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Quality optimization of Luja plant (*Peristhrophe bivalvis* Merrill) by Means of Light Intensity manipulation and identification of substance with pigment potential

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Luja plant is commonly known as a natural pigmenting plant which requires specific cultivation procedures. The most fundamental cultivation requirement is light intensity. This research aims to find out the best light intensity which can improve the plant's pigment quality. The field experiment was conducted using 100%, 65%, 35%, and 15% light intensity levels and randomized factorial design method followed by DMRT test and pigment identification through Luja plant extraction. The results showed that Luja plant with lower light intensity contained higher chlorophyll level compared to those of higher light intensity levels and that the production of chlorophyll level tended to decrease as the plant grew. Higher light intensity increased the lightness (L) of Luja extract and in order to obtain higher red pigment level Luja plant had to be placed in an environment with 65% or 35% light intensity level. Conversely, all light intensity levels did not affect the level of yellow pigment. The red pigment found in Luja plant is known as Sudan III pigment which is specifically used for dyes textiles.

Keywords: Luja, light intensity, pigment, red, sudan III

INTRODUCTION

Luja plant has been used and handed down for generations by some people who live on the island of Halmahera and the island of Morotai, North Maluku. Luja plant for the Halmahera community is used as woven crafts coloring, while the people on Morotai Island use the plant as medicine (Melati, 2016). Luja plant contains pigments, and this is justified by Thuy et al*.,* (2012) who found the structure of pigments from *Peristrophe bivalvis* plants or commonly known as "Cam", red and purple colored Peristrophine plants found in Vietnam, which are used as food coloring (Dang et al., 2014). Luja plant is a potential source of natural bioactive compounds, especially pigments. The color obtained from Luja plant extract is part of secondary metabolic results. Most of these compounds come from phenols and flavonoids (Quan et al., 2016).

Pigments are considered as secondary metabolic compounds and active metabolism when placed in a suitable environment. Modification of the cultivation environment such as light intensity modification can affect metabolic compounds. Like many other plants, Luja plant needs an optimal cultivation environment to produce good quality pigments. Environmental factors such as light intensity also play a role in photosynthesis and pigmentation (Paiva et al., 2003; Aryani et al., 2013). Furthermore, there is a positive correlation between light intensity with carotene and anthocyanin contents of the leaves of *Tradescantia pallida* (Rose) Hunt.cv. *purpurea* Boom (Commelinaceae). Roselle plants are also classified as coloring plants that grow better when planted using shades and without shades during the nursery phase, but only able to adapt to 25% shaded environmental conditions, only to stop growth activities in 50% shaded environments (Setyowati, 2011). Thus, the tolerance of plants to light intensity is different for each plant. Based on those findings, this study was conducted to determine the best light intensity that could improve the quality of pigments and the color produced.

Chlorophyll is directly involved in the absorption of solar radiation energy and the amount of light received by plants, which in turn can affect the quantity of pigments produced (Zhu et al., 2017). Therefore, chlorophyll can be used as an indicator of pigments produced by plants. According to Liew et al., (2008), chlorophyll is the dominant pigment that contributes to the leaves' red fluorescence, while chlorophyll *b* is an accessory pigment, the presence of which is one third of the total dominant chlorophyll. Furthermore, it is also explained that the excess energy from light obtained by dominant chlorophyll will be transferred to accessory chlorophyll (chlorophyll b) to form other pigments such as carotene and anthocyanin. Growth and accumulation of color extracts in Luja plants require fertile soil conditions with good drainage, clay with high humidity and low temperatures, and low light intensity (Dang et al., 2014). The conditions essential for optimum growth such as best light intensity have not been discussed exclusively, so it needs further investigation. Therefore, the results of this study are expected to be the references and other technical guidelines for cultivation, especially light intensity so as to support the quality of the color produced and find the dominant pigment compounds in the extract.

MATERIALS AND METHODS

This experimental study was carried out in the village of Dadaprejo, Dau Sub-district, Batu City in an altitude of 450 m above sea level in the laboratory of Plant Physiology, Biochemistry, and Agricultural Product Technology, University of Brawijaya, Malang. The study took place from September 2017 to January 2018. The study used a randomized block design consisting of 100%, 65%, 35% and 15% light intensity levels which were repeated 3 times. Lighting simulation used 2 types of nets, namely net A (hole size 0, 5 x 0, 5 cm) and net B (60% net mesh). The treatments tested in this experiments were treatment of 100% light intensity=no net / full sunlight (I0), 65% light intensity=1 sheet A, light intensity 35%=1 sheet net A+1 sheet B, and light intensity 15%=2 sheets B (I3). Analysis of variance and DMRT test to determine differences between treatments was tested using GenStat 12 software.

Luja plant was planted using shoots cuttings which were first seeded for one month in the same cultivation environment using 75% mesh. The seeds were removed from seeding beds according to the treatment at the age of 35 days after the seeding stage. Observation of color quality variables from leaf extracts was carried out 3 times with once a month observation intervals. The first observation was conducted one month after the treatment was given. The plant samples analyzed were leaf sections between the first segment and the fourth segment of the Luja plant tips (from top to bottom).

The method used to determine the total chlorophyll was extraction method which was analyzed using a spectrophotometer. The determination of total chlorophyll was done by grounding 2 grams of fresh leaf sample, then adding 10 ml 80% acetone solution. The solution and leaf samples were stirred and then left for 5 minutes. 1 ml sample solution was mixed back with 10 ml of acetone, then observed the absorbance value in the spectrophotometer. The absorbance value was calculated based on the equation of chlorophyll a and chlorophyll b values:

Chlorophyll a = 12.21. A_{663} – 2.81A_{646}
Chlorophyll b = 20.13. A_{663} – 5.03A_{646}.
Chlorophyll total (mg g^{-1}) = Chlorophyll a + Chlorophyll b

Color quality was obtained by extracting 5 grams of crushed Luja plant leaves with 10 ml methanol solution and leaving it for 30 minutes. As much as 5 ml of the extraction results was placed in a color reader and brought to the sensor. The reading was repeated three times and then the results seen on the monitor were then recorded. The color quality analysis was conducted using Color Reader based on the Hunter's Lab Colorimetric System. System symbols or notations used three values, namely Lightness (L), Redness (a), and Yellowness (b).

Leaf pigment identification was tested using the modified Electrospray Ionization Mass Spectrometry (ESI-LC) method while referring to Thuy et al., (2012) and Quan et al., (2016). The search for compounds was based on the molecular weight of these compounds and searches through the PubChem site and other supporting literature.

RESULTS AND DISCUSSION

Total Chlorophyll

The results of variance test showed that light intensity had a significant effect on the total chlorophyll content. Based on the findings, the total chlorophyll contained in the leaves varied according to the intensity of light received. Luja plant which was cultivated at 35% light intensity level had the highest total chlorophyll content in the first month at 59.21 mg g-1. This result was significantly different from Luja plant which was cultivated at 65% and 100% light intensity levels, but not different from the plants which were grown at 15% light intensity. Luja plant's growth phase in the second month showed that the highest chlorophyll content was shown by plant cultivated at 15% light intensity which was significantly different from other light intensity levels, except for 35% light intensity. Based on statistical analysis, chlorophyll content in the third month showed that Luja plant cultivated at 15% light intensity level was significantly different from plants in all treatments. Plants with 35% and 65% light intensity levels showed the same results, but both of these treatments showed different results with plants grown at 100% light intensity level. Generally, Luja plant with light intensity levels between 15-35% had higher total chlorophyll contents compared to those receiving more light. The finding also showed that chlorophyll content possessed decreased with age at all light intensity levels (Figure 1.)

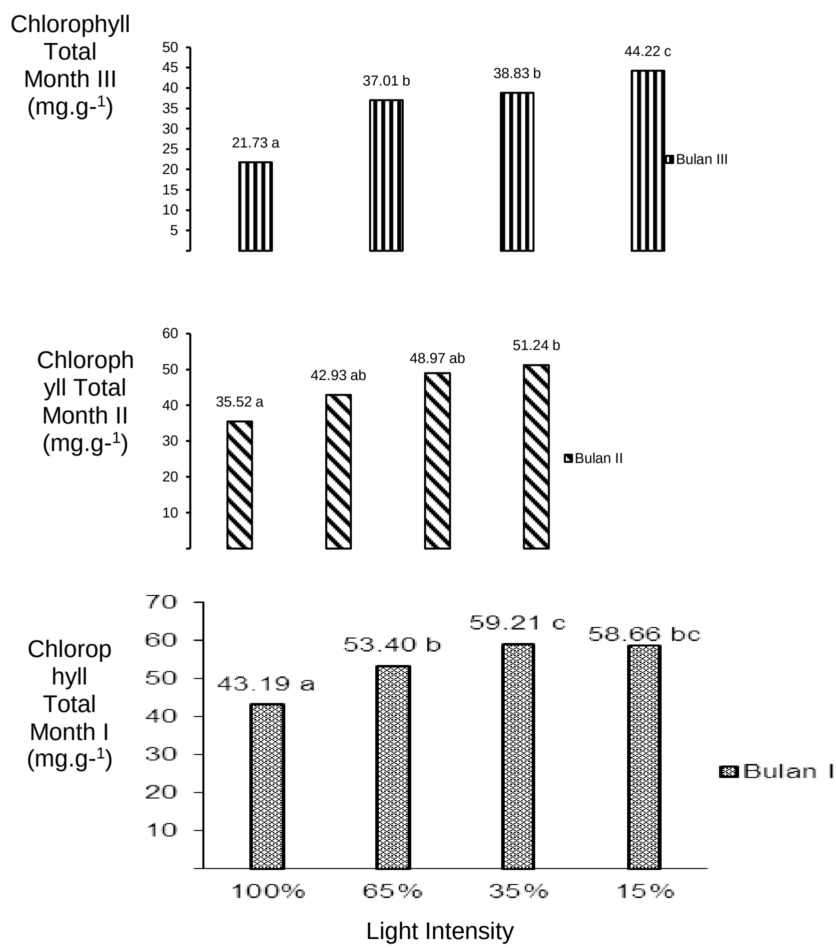

Figure 1. Total chlorophyll level of Luja plants in different light intensity levels in month I, II, and III. The figures followed by the same letters showed no significant difference based on Duncan's test at 0.05 level.

Luja plant grown in high light intensity levels was under stress which was characterized by increasingly fading leaf color. The condition caused the amount of chlorophyll produced to become low. Plants which were grown in high intensity level showed higher resistance to photo inhibition than plants of the same species grown in limited light (Keren et al., 2011). Contrastingly, Luja plant which was cultivated in low light intensity conditions showed an adaptation mechanism by changing leaf morphology to wider surface to maximize light absorption in limited conditions. The change in leaf morphology benefitted the plant in light absorption which caused chlorophyll to not decrease even though the light received by the plant was limited.

The results of this study are in line with studies in several low-light tolerant plants such as soybean plants (Chairudin et al., 2015). The total chlorophyll of Lithraea molleoides leaves was higher in shaded conditions compared to plants exposed to full light (Dias et al., 2007) and the limits for the light intensity of each plant were different depending on the genetic factors. Low light intensity varied for each type of plant to a certain extent (Sevik et al., 2012).

Luja which was shaded tended to have thin leaves because the plant applied physiological strategies to absorb light. The thinness of the leaves was related to the palisade layer, where low light intensity could reduce the palisade layer. The positive impact seen on the thin leaves was that the light absorption competition between leaves would be reduced because sunlight easily escaped or was passed to the part of the leaf at the bottom. The abovementioned use of light by leaves was a leaf physiology strategy that is commonly referred to as chlorophyll light harvesting (LHC). The high chlorophyll level was caused by the presence of chlorophyll a and chlorophyll *b* which acted as peripheral chloroplasts. This photosynthetic activity occurred in photosynthesis II by responding to light received by chloroplast going to the surface to maximize light absorption (Kisman et al., 2007; Zivcak et al., 2014; Grieco et al., 2015). This physiological mechanism was applied by Luja plant, so that at a minimum light intensity, such as at 15% light intensity level, the plant could grow normally, although the best light intensity of Luja plants was at a light intensity of 35%. Total chlorophyll would increase with the decrease of light intensity during growth phase. Thus, it can be said that the plant was more tolerant to low-light environments, and that the light conditions were similar to that of *Roxburghiana Peristrophe* found in Vietnam (Dang et al., 2014). The results of the study differed from previous studies in Calathea crotalifera Bracts plants which were put under 40% and 80% shaded conditions. The results showed that the plants suffered from decreased chlorophyll content, resulting in lower chlorophyll level compared to plants in full light in the tropical regions of Malaysia (Rozali et al., 2016).

Color Quality

The component of color quality in question is the lightness (L), redness (a*) and yellowness (b*) obtained from the extract of the Luja plant leaf. The results of variance test showed that different light intensities had an effect on the quality differences of the colors produced from time to time, especially the lightness and redness, while the yellowness level did not have a significant effect.

Color Lightness (L)

Light intensity had a significant effect on lightness levels in months II and III, but did not have a significant effect on month I. The difference in light intensity affecting lightness levels occurred in the second and third months. Plants that were in 100% and 65% light intensity levels had the highest values and were significantly different from the other two treatments (light intensity 35% and 15%) in month II. Observation in the third month showed that plants with 15% light intensity level had the lowest value and had a significant effect on the other three treatments. Thus it can be concluded that the greater the light intensity levels were, the higher the level of lightness or the more the color faded (Figure 2).

Redness (a)

Higher red pigment was found in shaded plants with light intensity of 65%, 35% and 15% levels compared to plants grown in 100% light intensity level in the first month. In the second month plant grown at 15% light intensity level had the lowest value and was significantly different from other treatments. Contrastingly, in the third month plants with 65% light intensity had more dominant red pigment and was significantly different from plants with 100% and 15% light intensity levels, but it was not different from 35% light intensity.

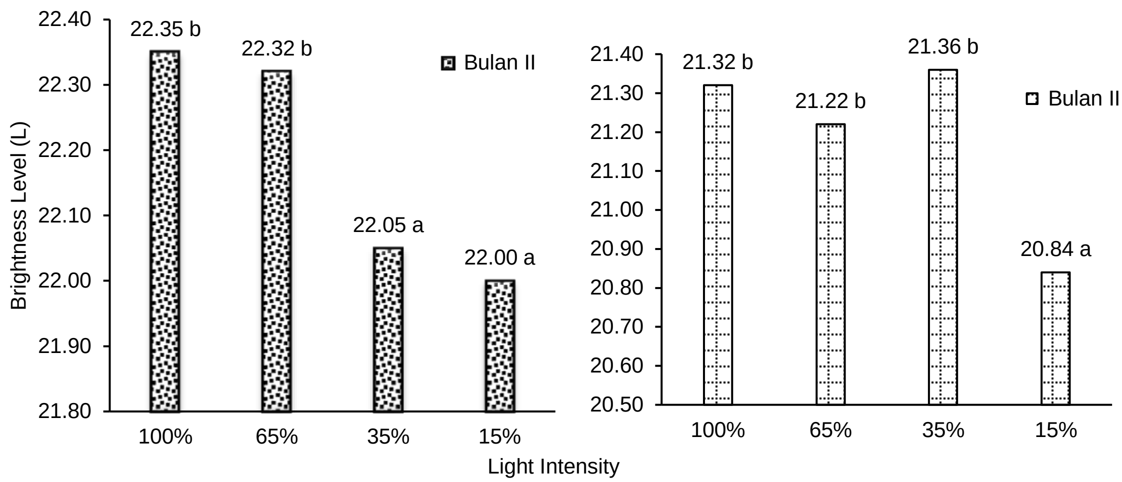

Figure 2. Lightness level of Luja plant extract in different light intensity levels were different in months I and II. The figures followed by the same letters showed no significant difference based on DMRT test at 0.05 level.

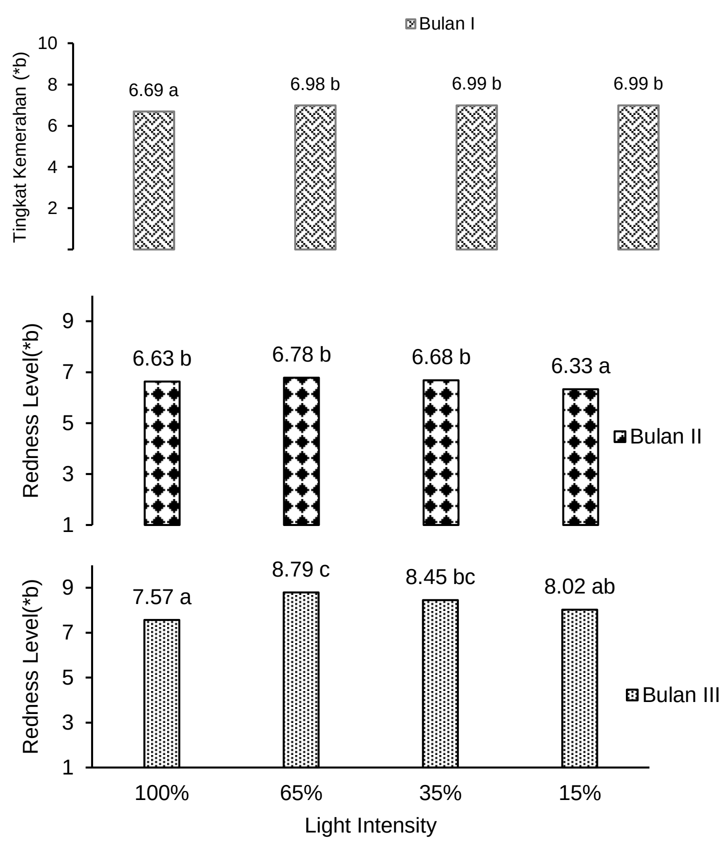

Figure 3. Redness level of Luja plant extract at different light intensity levels in months I, II, and III. The figures followed by the same letters showed that there was no significant difference based on DMRT test at 0.05 level

On the other hand, plants with 35% light intensity level differed significantly from plants grown at 100% light intensity, and had similarities with plants cultivated at 15% and 65% light intensity levels. In terms of quantity, a more prominent red color was seen in month III compared to the previous two months (Figure 3).

Color degradation was influenced by the light received by plants, in addition to genetic factors, media conditions, and the age of plants. Plants that received more light tended to have higher lightness and would show 0.13% - 1.57% decrease of lightness when they were in shaded conditions. When the pigment turned brighter, it indicated that the plant was out of stress or was at higher light intensity levels (100% and 65% light intensity levels) in the second month. This research is similar to previous studies on *Salvia officinalis* L. plants (Rezai et al., 2018), and a study which showed that high-level plants actually have certain pigments that give their own color naturally (Souripet, 2015).

More prominent red pigment appeared in Luja plant cultivated in shaded condition with 15%-65% light intensity levels. This was because the accumulation of red pigments derived from phenol compounds was greater in shaded conditions compared to plants exposed to full light. Similar studies on the leaves of the Red Osier Dogwood family of *Cornaceae* showed the physiological role of anthocyanins in overcoming excessive light (Feild et al., 2001). It is further explained that plants which received more light would turn brighter (senescent-yellow) resulting in red pigment degradation which was caused by physiological damage namely photo oxidative. Luja plant is a shade-tolerant plant because in addition to better growth variables, the pigments produced are higher than plants exposed to 100% light intensity level.

The red pigment in Luja plant extract is apparently derived from rare compounds which are found in animals. The leaf extract also contains a molecular weight of 272.9 m/z, indicated by purplish pigment called peristrophine found in *P. Roxburghiana* leaf extract (Thuy et al., 2012), but qualitatively it is not dominant compared to deep red pigmented compounds studied in this research. The compound from the red leaf extract of Luja plant that produces color is 1- (*4-phenylazophenylazo*)-2-*naphthalenol* which has a molecular weight of 352.7 m / z. The red pigmented compound is synonymous with Sudan III dyes with the molecular formula $C_{22}H_{16}N_4O$ (Figure 4).

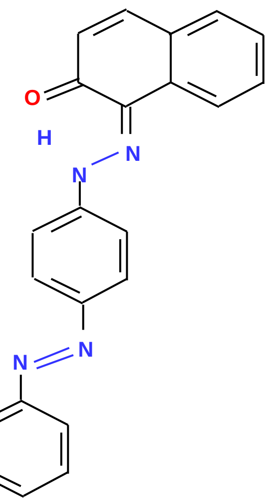

Figure 4. Red-pigmented Sudan III compound

Sudan III is a common name in the market and also known as synthetic dyes that cannot be used for food coloring (Fonovich, 2012; Alim-un-Nisa, 2016). This research found that the extract of the leaves also contained these compounds.

CONCLUSION

Luja plant has the potential to be used as the source of color because it contains pigment. Not only does the plant contain chlorophyll, Luja plant also produces red and yellow pigment leaf extract. The dominant color is red, which can be optimized through manipulating the cultivation environment. Luja plant grows best in an environment with 35%-65% light intensity level. Hence, Luja plant can be considered as a shade-tolerant plant which can be grown under tree stands with limited light intensity. Red pigment which is dominant in Luja plant extract is Sudan III compound, also known as textile coloring compound.

CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest.

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AUTHOR CONTRIBUTIONS

The contribution of each author in the research as follows as RM research designed, conducts research, analyzes data and writes scripts. YS, NA and EN designed the research and analyzed data. All authors read and approve the latest manuscript.

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