Asian Journal of Plant Sciences

ISSN 1682-3974

science alert

ANSInet
an open access publisher
http://ansinet.com

OPEN ACCESS Asian Journal of Plant Sciences

ISSN 1682-3974
DOI: 10.3923/ajps.2019.117.122

Research Article
Physiological Characteristics of Binahong (*Anredera cordifolia* (Ten.) Steenis) on Application of Natural Plant Growth Regulator

Yaya Hasanah, Lisa Mawarni and Herla Rusmarilin

Faculty of Agriculture, Universitas Sumatera Utara, Jl. Prof. A. Sofyan No. 3, Kampus USU, Padang Bulan, Medan, 20155, Indonesia

Abstract

Background and Objective: Natural Plant Growth Regulator (PGR) not only affects the growth and yield of binahong, but also affects the physiological characteristics of binahong. The objective of the research was to determine the impact of the foliar application of natural PGR (shallot extract, banana hump extract and bamboo shoot extract) on physiological characteristics of binahong. **Materials and Methods:** The research use a non-factorial randomized block design with 7 treatment and 4 replication. The natural PGR treatment in the research was without natural PGR ; shallot extract 40 mL nL^{-1}; bamboo shoots extract 40 mL L^{-1}; banana hump extract 40 mL L^{-1}; shallot extract (20 mL L^{-1})+bamboo shoot extract (20 mL L^{-1}); shallot extract (20 mL L^{-1})+banana hump extract (20 mL L^{-1}); bamboo shoots extract (20 mL L^{-1})+banana hump extract (20 mL L^{-1}). The variables observed were chlorophyll a, chlorophyll b, total chlorophyll, density of stomata and cuticle thickness. Data were analyzed by using analysis of variance at $p = 0.05$. **Results:** The result showed that the application of natural PGR decreased stomatal density compared to control (without natural PGR application), but increased chlorophyll a, chlorophyll b and total chlorophyll. Natural PGR of shallot extract 40 mL L^{-1} application increased total chlorophyll. Banana hump extract 40 mL L^{-1} application increased the cuticle thickness compared to other natural PGR. **Conclusion:** It was concluded that application of natural PGR decreased the stomatal density, but increased the total chlorophyll. Banana hump extract 40 mL L^{-1} application increased the cuticle thickness compared to other natural PGR.

Key words: Natural plant growth regulator, bamboo shoot, binahong, shallot extract, cuticle thickness, banana hump, stomatal density

Citation: Yaya Hasanah, Lisa Mawarni and Herla Rusmarilin, 2019. Physiological characteristics of binahong (*Anredera cordifolia* (Ten.) Steenis) on application of natural plant growth regulator. Asian J. Plant Sci., 18: 117-122.

Corresponding Author: Yaya Hasanah, Faculty of Agriculture, Universitas Sumatera Utara, Jl. Prof. A. Sofyan No. 3, Kampus USU, Padang Bulan, Medan, 20155, Indonesia Tel: +6261-8213236

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Binahong is one of the species from the family Basellaceae which has health benefits. The Basellaceae family has several species such as *Anredera baselloides* (Kunth) Baill, *Anredera cordifolia* (Ten.) Steenis and *Anredera diffusa*, *Anredera leptostachys* (Moq.) Steenis, *Anredera vesicaria*, *Anredera spicata* and others[1]. The species that is widely known in Indonesia is *Anredera cordifolia* with the local name "binahong". This plant was known to have extraordinary benefits and has been consumed more than thousands of years ago in China, Korea and Taiwan. Almost all parts of binahong plants such as; tubers, stems and leaves can be used as herbal therapy[2,3].

Binahong is one of the medicinal plant used to be a raw material for phytopharmaca. Previous studies reported that binahong contains several flavonoids, alkaloids, terpenoids, saponins, anthocyanins, glucans, carotenes, organic acids, mucopolysaccharides such as; L-arabinose, D-galactose, L-rhamnosa, acid aldonat, also contains saponins, vitamins[4-6] A, B and C. It was reported that binahong leaf paste can be used to heal wounds[7]. Binahong leaf extract has analgesic activity[8]. Binahong leaves are also proven to be able to improve kidney function that has been damaged[9]. In addition, binahong is effective for accelerating wound healing, burn wound healing, smoothing and normalizing circulation and blood, healing internal and external wounds after surgery, swelling and blood clots, restoring weak conditions after illness and also preventing strokes[10,11].

Nowadays, binahong has been used as a raw material for the phytopharmaca industry. According to Balai Penelitian Tanaman Rempah dan Obat (Research Institution of Medicinal Plant and Spice)[10], only about 20% of the raw material of binahong for industry is obtained from cultivation, while the rest is obtained from the forest. Therefore, in order to fulfil the needs of binahong as a medicinal ingredient, it is necessary to conduct cultivation in a directed manner, so that a quality of binahong can be obtained.

In fulfilling the needs of large-scale binahong plant material, it is necessary to apply the treatment to improve the physiological characteristics of binahong. The application of natural PGR is one treatment to improve the physiological characteristics of binahong such as; chlorophyll content, density of stomata and cuticle thickness. Natural PGR is the right choice because it is environmentally friendly, often the natural PGR that is naturally present in plants is below optimal, so external sources are needed to produce a maximum response[12]. Chlorophyll is the most important pigment for green plants because it can generate solar radiation and energy into chemical energy. Chlorophyll a plays an important role in the process of photosynthesis. The status of chlorophyll pigments in leaf tissue is very influential in the overall photosynthesis that occurs directly on plant growth, development and yield[13-20].

Until now there is still very little information about the role of natural PGR on the physiological characteristics of binahong (chlorophyll content, density of stomata and cuticle thickness). Based on the background, the study was aimed to identify the physiological response of binahong as a potential medicinal plant through the application of natural PGR.

MATERIALS AND METHODS

Research area and materials: A pot experiment was conducted at the Experimental Field, Faculty of Agriculture Universitas Sumatera Utara, Medan on April-July, 2018. The materials used in this research were seedlings of binahong, shallot, bamboo shoots, banana hump, 2 kg polybag size, top soil, chicken manure, compost, acetone, kutex. The equipment used namely scale, sieve, bucket, hand sprayer, broach, microscope, spectrophotometer.

Procedures: This research was a pot experiment using a non- factorial randomized design with 7 types of treatment and 4 replications. There were 28 experimental units. The natural PGR treatments in the research was:

P_0 : Without natural PGR
P_1 : Shallot extract 40 mL L^{-1}
P_2 : Bamboo shoots extract 40 mL L^{-1}
P_3 : Banana hump extract 40 mL L^{-1}
P_4 : Shallot extract (20 mL L^{-1})+bamboo shoot extract (20 mL L^{-1})
P_5 : Shallot extract (20 mL L^{-1})+banana hump extract (20 mL L^{-1})
P_6 : Bamboo shoots extract (20 mL L^{-1})+banana hump extract (20 mL L^{-1})

The variables observed were chlorophyll a, chlorophyll b, total chlorophyll, density of stomata and cuticle thickness. Determination of chlorophyll content is carried out by taking sample of 0.1 g of fully expanded leaves for each treatment and then macerated in mortar with 10 mL of aqueous acetone (v/v). Determination of chlorophyll a, chlorophyll b and total chlorophyll was done using the formula to the specific absorption coefficient as reported by Henry and Grime[21]. The content of chlorophyll were expressed as mg g^{-1} of fresh leaf:

$$\text{Chlorophyll a} = \{(12.7\times A663\}-(2.69\times A645)\}/10$$

$$\text{Chlorophyll b} = \{(22.9\times A645\}-(4.68\times A663)\}/10$$

$$\text{Total of chlorophyll} = \{(8.02\times A663)+(20.2\times A645)\}/10$$

Determination of stomatal density used leaf impression method. This method used clear nail polish to make an impression or cast of the leaf surface. The cast is removed with sticky tape and placed on a microscope slide. The stomatal density expressed as Unit mm^{-2}.

Statistical analysis: Data were analyzed by two way analysis of variance (ANOVA) procedures the SAS version 12 computer program, if there were significant differences then proceed with Duncan's Multiple Range test at the level of $\alpha = 5\%$.

RESULTS AND DISCUSSION

Analysis of natural PGR: Based on Table 1, it can be seen that bamboo shoot contained the highest indole acetic acid (0.0084%), GA_3 (0.0058%) and cytokinins (0.0045%) than shallot and banana hump. While, banana humps contained the lowest indole acetic acid (0.0022%), GA_3 (0.0016%) and cytokinins (0.0021%).

Stomatal density: Based on Table 2, it can be seen that without natural PGR (P0) treatment produced the highest stomata content of 112 Unit mm^{-2}. Natural PGR treatment decreased stomata density, where the lowest stomata density was found in treatment P6 (bamboo shoots extract (20 mL L^{-1})+banana hump extract (20 mL L^{-1}). This showed that bamboo shoot extract and extract banana humps containing GA_3 and cytokinin suppress stomata formation. This is in line with previous study that the application of GA_3 resulted in a decrease in stomata in the epidermis[12].

It was contrary to the previous reports that the stomata density was stimulated by cytokinin, the effect of specifics is correlated with the concentration of cytokinin and the method of application[22,23].

Chlorophyll a, chlorophyll b and total chlorophyll: Based on Table 3, it can be seen that each natural PGR treatment has a different influence on the chlorophyll a, chlorophyll b and total chlorophyll. The highest of total chlorophyll content is found in the treatment P1 (shallot extract 40 mL L^{-1} water). This showed that GA_3, cytokinin and IAA contained in shallot play a major role in the formation of chlorophyll. Natural PGR increased the changes in physiology and biochemistry and improved the plant productivity. Previous research also found the similar result that GA_3 increases chlorophyll content in apple plants and *Mentha piperita*[24,25]. It was also reported that application of mepiquat chloride and GA_3

Table 1: Content of auxin, gibberellin and cytokinin in bamboo shoots, shallots and banana hump

Source of natural PGR	Indole acetic acid (%)	GA_3 (%)	Cytokinin (%)
Bamboo shoot	0.0084	0.0058	0.0045
Shallot	0.0027	0.0021	0.0022
Banana hump	0.0022	0.0016	0.0021

Source: Research Institution of Medicinal Plants and Spice (Bogor)

Table 2: Stomatal density of binahong with application of natural PGR

Treatments	Stomatal density (Unit mm^{-2})
P0: Without natural PGR	112.30±11.78[a]
P1: Shallot extract 40 mL L^{-1} water	93.90±26.10[b]
P2: Bamboo shoots extract 40 mL L^{-1}	74.75±9.64 [c]
P3: Banana hump extract 40 mL L^{-1} water	88.30±19.74[c]
P4: Shallot extract (20 mL L^{-1})+bamboo shoot extract (20 mL L^{-1})	92.50±10.51[b]
P5: Shallot extract (20 mL L^{-1})+banana hump extract (20 mL L^{-1})	71.85±32.62[c]
P6: Bamboo shoots extract (20 mL L^{-1})+banana hump extract (20 mL L^{-1})	68.20±4.96[c]

Means followed by the same letter indicated not significantly different based on Duncan's Multiple Range test (p = 0.05)

Table 3: Chlorophyll a, Chlorophyll b and total chlorophyll content of binahong with application of natural PGR

	Fresh leaf (mg g^{-1})		
Treatments	Chlorophyll a	Chlorophyll b	Total of chlorophyll
P0	1.38±0.34	1.57±0.48	2.95±0.13
P1	1.54±0.51	1.81±0.90	3.52±1.21
P2	1.68±0.71	1.51±0.64	3.20±1.35
P3	1.34±0.09	1.83±0.36	3.16±0.94
P4	1.01±0.09	1.20±0.27	2.20±0.19
P5	1.45±0.65	1.78±0.49	3.32±1.15
P6	1.39±0.03	1.57±0.19	3.10±0.05

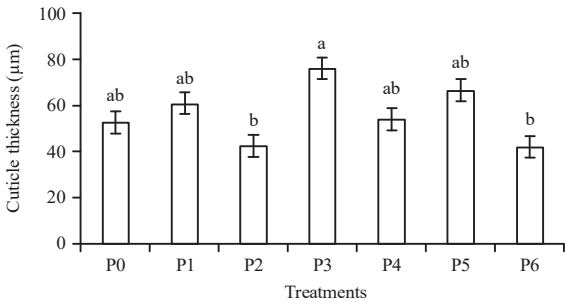

Fig. 1: Cuticle thickness of binahong on application of natural PGR

combined or alone, improved the chlorophyll content and leaf area in grapes[26]. Previous research also stated that the application of foliar GA_3 increased chlorophyll levels in corn plants under saline condition[27]. Another researcher reported that application of foliar application of PGR can increase growth, photosynthesis and flowering in cashew[28].

Cuticle thickness: Based on Fig. 1, it can be seen that the treatment of P_3 (banana hump extract 40 mL L^{-1} water) produced the highest cuticle thickness, while treatment P_2 (bamboo shoots extract 40 mL L^{-1}) and P_6 bamboo shoots extract (20 mL L^{-1})+banana hump extract (20 mL L^{-1}) produced the lowest cuticle thickness.

Bamboo shoots contained GA_3, Indole Acetic Acid and cytokinin were the highest among other natural PGR (Table 1), resulted in lower cuticle thickness. This indicated the influence of higher levels of natural PGR on the decrease in cuticle thickness. The banana hump contained the lowest IAA, GA_3 and cytokinin produced the highest cuticle thickness. The results of this research were in line with previous study stated that an increase in GA_3 application on radish seedlings increased the thickness of the cuticle[29]. Although, the results was not appropriate with previous literature that stated the foliar application of GA_3 enhanced thickness of the cuticle as compared to the control and 2,4-D[30]. The thickness of the cuticle can affect the transpiration process in plants. Modification of cuticle thickness is a response to reduce the transpiration and reaction of plants to the entry of pollutants. It was studied that the cuticle is located in the outer layer of the epidermis consisting of two layers, the outermost layer which only consists of the layer of cutin (true cuticle) and the inner layer (the cuticular layer) which contains the cutin and other cell wall material. The outermost layer of leaves is used to maintain leaf moisture, because the cuticle layer can control transpiration or evaporation, so as to minimize water loss[31]. In addition to maintaining moisture, the cuticle also served as an initial defense against the entry of foreign objects including pollutants from the air into the leaves.

CONCLUSION

The use of natural PGR decreased stomatal density compared to control (without natural PGR application), but increased the chlorophyll a, chlorophyll b and total chlorophyll contents. Natural PGR shallot extract 40 mL L^{-1} application increased the total chlorophyll. Banana hump extract 40 mL L^{-1} application increases the cuticle thickness compared to other natural PGR.

SIGNIFICANCE STATEMENT

This research has discovered findings that there are differences in the response of stomatal density, chlorophyll content and cuticle thickness of binahong on application of natural plant growth regulator. Based on this research a new theory has been obtained that, the use of natural PGR decreases stomatal density compared to control (without natural PGR application), but increases chlorophyll a, chlorophyll b and total chlorophyll. Natural PGR shallot extract 40 mL L^{-1} application increases total chlorophyll. Banana hump extract 40 mL L^{-1} application increases the cuticle thickness compared to other natural PGR. It is concluded that application natural PGR decreased the stomatal density, but increase the total chlorophyll. Banana hump extract 40 mL L^{-1} application increased the cuticle thickness compared to other natural PGR. This research will help the researchers to cope the best natural PGR to increase the chlorophyll content that play an important role for photosynthetic process.

ACKNOWLEDGMENT

The author would like to thank the Research Institution, Universitas Sumatera Utara for funding this research through a Research Contract TALENTA Universitas Sumatera Utara, Fiscal Year 2018 Number: 2590/UN5.I.R/PPM/2018 dated March 16, 2018. The author also thank Panca Ramadhan for his assistance in this research.

REFERENCES

1. Abou-Zeid, A.H.S., F.M. Soliman, A.A. Sleem and M.N.R. Mitry, 2007. Phytochemical and bio-activity investigations of the aerial parts of *Anredera cordifolia* (Ten.) Steenis. Bull. Natl. Res. Centre Cairo, 32: 1-33.

2. Astuti, S.M., A.M.M. Sakinah, B.M.R. Andayani and A. Risch, 2011. Determination of saponin compound from *Anredera cordifolia* (Ten) steenis plant (Binahong) to potential treatment for several diseases. J. Agric. Sci., 3: 224-232.
3. Syahputra, A., Y. Hasanah and A. Barus, 2018. [Response growth of Anredera (*Anredera cordifolia* (Rens) Stennis) on differences of planting material and planting media composition]. Jurnal Agroteknologi, 6: 14-19, (In Indonesian).
4. Ozela, E.F., P.C. Stringheta and M.C. Chauca, 2007. Stability of anthocyanin in spinach vine (*Basella rubra*) fruits. Cienc. Invest. Agric., 34: 115-120.
5. Rachmawati, S., 2008. [Macroscopic and microscopic studies and phytochemical screening of *Anredera cordifolia* (Ten.) Steenis leaves]. Fakultas Farmasi, Airlangga University, Indonesia, (In Indonesian).
6. Djamil, R., P.S. Wahyudi, S. Wahono and M. Hanafi, 2012. Antioxidant activity of flavonoid from *Anredera cordifolia* (Ten) steenis leaves. Int. Res. J. Pharm., 3: 241-243.
7. Hanafiah, O.A., D.S. Hanafiah, E.S. Bayu, T. Abidin, S. Ilyas, M. Nainggolan and E. Syamsudin, 2017. Quantity differences of secondary metabolites (saponins, tannins and flavonoids) from Binahong plant extract (*Anredera cordifolia* (Ten.) Steenis) treated and untreated with Colchicines that play a role in wound healing. World J. Dent., 8: 296-299.
8. Yuziani, Y., U. Harahap and K. Karsono, 2014. Evaluation of analgesic activities of ethanolic extract of *Anredera cordifolia* (Ten) steenis leaf. Int. J. PharmTech Res., 6: 1608-1610.
9. Takahashi, M., Y. Asikin, K. Takara and K. Wada, 2012. Screening of medicinal and edible plants in Okinawa, Japan, for enhanced proliferative and collagen synthesis activities in NB1RGB human skin fibroblast cells. Biosci. Biotechnol. Biochem., 76: 2317-2320.
10. Balai Penelitian Tanaman Rempah dan Obat (BALITTRO), 2006. Rencanadan strategis Balai Penelitian Tanaman Obat dan Aromatik 2006-2009. Balai Penelitian Tanaman Obat dan Aromatik (BALITTRO), Bogor, Indonesia.
11. Sukrama, D.M, D.M. Wihandani and A.P. Manuaba, 2016. Topical Binahong (*Anredera cordifolia*) leaf extract increases interleukin-6 and VEGF during burn wound healing in Wistar rats infected with *Pseudomonas aeruginosa*. Biol. Med., Vol. 9. 10.4172/0974-8369.1000369.
12. Janowska, B. and M. Stanecki, 2013. Effect of rhizome soaking in a mixture of BA and GA_3 on the earliness of flowering and quality of the yield of flowers and leaves in the calla lily (*Zantedeschia* Spreng.). Acta Sci. Pol. Hortorum Cultus, 12: 3-12.
13. Hanelt, D., 2018. Photosynthesis Assessed by Chlorophyll Fluorescence. In: Bioassay: Advanced Methods and Applications, Hader, D.P. and G.S. Erzinger (Eds.). Chapter 9, Elsevier Inc., New York, USA., ISBN: 978-0-12-811861-0, pp: 169-198.
14. Frankenberg, C. and J. Berry, 2018. Solar Induced Chlorophyll Fluorescence: Origins, Relation to Photosynthesis and Retrieval. In: Comprehensive Remote Sensing: Volume 3: Terrestrial Ecosystems, Liang, S. (Ed.). Chapter 3.10, Elsevier Inc., New York, USA., ISBN: 978-0-12-803221-3, pp: 143-162.
15. Taiz, L. and E. Zeiger, 2006. Plant Physiology. 4th Edn., Sinauer Associates Inc. Publishers, Sunderland, MA., USA., ISBN-10: 0-87893-856-7, Pages: 764.
16. Hailemichael, G., A. Catalina, M.R. Gonzalez and P. Martin, 2016. Relationships between water status, leaf chlorophyll content and photosynthetic performance in Tempranillo vineyards. S. Afr. J. Enol. Viticult., 37: 149-156.
17. Baker, N.R. and E. Rosenqvist, 2004. Applications of chlorophyll fluorescence can improve crop production strategies: An examination of future possibilities. J. Exp. Bot., 55: 1607-1621.
18. Inanc, A.F., 2011. Chlorophyll: Structural properties, health benefits and its occurrence in virgin olive oils. Akademik Gida, 9: 26-32.
19. Blankenship, R.E., 2002. Photosynthetic Pigments: Structure and Spectroscopy. In: Molecular Mechanisms of Photosynthesis, Blankenship, R.E. (Ed.). Chapter 4, Blackwell Science, Oxford, UK., ISBN-13: 9780632043217, pp: 42-60.
20. Hasanah, Y. and M. Sembiring, 2018. Role of elicitors in chlorophyll content and stomatal density of soybean cultivars by Foliar application. J. Agron., 17: 112-117.
21. Hendry, G.A. and J.P. Grime, 1993. Methods in Comparative Plant Ecology: A Laboratory Manual. Chapman and Hall, London, ISBN-13: 9780412462306, Pages: 252.
22. Farber, M., Z. Attia and D. Weiss, 2016. Cytokinin activity increases stomatal density and transpiration rate in tomato. J. Exp. Bot., 67: 6351-6362.
23. Carey, Jr. D.J., 2008. The effects of benzyladenine on ornamental crops. M.S. Thesis, Graduate Faculty of North Carolina State University, Raleigh, NC., USA.
24. Moneruzzaman, K.M., A.B.M.S. Hossain, W. Sani and M. Saifuddin, 2008. Effect of stages of maturity and ripening conditions on the physical characteristics of tomato. Am. J. Biochem. Biotehcnol., 4: 329-335.
25. Kavina, J., R. Gopi and R. Panneerselvam, 2011. Plant growth regulators and fungicides alters growth and biochemical contents in *Mentha piperita* Linn. Int. J. Environ. Sci., 1: 2096-2108.
26. Lim, S.C., S.K. Kim, Y.H. Kim, C.K. Youn and T. Yoon, 2004. Vine growth and fruit quality of 'kyoho' grapes as affected by mepiquat chloride and GA. Acta Hortic., 653: 145-149.
27. Tuna, A.L., C. Kaya, M. Dikilitas and D. Higgs, 2008. The combined effects of gibberellic acid and salinity on some antioxidant enzyme activities, plant growth parameters and nutritional status in maize plants. Environ. Exp. Bot., 62: 1-9.

28. Bhat, M.G., K.V. Nagaraja and T.R. Rupa, 2010. Cashew research in India. J. Hortic. Sci., 5: 1-16.
29. Cavusoglu, K., S. Kılıc and K. Kabar, 2008. Effects of some plant growth regulators on stem anatomy of radish seedlings grown under saline (NaCl) conditions. Plant, Soil. Environ., 54: 428-433.
30. Doaigey, A.R., M.H. Al-Whaibi, M.H. Siddiqui, A.A. Al Sahli and M.E. El-Zaidy, 2013. Effect of GA_3 and 2,4-D foliar application on the anatomy of date palm (*Phoenix dactylifera* L.) seedling leaf. Saudi J. Biol. Sci., 20: 141-147.
31. Fahn, A., 1991. Plant Anatomy. 4th Edn., Pergamon Press, UK., ISBN-13: 9780080374901, Pages: 588.