Morph-physiology, Sterol and Chlorophyll Content of Bangun-Bangun (*Plectranthus amboinicus* (Lour.) Spreng) Accessions

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Abstract: Research was purposed to know the morph-physiology characteristic, sterols and chlorophyll content of bangun-bangun (Indian borage) accessions. This research was held at home screen Agriculture Faculty, University of Sumatera Utara, Medan. The study used Medan (Krakatau), Medan (Tuntungan), Sibolangit, Simalungun and Brastagi accession. The results showed that morphology character of accession plants Medan (Tuntungan), Sibolangit, Simalungun and Brastagi not significantly different, but significantly different with accession Medan (Krakatau) for stem color, leaf surface color, leaf size, petiole length and space interleaf. Accession did not significantly for sterols and total chlorophyll content. Accession Brastagi have the highest average root length parameter (31 cm) and shoot dry weight (12.2 grams), and for number of leaves (6 week after culture) contained in accessions Medan (Krakatau) (68 leaves).

1 INTRODUCTION

Bangun-bangun is a tropical plant whose leaves have a certain aroma so known as aromatic plants. These plants is widely found in India and Ceylon and South Africa, has a sharp flower and contain essential oils so-called *C. aromaticus*. In India, this plant has also long been known as medicine for malarial fever, hepatopati, kidney and bladder stones, cough, chronic asthma, gibbing, bronchitis, intestinal worms, colic and seizures. This plant contains various types of flavonoids i.e.; quercetin, apigenin, luteolin, salvigenin, genkwanin. This plant leaf has also been proven as anti-inflammatory because it inhibits the inflammatory response induced by cyclooxygenase, also proven as anti-cancer and anti-tumor (Kaliappan and Viswanathan, 2008).

This plant is found almost in all regions of Indonesia with different names. In Central Java called cumin leaves, the Sundanese call it ajeran leaves, in Madura called goat leaves, and in Bali called *iwak* leaf. In North Sumatra Batak region it is referred to as leaves bangun-bangun or torbangun (Priyatno, 2013). Bangun-bangun has three important components namely; the first component is lactagogue compounds, which are components that can stimulate the production of mammary glands in the induction process lactation. The second component is the nutrient component and the third component is the pharmacoseutics component of buffer, antibacterial, anti oxidant, lubricant, plasticizer, colorant and stabilizer. High milk production can be characterized by high nutrient absorption due to high nutrient absorption. High milk production can increase the growth of child weight and increase weaning weight (Khajarern and Khajarern, 2002). Scientifically, the efficacy of Bangun-bangun leaves has been raised by several researchers. Silitonga (1993) reported that bangun-bangun leaf use may increase lactation production of lactating white lactate milk by up to 30%.

Natural materials, including terpenoids, have previously been used in pharmaceuticals, agriculture, and other commercial uses, such as cancer treatments. Terpene as small molecular weight are always used for perfumes and tasters. Steroids are thought to be derived from terpenoids, because in their biosynthetic pathways, steroids are derived from squalene, which is also a triterpene-forming compound. Over the last three decades β-sitosterol has been known to reduce cholesterol levels. Although β-sitosterol is not well absorbed by the body (5-10%), when taken with cholesterol effectively blocks the absorption of cholesterol, resulting in lower serum cholesterol (Tisnadjaja *et al.,* 2006). Three kinds of compounds

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commonly referred to as "phytosterol" ie sitosterol (better known as beta-sitosterol), stigmasterol and campesterol proved to be found in some high plant species. Some research results show that phytosterol can reduce total cholesterol and LDL cholesterol in the blood. Certain types of sterol compounds such as ergosterol, can be found in low level plants such as yeasts and fungi (Harborne, 1998).

Bangun-BAngun contains phenolic, terpenoids, chlorophyll, nitrogen, vitamins, and secondary metabolites that function as antioxidants, antimicrobials, anti-inflammatory, antitumor, antimutagen, anticancer, and diuretics (Sahay *et al*., 2011). Chlorophyll or pigment main plant is widely used as a food supplement that is useful to help optimize metabolic functions, immune system, detoxification, relieve inflammation (inflamatorik) and balance the hormonal system. Chlorophyll also stimulates blood formation because it provides the basic ingredients of the hemoglobin-forming agent. This is due to the chlorophyll structure resembling blood hemoglobin with a difference in the nucleating atoms of the core of its porphyrin ring (Setiari and Nurchayati, 2009).

Efforts to assemble superior varieties can be done through plant breeding activities and one of the determinants of the success of the superior variety assembly program is the availability of genetic diversity. Efforts to generate genetic diversity can be done through domestication, exploration, hybridization, polyploidization, mutation, or other techniques and to support breeding activities requires an attempt to assess genetic diversity. There are several methods that can be used to assess genetic diversity, one with morphology and cytology analysis, so that the genetic and phenetic information of an individual can be known (Suntoro, 1983).

Based on the above description the authors are interested to know the differences of vegetative growth characteristics, sterol content and chlorophyll from some plant wake accession.

2 MATERIAL AND METHODS

This research was conducted at the screen house of Faculty of Agriculture, University of Sumatera Utara, Medan with height of place ± 25 meter above sea level. The plant material was collected from 5 locations namely Medan (Krakatau) which is a wild plant, and the garden yard of the people of Medan (Tuntungan), Sibolangit, Brastagi and Simalungun. Part of the plant taken is a whole plant, namely the roots and canopy plants or branches of plants that have touched the ground and have roots. After collection of plant material next the plant is planted in polybag, where this plant will be used as plant source for propagation. The plant material used in this research is cuttings of shoots of Bangun-Bangun plants from Medan (Krakatau), Medan (Tuntungan), Sibolangit, Simalungun and Brastagi. Plant parts used for propagation are plant shoots, where in the shoot cuttings for all accessions are uniformed as 5 leaves open, and stem diameter uniformed for each accession. The cuttings to be used are removed all the leaves and the base of the cuttings are cut near the leaf's armpits.

Other materials used are compost, water, top soil, polybag, scissors, label, methanol, chloroform, acetic anhydride, concentrated sulfuric acid, and standard β-sitosterol. Tools used include analytical scales, ovens, and water baths. The instrument used was UV-Vis Spectrophotometer 25 Lambda.

The research used non factorial Randomized Block Design (RBD) with 5 treatments and 6 replications. Factor of treatments was accession plant (A) which consists of 5 types, namely: A1 : plant origin Medan (Krakatau); A2 : plants from Medan (Tuntungan); A3: plant origin Sibolangit; A4: plant origin Simalungun; and A5 : plant origin Brastagi.

2.1 Implementation of Research

Planting medium used is a mixture of soil and cow manure with a ratio of 1: 1 and stirred evenly and then put into polybags. Planting media is then arranged on the experimental grounds. Planting is done by planting shoots cuttings into the planting medium that has been perforated then pressed to become more dense then watered with clean water. Watering is done every day or if needed by looking at the condition of planting media in the field. Harvesting was done after the plant aged 8 week after culture. The harvested crop has a minimum of two branches and the leaves of the lower plants begin to turn yellow. Parameter observation was number of leaves (strands), root length (cm), fresh weight of shoot per plant (g), dry weight of shoot per plant (g), shoot/root ratio (g). Determination of dry weight was done by drying ovenkan top of the plant that has been calculated the weight of fresh crown at 70ºC for 48 hours to obtain a constant dry weight

2.2 Sterol Content

Determination of sterol levels is done by Liebermann-Burchard test used to determine the presence of triterpenoid and sterol compounds in the material.

Leaves weighed as much as 2 grams and then crushed by using mortal until smooth. The extraction was done by adding methanol two times where the first extraction was added 10 ml of methanol then the residue was extracted again by adding 5 ml of methanol. Two ml of methanol extract evaporated to dry using a water bath. The residue was extracted two times using chloroform of 2.5 ml each time the extraction. Then as many as 3 test tubes prepared. Tube 1 (Sample) contains 5 ml of chloroform extract; 2 ml acetic acid anhydride and 0.1 ml of concentrated sulfuric acid. Tube 2 (Standard) contains 5 ml of standard β-sitosterol (0.1 mg / ml); 2 ml acetic acid anhydride and 0.1 ml of concentrated sulfuric acid. Tube 3 (blank) contains 5 ml of chloroform; 2 ml acetic acid anhydride and 0.1 ml of concentrated sulfuric acid. Each tube is shaken, then stored in a dark room for 15 minutes. After that the absorbance is measured at a wavelength of 254 nm.

2.3 Chlorophyl Content

The fresh leaves of the plant are washed and then dried with a paper tissue. Leaves weighed as much as 1 gram then crushed by using mortal until smooth (Harborne, 1998). Then extracted by adding 10 ml of methanol and let stand for 3 days. The extract was filtered and then measured its absorbance at 645 and 663 nm wavelengths. Calculation of chlorophyll content is done by the formula:

Total chlorophyll (mg / L) = 20.2 A645 nm + 8.02 A 663 nm

Chlorophyll a (mg / L) = 12.7 A663 nm - 2.69 A 645 nm

Chlorophyll b (mg / L) = 22.9 A645 nm - 4.68 A 663 nm

3 RESULT AND DISCUSION

Based on observation data and the variance it is known that the accession of plants has a significant effect on the number of leaf, root length, and dry weight of shoot at 6 weeks after planting. While plant accession has no significant effect on wet weight of plant canopy and shoot/root ratio (Table 1).

The accession of plant origin Medan (Krakatau) has the highest average number of leaves (68 pieces) and significantly different from the accession of the plant from Medan Tuntungan, Sibolangit, Simalungun and Brastagi. The accession of plant origin Brastagi has the highest average root length that is (31 cm) and is different with accession of plant origin Medan (Krakatau), Medan (Tuntungan), Sibolangit and Simalungun. Accession of plant origin Brastagi has the highest dry weight of crown (12,12 g) not significantly different from accession of plant origin Sibolangit and Simalungun, and different from accession of plant origin Medan (Tuntungan) and origin of Medan (Krakatau) which have average dry weight lowest (8.92 g) (Table 1).

Based on the statistical analysis data, it was found that on the leaf number (6 weeks after planting), the highest was on the accession of plant origin of Medan (Krakatau) that is (68 pieces), it could be seen that the size of the accession leaf from Medan (Krakatau) (length was 48.33 mm and width 47.50 mm) was the smallest compared to the accession of plants from Brastagi (58.7 mm; 62.6 mm), Medan (Tuntungan) (68.90 mm; 63.00 mm), Sibolangit (72.60 mm; 67.60 mm), and Simalungun (66,10 mm; 65,90 mm). It was known that Medan (Krakatau) is a lowland area, and in soil surface area sampling is quite shallow so that the plants adapt to survive in the environment with decreasing the nature of the number of leaves many small and thin to the next generation that can benefit plants in the process of transpiration. The number of leaves of Bangun-bangun plants was more influenced by genetics, it was seen from high heritability, so it was known that this number of leaves nature will be inherited in the next generation, this is in accordance with Rahayu (2011) which states that the adaptation of plants to the environment causes the inherited properties useful for the survival and reproduction of organisms become more common in a population and vice versa, the detrimental properties become lessened, so that more individuals in the next generation inherit these favorable traits.

In the root length and dry weight of the canopy, the accession of plants from Brastagi has the highest average (31 cm) and (12.2 gram). Root crop deep enough to allow plants to absorb water and more nutrients so that the mass of plant canopy produced higher. The root length and dry weight of the canopy has a high heritability value (Table 3), so the probability of this trait will be inherited to a larger offspring due to greater genetic influence than its environment, in accordance with Poespadorsono (1988) which states heritability can be used to measure the ability of a genotype in plant populations to bequeath characteristics possessed. The higher the heritability values of a trait the greater the genetic influence of the environment.

Based on the Liebermann-Burchard test it is known that all accessions show positive results on sterol and triterpenoid contents (Harborne, 1998). The color changes that occur after the addition of sulfuric acid and acetic acid anhydride are blue to green and then

become dark green. Based on observation data and analysis of variance known that accessions of plant have no significant effect on β-sitosterol and chlorophyll content (Table 2). The highest sterol content was found in the accession of the plant originating from Medan (Krakatau), namely (47.44 mg/L) and the lowest in accession from Medan (Tuntungan) (45,21 mg/L). Based on observation data and the variance known that the accession of plants has no significant effect on the content of plant chlorophyll. However, the highest content of chlorophyll was found in the accession of plant origin Medan (Tuntungan) that is (37,18 mg/L) and the lowest in accession of plant origin of Brastagi (35,32 mg/L). The total content character of β-sitosterol and chlorophyll in all plant accessions shows a low heritability value which means that the environmental influence is greater than the genetic effect (Table 3).

Table 1. Number of leaves, root length, fresh weight of shoot, dry weight of shoot per plant and shoot/root ratio of Bangun-bangun plant accessions at 6 weeks after planting.

Accession	Number of leaves (strands)	Root length (cm)	Fresh weight of shoot per plant (g)	Dry weight of shoot per plant (g)	Shoot/ Root ratio
Krakatau	68,00a	19,50b	90,97	8,92b	6,84
Tuntungan	34,00b	20,75b	88,00	9,75b	5,81
Sibolangit	34,00b	20,92b	81,38	11,43a	5,50
Simalungun	33,33b	21,58b	93,15	11,32a	7,25
Brastagi	36,67b	31,00a	102,87	12,12a	5,79

The numbers followed by different notations in the same column show significant differences based on the Duncan Multiple Range Test (DMRT) test at 5%

Table 2. β-sitosterol and chlorophyll content of Bangun-bangun plant accessions at 6 weeks after planting.

Accession	β-sitosterol (mg/L)	Chlorophyll (mg/L)
Krakatau	47,44	35,39
Tuntungan	45,21	37,18
Sibolangit	46,30	35,43
Simalungun	46,32	36,05
Brastagi	47,06	35,32

The numbers followed by different notations in the same column show significant differences based on the Duncan Multiple Range Test (DMRT) test at 5%

Table 3. Heritability of observation parameters

Observation Parameters	h (heritability)	Note
Number of leaves (6	0.97	high
weeks after planting)	0.98	high
Distance between	0.94	high
leaves	0.94	high
The length of the	0.96	high
petiole	0.89	high
Leaf width	0	low
Leaf length	0.71	high
Root length	0.06	low
The wet weight of the	0.19	low
canopy	0	low
Dry crown weight		
Shoot / root ratio		
The content of sterols		
Total chlorophyll		
content		

4 CONCLUSION

The accession of Bangun-Bangun plants has a significant effect on the number of leaf, root length, and dry weight of shoot at 6 weeks after planting. Brastagi accession has the highest average of root length and dry weight of shoot, while the highest number of leaf (6 weeks after planting) is found in the accession of plant origin Medan (Krakatau). Accession of plants has no significant effect on sterol content and total chlorophyll.

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