

4. Dormancy intensity. Dormancy intensity is a percentage of un-growing seeds until the end of observation.

Data analysis

Data of morphological and anatomical structure of

seeds and the seed germination were processed by using a t-test and variance analysis, respectively. When the test result indicated a significant effect, a post hoc test of the Duncan Multiple Range Test (DMRT) with a 95% confidence level was then taken.

Results and Discussion

Morphological and Anatomical Seeds of *C. buruana* Miq.

The fruit of *C. buruana* Miq. is almost similar with rambutan (hairy fruit), thorned fruit, sharp and woody. It has a rounded form with spines appearing from four sides of cupules and its diameter is in a range of 2-3 cm (Fig. 1a). The ripe fruit has cupules dividing dehisces irregularly. Its cupules are green when in young and becoming yellow until brownish yellow when in ripe (Fig. 1b). Each cupule contains one seed with oval, pointed and even concave forms (Fig. 1c), brown color and different sizes (Fig. 1d).

The variation of *C. buruana* Miq. seed size based on seed weight is able to occur in either a tree stand population or among tree stand population can be showed from t-test results. *C. buruana* Miq. seed size was various based on length (17.46 \pm 0.40-19.34 \pm 0.28 mm), diameter (12.84 \pm 0.31-15.03 \pm 0.13 mm), weight (0.87 \pm 0.015-1.68 \pm 0.015 g) and testa thickness (169.0 \pm 24.92-231.9 \pm 34.45 μm).

The Chemical Composition of Seeds

The chemical composition of *C. buruana* Miq. seed consisted of fat, lignin, cellulose and hemicellulose. The result showed that *C. buruana* Miq had a fat level of



Fig. 1: The morphological features of *C. buruana* Miq. Fruits and seeds. (a) young cupule, (b) opening cupule, (c and d) size variations of *C. buruana* Miq.

0.80% for big seeds and 0.78% for small seeds. The contents of lignin, cellulose and hemicellulose were 16.61%, 59.78% and 44.53% (big seeds), and 15.72%, 60.25% and 44.98% (small seeds), respectively.

The Breaking of Seed Dormancy

The IBA treatment indicated a significant effect on all parameters of seed germination, except the germination percentage which had not any significant influence. Moreover, the seed size treatment was not significantly affected all parameters of seed germination. An interaction between IBA treatment and seed size had an important effect on all parameters of seed germination (Table 1).

The results of the least significant difference ($\alpha=5\%$) based on the DMRT of the interaction effects of IBA treatment and seed size toward the seed germination percentage, potential maximum growth, germination rate, and dormancy intensity of *C. buruana* Miq. is presented in fig. 2, 3, 4 and 5. The large seed in the treatment IBA with 150 ppm yields the highest seed germination percentage (84.44%) and had un-significant effect on the treatment IBA with 500 ppm and 100 ppm, however, it showed a significant result on the control (IBA 0 ppm). Further, the small seed in treatment IBA with 100 ppm generated the highest seed germination percentage (75.56%) and had an un-significant effect on all other treatments (Fig. 2a).

The large seeds in the treatment IBA with 150 ppm obtained the highest potential maximum growth (90%) and had un-significant differences with two other treatments (IBA with 50 ppm and IBA with 100 ppm), however, they had significant differences with the control (IBA with 0 ppm). The small seeds in the treatment IBA with 100 ppm generate the highest potential maximum growth and possess un-significant difference with other treatments (Fig. 2b).

The large seeds in the treatment IBA with 50 ppm produced the highest seed germination rate of 1.33% KN/etmal and had un-significant differences with other

Table 1: The variance results of IBA and seed size treatment effects in all parameters of *C. buruana* Miq. seed germination.

Parameter	Treatment			CV (%)
	IBA (A)	Seed Size (B)	Interaction A*B	
Germination percentage	ns	ns	*	10.55
Potential maximum growth	*	ns	*	12.80
Germination rate	*	ns	*	7.16
Dormancy intensity	*	ns	*	27.40

Note: *a significant effect in the confidence level of 5%, ns = not a significant influence, CV = coefficient variance.

treatments, except both control treatments (IBA with 0 ppm) in the two types of seeds and treatment IBA with 50 ppm at small seed size group (Fig. 2c). The large seeds without conducting the treatment IBA gain the highest intensity of seed dormancy (42.22%) and they were different significantly with other treatments on both two seed groups, large and small (Fig. 2d).

The relationships between morphological, anatomical and germination characters

The seed of *C. buruana* Miq. encounters problems in germination causing by the seed morphological and anatomical characters. The correlation analysis results exhibit that the weight and thickness of testa possess a close relationship on germinating seed (Fig. 6).

The seed *C. buruana* Miq. morphologically has an oval until to be concave features and brown color with varied sizes. The seed variation is supposed due to genetic factors and quality of habitats. The genetic diversity of a certain plant is able to be caused by, obiter, the diversity among provenances, habitats, among trees and in side of the tree itself (Yulianti, 2011). The quality of habitats is highly related with environment and climate. According to Owens (1995), flowering and fertilization are influenced by environmental factors such as light intensity, temperature, water availability and nutrient content. Furthermore, number of trees or tree’s stand density also will affect to the production of fruits. This density can alter the resulted seeds production. The more density, the more seed production that will be resulted. This research is in line with Nugroho, (2010) that a diversity of merbau seed size is not only due to a narrow seed forming room but also is caused by genetic factors and even though a habitat factor. The seed size difference in a certain population or among population signifies that an individual tree factor (genetic) and habitat factor plays an important role in shaping and striving a seed. This differentiation is a commonly event, such as a different seed weight in *Pinus caribaea* (Evans, 1986) *Eucalyptus regnans*, *E. delegatensis* (Close and Wilson, 2002).

Germination is a complex physiological process, starting with an imbibition process, followed by embryo development, and ending with a radicula appearance (Hartman *et al.*, 2002). Due to a happened imbibition process, the seed coat will be soft and cracks (Kusawanto, 1996). The thickness of seed coat will result in the seed germination process in this case, becoming a barrier for water and oxygen intrusion and the inhibitor is stack inside the seed (Bewley and Black, 1986). An enough water and oxygen availability are very helpful for an embryo to puss out a radicula and new bud. An

obstacle due to the thickness and hardness of seed coat, makes the seed undergoes physical dormancy. A research result of Yulianti, (2011) exhibits that there is a trend of endocarp thickness does not become an obstacle on either the germination or growth rate of mindi seed. This is presumed because of an effectively pre-treatment in breaking a dormancy due to the seed coat thickness. The density of seed composing cell is assumed to be one of obstacles in germinating process of *C. buruana* Miq. seed. According to Pramono and Danu, (1998), sowing mindi seed without any dormancy breaking treatment needs more time to be germinated (± 3 months). Reciprocally, the candlenut seed without any pre-treatment needs more time to be germinated after six months (Murniati, 1995).

A seed germination is not only affected by physical factors, but also influenced by chemical compound inside the seed. The chemical compound inside the seed affects the psychological and germination process of the seed. The analysed chemical content in this research consists of fat, lignin, cellulose and hemicellulose level. The fatty level in a seed becomes one of energy reserves belongs to the seed to be germinated, as well as carbohydrate and protein (Bewley and Black, 1986). A high level of fat inside of a seed is able to be a characteristic guide of the seed which is categorized into a recalcitrant or very sensitive on water level decline (Yulianti, 2011).

The *C. buruana* Miq seed has 0.34% fat. This is categorized into a low level than fat levels in mindi 2-5.6% (Yulianti, 2011), oil palm 40-60% (Bewley and Black, 1986) and candlenut 58.25% (Murniati, 1995). Lowering total fat level and increasing free fatty acid causes a decrease of seed viability and vigor. A setback symptom biochemically in a seed is resulted in by changes in enzyme activities, respiration rate, increase fatty acid, and decreasing of food reserve stock (Yulianti, 2001). A high fatty acid compound inside a seed is an indication of fat accumulation occurrence because it is not processed further to be energy that the seed lost energy to be germinated (Tresniawati *et al.*, 2014).

Further, this research also analysed lignin, cellulose, and hemicellulose content with different values namely 16.16%, 60.01% and 44.75%, respectively. Results of these three contents were conducted on the seed coat part (pericarp) because some forestry plant species possess seed coats shapping structures like a wood (woody seed) and undergoes signification which is commonly linked with a stop metabolism process and cell death (Puspitarini, 2003). The chemical composition of a wood consists of holosellulose, cellulose, lignin, dust, and water (Martina *et al.*, 2002). This chemical component causes a hardness or weakness level of lignin contents of the wood. Lignin is a structural component and influences on the wood robustness or hardness.

According to Pereira *et al.*, (2003), commonly lignin compound in a wood is in a range of 20-35%. A wood contains lignin in a range of 18-33% is categorized into a medium group. The current research indicates that the lignin, cellulose and hemicellulose contents are in ranges of 16.16%, 60.01% and 44.75%. Therefore, *C. buruana* Miq. in is distinguished into a medium lignin content. If this compound is linked with the seed anatomical structure, it has indicated the pericarp thickness and weight are 231.9 μm and berat 1.03 g. This points out that there is not any relationship between the seed coat thickness and lignin level on the seed weight. However, the seed coat thickness and lignin compound in the *C. buruana* Miq. are two limiting factors in the germination process.

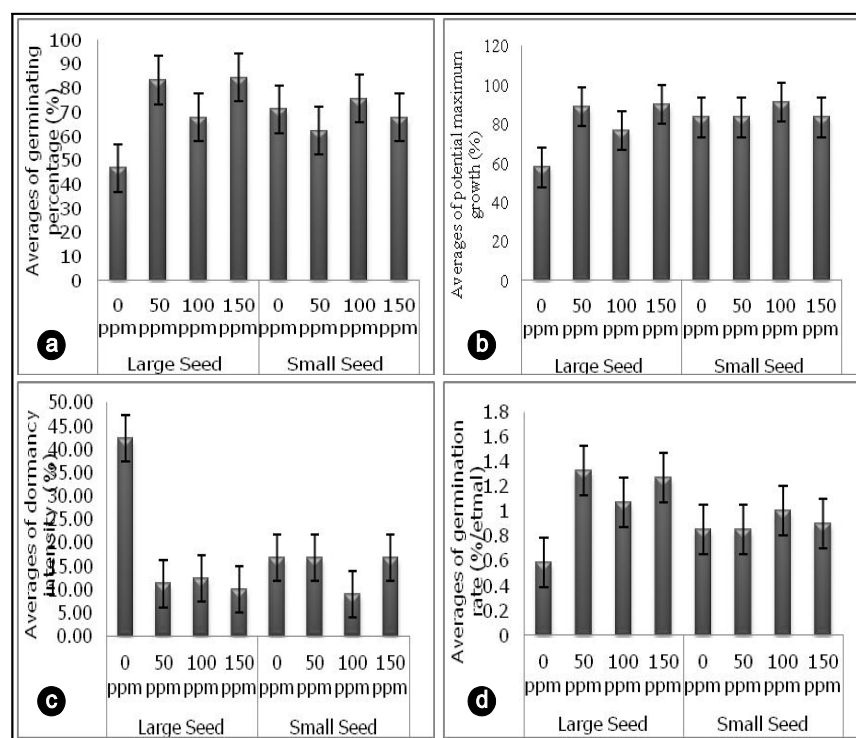


Fig. 2: The interaction effects of IBA and seed size treatments. (a) germination percentage, (b) potential maximum growth, (c) germination rate (d) dormancy intensity of *C. buruana* Miq.

The required attempts in overcoming those obstacles are how in order to the needed water and gas in the germinating

process can pass through the seed coat. One of efforts that can be carried out is through delignification that defines lignin with a pre-treatment such as soaking in the hot water and IBA aphrodisiacs grow.

The results analysis of anatomical structure and chemical seed of *C. buruana* Miq seed significates that the seed of *C. buruana* Miq has dormancy phases. The seed dormancy exhibits a condition normally good for germanation (Schmidt, 2002). The causes of seed dormancy are many and varied, most of them are due to impermeability of the seed coat on water and gas, embrio is not ready and presence of inhibitor (Murray, 1984). In

order to resolve those problems, a pre-treatment is taken prior the seed took to the seedling bed in order to add a readiness and uniformity of germination.

The test result of DMRT with 95% confidence level indicates that both IBA treatment and seed size provide the significant differences on germination percentage, potential maximum growth, seed germination rate and dormancy index. The germination percentage reflects the seed ability to grow and trive becoming normal organism. The current research also exhibits that the larger seed size with IBA 150 ppm resulted a germination percentage of seed as high as 84.44% and possesses insignificant

different with other treatments, except control with larger seed size (Fig. 2a). This is due to the soaking process is able to accelerate imbibition process marking out by an increasing water level in the seed. This result is in line with a research result of Basin and Baksin, (2001) that soaking in the hot water as long as 60 sec. can generater a seed germination percentage of *Acacia falcata*, *A. terminalis*, and *A. Suaveolens*. While IBA auksin is able to induce root shapping with breaking root apical domination which is caused by sitokini (Kumari *et al.*, 2010). The IBA hormone is able to be used for clearing up the dormance and uplifting the seed's germination (Guney *et al.*, 2016). An escalation of IBA Auksin concentration gradually from 5 to 20 ppm can boost morphological parameters of cucumber's seed root than the control treatment (Balliu and Sallaku, 2017). Larger seed size taking treatment soaking into the water as long as 24 hours without any IBA treatment generates the lowest germination percentage namely 46.67%. This lowering power is presumed due to the water in temperature 80°C is not able to break the seed dormancy well and effectively ude to the seed *C. buruana* Miq. has a strong and thick seed coat. The similar condition also occurs on the potential maximum growth and seed growth rate. The research results show that the smaller seed size with IBA 100 ppm treatment yields the highest seed potential maximum growth namely

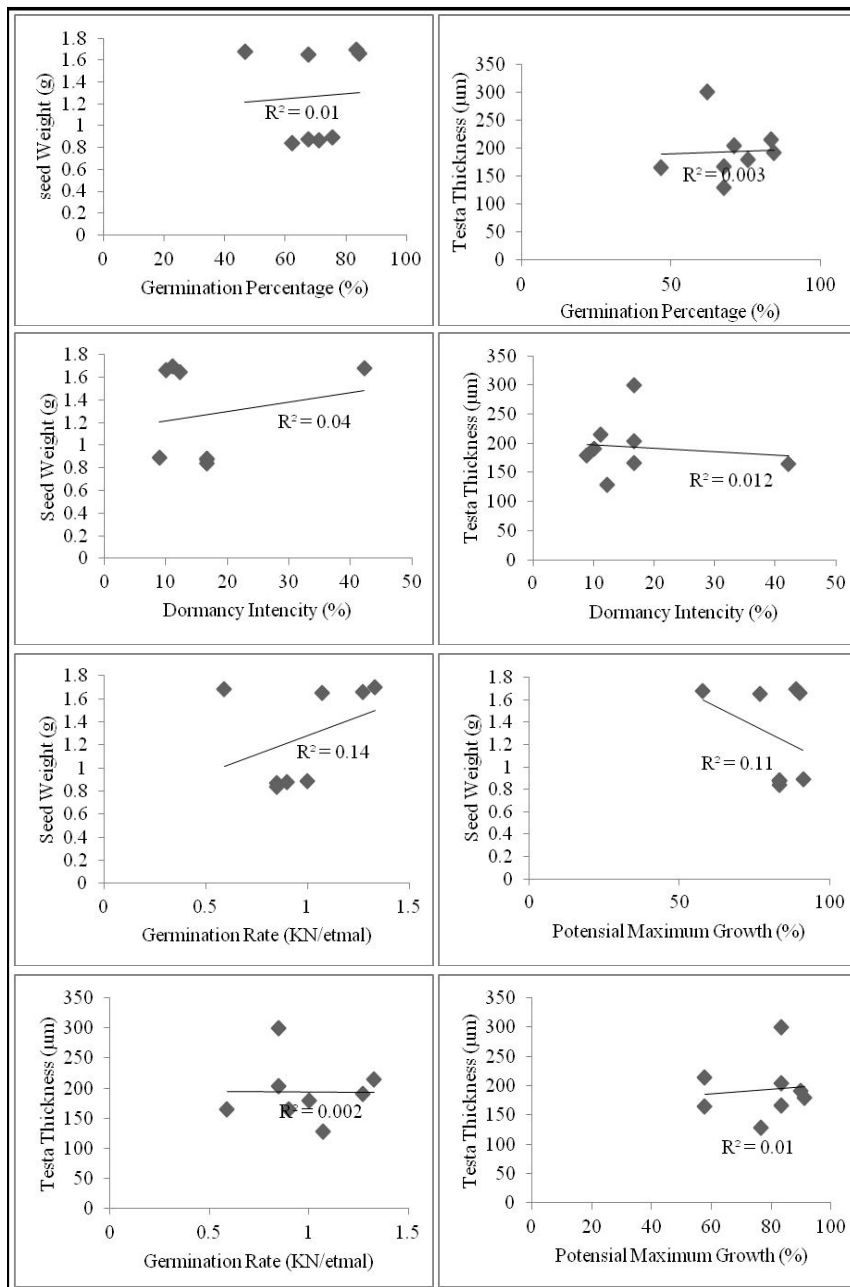


Fig. 6: The relationships among seed weight and testa thickness towards the seed germination variables of *C. buruana* Miq.

91.11% and insignificant different with other treatments, except the control with larger seed size (Fig. 2c). This happened due to the potential maximum growth is a benchmark in order to seek a viability of *C. buruana* Miq seed. All germinating seeds both normal and abnormal growing are counted as the potential maximum growth.

The germination rate is an indicator to measure a vigor of growth strength. It was observed daily during 42 hours. The research result exhibits that the larger seed size with IBA 50 ppm treatment generates the highest germination rate (1.33%/etmal) and insignificant different with three treatments (IBA with 100 ppm treatment and IBA with 150 ppm in larger seed size and 100 ppm in smaller seed size), however, it is significant different with other treatments (Fig. 2d). This is surmised that the larger seed size has a better vigor index than the smaller one. Size and weight of seed are to be the main characteristics of high and low food reserve existing inside the seed as an energy source in germination process. Further, IBA plays an important role in mobilizing carbohydrate in leaves and stems and also increases transportation to root's zone. An auksin hormonal signal also is belived highly affecting the morphology of roots, escalating the lateral root production and inducing adventive roots (Aloni *et al.*, 2008, Overvoorde *et al.*, 2010, Woodward).

A dormancy intensity figures out a seed dormancy level after conducting dormancy breaking. A high percentage of dormancy intensity reflects a high percentage of ungerminating seed after taking dormancy breaking. The results of this research signify that the larger seed size without any IBA treatment produces an intensity of seed dormancy as high as 42.22% and significantly different with three IBA treatments (50 ppm, 100 ppm, and 150 ppm) both larger and small seed sizes. This is due to the treatment such soaking into a hot water in temperature 80°C without any IBA treatment is not able to increase the germination of *C. buruana* Miq. seed because water can not capable to be absorbed well into the seed of *C. buruana* Miq. possessing a thick and strong seed coat.

The larger seed size of *C. buruana* Miq. tends to uplift the germination, potential maximum growth and growing speed of the seed (Fig. 6). This is due to it is regarded with the food supply mainly carbohydrate storing in the cotyledons are higher than the smaller seed size. Result of this research is inline with researches conducting by Close and Wilson, (2002) for the seed of *Eucalyptus regnans*, Uphadaya *et al.*, (2007) for the seeds of *Prunus* and Nugronho, (2010) for the seed of merbau. Furthermore, the testa thickness tends to reduce the germination of *C. buruana* Miq. seed (Fig. 6). A

thickness of seed's skin will be impacted to the germination process, it will be a barrier for water and oxigen intrusion and an inhibitor to be restricted in the seed (Bewley and Black, 1986, Yulianti, 2011).

Conclusion

This research infers that *C. buruana* Miq. seed showed a high variation both physical feature and size. The length, diameter, weight and testa thickness of the seed were in ranges of 17.46±0.40-19.34±0.28 mm, 12.84±0.31-15.03±0.13 mm, 0,87±0,015-1,68±0,015 g, 169.0±24.92-231.9±34.45 µm, respectively. The larger size of the seed in treatment IBA with 50 ppm was the best treatment due to it generates germination percentage, potential maximum growth, germination rate of *C. buruana* Miq. seed are 83.33%, 88.89% and 1.33% KN/etmal.

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