The Effect of Gibberellin on Growth and Content of Bioactive Compounds of Kale (Brassica Oleracea Var. Acephala)

Hanifatus Sholihah[1], Kumala Dewi[2]

[1,2]Faculty of Biology, Universitas Gadjah Mada, Yogyakarta, Indonesia

Abstract

Kale (Brassica oleraceae var. acephala) is a green leafy vegetable dubbed a "superfood" because of its high nutritional value that provide health benefits. Gibberellins (including GA_3) are one of the growth regulators that play significant roles in plant growth and development, as well as metabolism. Although the effects of gibberellin on plant growth and development are well known, its impact on the level of kale's biologically active compounds (total phenol and vitamin c content) has yet to be thoroughly investigated. This study aims to examine gibberellins' effects on the growth and content of biologically active compounds in kale. A completely randomized design (CRD) is used in this study, with four replications for each treatment. Gibberellin was sprayed on the entire plant at four different dose levels (0 ppm, 50 ppm, 100 ppm, and 200 ppm). The obtained data were analyzed using one-way ANOVA (Analysis of Variance), followed by Duncan Multiple Range Test (DMRT) at 5%. This study found that increasing the concentration of GA_3 to 100 ppm had the best effects on kale growth (leaf area and wet weight) and the content of bioactive compounds, specifically total phenol content and vitamin C content.

Keywords: Gibberellins, Kale, Growth, Bioactive Compounds.

Introduction

The Brassicaceae family, known as cruciferous vegetables, has been under scientific attention for several decades. Numerous studies have shown that vegetables under the genus Brassica (kale, broccoli, cauliflower, and cabbage) are high in nutritional value and bioactive substances such as glucosinolates, carotenoids, vitamins, and polyphenols (Podsedek, 2007; Šamec et al., 2019). Diets high in bioactive compounds have been linked to reduced risks of several chronic diseases due to their anticarcinogenic, antibacterial, and antioxidant properties (Šamec et al., 2019; Makmur et al. 2021). Kale (Brassica oleracea var. acephala), a Brassica vegetable member characterized by its headless form, is well known for frequently appearing on lists of "superfoods." Although kale has been cultivated for centuries around the world, it has only begun to gain popularity in Indonesia for its health benefits in the last couple of years. The health benefits of kale are mainly correlated to the presence of health-promoting substances such as polyphenols and vitamin C. The consumption of kale has become essential for a healthy diet. Kale has a high polyphenol content that contributes to its biological activity. According to Korus (2011), the polyphenol content of kale is significantly higher than that of broccoli, cabbage, and cauliflower, reaching 531 mg per 100 g. On the other hand, kale reported to contains more than 40% of the recommended daily vitamin C intake in one serving, which makes it an excellent source of vitamin C (Becerra-Moreno et al., 2013).

Originating from the Mediterranean region, kale is known for its incredible adaptability that allows for cultivation around the world (Šamec et al., 2019). However, climates have significant impacts on kale cultivation. In Indonesia's tropical climate, kale has difficulty blooming, producing seeds, and expanding leaves to optimal width, resulting in reduced yields below optimal levels (Fajri and Soelistyono, 2018). In such a case, efforts are needed to increase kale value, which can be done through the provision of plant growth regulators (PGRs).

Natural and synthetic growth regulators have been widely used to modify plants by controlling their developmental processes. Gibberellins (GAs) are one of several essential hormones that regulate various physiological processes in plants, fungi, and some microbes. Exogenous gibberellin application affects wide range of plant physiological processes, such as seed dormancy, germination, flowering, internode elongation, leaf expansion, and root elongation (Hedden and Sponcel, 2015). It triggers the transcription of genes involved in cell division and elongation during plant growth. GAs promotes the expression of hydrolytic enzymes necessary for the conversion of starch to sugar. Gibberellin can affect plant growth in general by regulating the accumulation and utilization of starch (Oktaviani et al., 2021).

Gibberellin has been shown to have documented effects on the growth and quality of brassicas and other crops. Gibberellin positively affects plant height, leaf area, and root length of Chinese broccoli and cabbage (Maharani et al., 2018, Mazed et al., 2015). It also caused the broccoli leaf expansion rate and flower mass weight to increase (Onggo and Kusumiyati, 2017). Gibberellin treatment at 100 ppm concentrations is stated to have positive effects on the content of bioactive compounds correlated to camellia tea antioxidant activity (Wen et al. 2018). According to Kaplan et al. (2021), gibberellins had a significant effect on increasing yield size and the total phenolic compound of grapevine fruit. Among the PGR treatments tested on paprika, foliar spraying with gibberellin resulted in the highest increase in vitamin C in paprika fruit (Ouzounidou et al., 2010). These effects can vary widely depending on the plant species, application method, hormone requirements, cultivation techniques, and plant response at different growth stages. The quality of leafy vegetables is seen in the appearance of the leaves and the nutritional value they contain. Gibberellins are thought to have critical roles in many metabolic pathways that affect this characteristic. Therefore, this study was conducted to characterize the effect of gibberellins' provision on the growth and content of bioactive compounds in kale (Brassica oleracea var. acephala DC).

Methods

The research was conducted at Kalirejo village in Malang, East Java. This work used a completely randomized design, with four replications for each treatment. The applications of various gibberellin (GA_3) concentrations has used as the treatments (0, 50, 100, and 200 ppm). Foliar application using a manual sprayer is the method used, which is applied twice in the fourth and sixth weeks after planting (WAP). A 1:1 mixture of compost and husk charcoal was used as the media. Watering was done daily, and 10 g of NPK fertilizers were applied regularly at once-a-week intervals.

Plant height (cm) was measured from the base of the stem to the top of the highest leaf using a ruler. Each fully developed leaf was counted in order to calculate the total number of leaves. The grid count method was used to calculate the leaf area, which was then expressed in (cm^2). Wet weight (g) is calculated by weighing all parts of the plant immediately after harvest.

The vitamin C levels were determined using a modified iodometric titration method (Nweze et al., 2015). Kale leaves were weighed 20 grams and mashed, then milled with 50 mL of distilled aquades. Filter clothes were used to separate the filtrate from the residue. The filtrate obtained was then poured into a 100 mL volumetric flask, and distilled aquades were added to fill the flask to the line. The kale extract obtained is suitable for iodometric measurement. A total of 25 mL of kale extract sample was placed in a 125 mL volumetric flask, followed by 6 mL of 10% sulfuric acid (H_2SO_4) and 1 mL of 1% starch. The mixture was titrated with a 0.01 N iodine solution until a blue-black color remained visible over 1 minute. The following equation is used to calculate vitamin C levels:

$$\text{Vitamin C (mg/100g)}: \frac{\text{V Iodine x } 0{,}88 \text{ x } \frac{100}{25} \text{ x } 100}{\text{sample mass}}$$

The Follin-Ciocalteu method was used to determine the total polyphenol contents of kale leaf (Wang et al., 2000). The test tube is filled with 0.05 mL of kale extract sample, followed by 1 mL of ethanol, 5 mL of distilled water, and 0.5 mL of the Folin-Ciocalteu reagent, then vortexed to homogenize the mixture. 1 mL of 5% Na_2CO_3 was added after 5 minutes and vortexed to homogenize. The mixture was kept in the dark for 60 minutes while wrapped in aluminum foil, and the absorbance at 725 nm wavelength was measured. Standard curves are created by replacing samples with various concentrations of gallic acid.

Data obtained on growth and bioactive content in kale were statistically analyzed using a one-way ANOVA with a significance level of 5%. Furthermore, Duncan's test was then used to determine the significant differences between the treatments of GA_3 concentrations.

Result

Table 1: The Effect of Gibberellins on Plant Height of Kale

GA_3 treatmet (ppm)	**Plant height (cm)**
G0 (0)	13,72 a
G1 (50)	**20,95** c
G2 (100)	18,55 bc
G3 (200)	17,45 b

*Mean values marked with the same notation represent no significant difference Tukey's test ($p \leq 0.05$)

The results indicated that gibberellin significantly affects plant height (Table 1). Gibberellin at low concentrations (G1 = 50 ppm) can accelerate plant growth, but increasing concentrations give a less noticeable effect. The concentration of gibberellin at 50 ppm shows the average plant height increased to nearly 48% higher than the control of 20,95 cm.

Table 2: The Effect of Gibberellins on Kale Leaf

GA$_3$ treatmet (ppm)	Leaf area (cm^2)	Number of leaves
G0 (0)	66,84 a	**24,5** a
G1 (50)	68,37 ab	23,5 a
G2 (100)	**71,06** c	23,25 a
G3 (200)	69,73 bc	23,75 a

*Mean values marked with the same notation represent no significant difference Tukey's test ($p \leq 0.05$)

The leaf area of kale is significantly affected by the exogenous application of gibberellin (Table 2). The highest value of leaf expansion was found under G2 (100 ppm) treatment reaching 71,06 cm2. Notably, there was no significant difference in the number of leaves after the provision of gibberellin.

Table 2: The Effect of Gibberellins on Fresh Weigh of Kale

GA$_3$ treatmet (ppm)	Fresh weight (g)
G0 (0)	215,17 ± 1,309 **a**
G1 (50)	238,26 ± 1,888 **c**
G2 (100)	**239,24 ± 1,86** c
G3 (200)	224,47 ± 1,892 b

*Mean values marked with the same notation represent no significant difference Tukey's test ($p \leq 0.05$)

Gibberellin treatment in kale showed significant increase of fresh weight compared to the control. Various concentrations of gibberellin a significant effect on increasing the average fresh weight of kale plants, with the highest yield found in the G2 (100 ppm) treatment of 239.24 g.

Table 2: The Effect of Gibberellins on Total Phenol and Vitamin C Content of Kale

GA$_3$ treatmet (ppm)	Total phenol content (mg/kg)	Vitamin C levels (mg/100g)
G0 (0)	742,51 ± 2,702 b	123,96 ± 0,710 b
G1 (50)	729,70 ± 6,190 a	119,15 ± 0,839 a
G2 (100)	**869,22 ± 7,245 c**	**127,85 ± 0,877** c
G3 (200)	842,08 ± 7,912 d	126,96 ± 0,814 c

*Mean values marked with the same notation represent no significant difference Tukey's test ($p \leq 0.05$)

Total phenolic contents of kale significantly affected by exogenous application of gibberellic acid. Application of G2 (100 ppm) and G3 (200 ppm) increased the average total phenolics by 17% and 13.4%, respectively, compared to the control. Maximum total phenolic contents were observed in 100 ppm g3 treatment reaching 869.22 mg/kg level. The study reveals that as the gibberellin concentration increased, this was accompanied by increase of vitamin C content. Samples from G0 treatment (0 ppm) group showed the lowest vitamin C content of among all treatments. The content of vitamin C was not significantly different in G2 (100 ppm) and G3 (200 ppm) treatment, with the highest found in G3 (100 ppm) treatment reaching 127.85 ± 0.877 mg/100 g.

Discussion

The increase in plant height was the most common physiological response found in gibberellin-treated plants. Increased levels of gibberellins in plants are synergistically followed by increases in other growth hormones such as auxins, cytokinins, and brassinosteroids, which then work together in influencing plant growth and development even though they work at different growth and development phases. (Maharani et al. 2018). Asra and Ubaidillah (2012) stated that gibberellins support the formation of proteolytic enzymes that can liberate the amino acid tryptophan as an auxin precursor so that auxin levels increase.

Auxin plays a role in plant growth, especially at the tip of the plant canopy, by inducing the entry of protons in the acid growth mechanism. Gibberellins induce cell expansion by increasing the activity of the enzyme xyloglucan endotransglucosylase, which can cause the rearrangement of the cell wall matrix. Gibberellins facilitate the entry of expansion so that the cell wall stretches and the cell can elongate, which leads to an increase in plant height (Matos et al., 2020). Gibberellins also promote cell elongation by promoting the formation of the starch hydrolysis enzyme -amylase. As a result of this process, the concentration of reduced sugar in the cell rises, as does the osmotic potential, enabling water to enter easily and thus the cell to prolong (Oktaviani et al., 2021).

Leaves are the main organs in plants that have a special function to carry out photosynthesis. Carbohydrates produced through the process of photosynthesis are translocated to parts of the plant that need them to support growth and development, so the higher the level of carbohydrate assimilation, the more overall plant growth becomes optimal. (Matos et al., 2020). Gibberellins are also known to affect the increasing leaf area of several other leaf vegetable plants, such as kailan (Maharani et al., 2018; Riko et al., 2019), Pak Choy (Mutryarny and Lindar, 2018), and mustard greens (Oktaviani et al., 2021). Wu et al. (2017) stated that, in general, leaf area is determined by cell division in the young leaf phase and cell expansion in the old leaf phase. Both can be influenced by the function of gibberellins in increasing cell division so that the number of cells and leaf area increase. Gibberellins promote leaf cell elongation by interfering with the α-amylase enzyme (Oktaviani et al., 2021). The response to the increase in cell length by gibberellins in stems is usually an increase in length between plant segments and does not increase the number of internodes formed so that the number of leaves produced does not increase (Hedden and Sponcel, 2015).

Fresh weight is one of the determinants of whether or not the growth of a plant is good. This is because the overall fresh weight indicates the accumulation of photosynthetic products and the water content of the plant, which are influenced by metabolic activity and environmental factors during growth. Gibberellins can induce the transcription of genes involved in cell elongation and cell division during cell growth (Soad et al., 2010). In addition, gibberellins can stimulate the expression of the hydrolytic enzyme α-amylase involved in the conversion of starch to sugar (Miceli et al. 2019). According to Matos et al. (2020), increased leaf growth due to gibberellins can cause the effectiveness of light absorption by chlorophyll and the absorption of water and carbon dioxide by stomata to be higher, so that the results of photosynthesis and carbohydrate assimilation increase. By controlling the conversion and accumulation of starch, gibberellins can affect overall plant growth. Thus, GA signals in plant tissues can influence gene expression, plant physiology, and morphology.

Phenolic compounds are a group of secondary metabolites that play an important role in plant defense mechanisms against pests and external stimuli. Not only that, phenolic compounds are known to have antioxidant abilities, counteract free radicals, and have the potential to prevent chronic diseases in humans (Sardoei et al., 2014). The concentration of GA_3 at 2 mg/L is the best concentration to increase the total phenol content and antioxidant activity of the plant Stevia rebaudiana (Ahmad et al., 2020). Gibberellins. According to Ahmad et al. (2020), elicitors are compounds that can stimulate the formation of secondary metabolites in plants as a self-protection response when applied to plant tissues.

Gibberellins are also used as elicitors in increasing the biosynthesis of secondary metabolites in the form of phenolic acids. According to Treutter (2010), the biosynthesis of phenolic compounds occurs through the shikimate pathway, which produces the amino acid phenylalanine, Phenylalanine is deaminated by the enzyme phenylalanine ammonia-lyase (PAL) into cinnamic acid derivatives, which are then converted into phenolic compounds and their derivatives. Research by Khandaker et al. (2015) reported an increase in PAL activity in guava after gibberellin induction at a concentration of 50 mg/L. So, there is a possibility that gibberellins can increase the total phenolic content of kale leaves by influencing the performance of the PAL enzyme in the biosynthetic pathway of phenol bioactive ingredients.

Research by Singh and Singh (2021) stated that the application of 150 ppm gibberellins increased the vitamin C content in chili plants. Meanwhile, the results of Miceli et al. (2019) showed that there were differences in the responses of different hydroponically grown leaf vegetable species to the use of gibberellins. Lettuce leaves experienced a slight decrease in vitamin C content after gibberellin induction, while the vitamin C content of rocket plants increased significantly. This shows that there are differences in plant responses to the application of exogenous gibberellins that vary depending on differences in species, plant growth stages, dose, and method of gibberellin application and cultivation methods used. The gibberellin hormone is thought to affect the increase in vitamin C content by increasing the activity of enzymes involved in ascorbic acid biosynthesis or by suppressing the activity of the ascorbate oxidase enzyme, which can cause damage to vitamin C in plants (Netam and Sharma, 2014).

CONCLUSION

Based on the results of studies investigating the impact of gibberellin concentration on the growth and content of bioactive compounds in kale (Brassica oleraceae var. acephala), it can be concluded that the concentration of GA_3 at 100 ppm was able to provide the best results for increasing leaf area and fresh weight of kale plants, as well as increasing bioactive compound content as measured by the total phenolic content and vitamin C content in kale leaves.

REFERENCES

1. A. Ahmad, H. Ali, H. Khan, A. Begam, S. Khan, S.S. Ali, N. Ahmad, H. Fazal, M. Ali, C. Hano, N. Ahmad, B.H. Abbasi, Effect of Gibberellic Acid on Production of Biomass, Polyphenolics and Steviol Glycosides in Adventitious Root Cultures of Stevia rebaudiana (Bert.), 2020, Plants, 9(420): 1-14.
2. Asra, R. and Ubaidillah. Pengaruh Konsentrasi Giberelin (GA_3) Terhadap Nilai Nutrisi Calopogonium caeruleum. 2012, Jurnal Ilmu-Ilmu Peternakan. 15(2): 81-85.
3. Becerra-Moreno A, P.A. Alanís-Garza, J.L. Mora-Nieves, J.P. Mora-Mora and D.A. Jacobo-Velázquez. Kale: An excellent source of vitamin C, pro-vitamin A, lutein and glucosinolates. 2013, CyTA - Journal of Food 12; 298-303.

4. Fajri, L. N. and Soelistyono, R. Pengaruh kerapatan tanaman dan pupuk urea terhadap pertumbuhan dan hasil tanaman kale (brassica oleracea var. achepala). 2018, Plantropica 3(2): 133-140
5. H. Wang, G.J. Provan, Halliwell, Tea flavanoids: their function, utilization and analysis, 2000, Journal of Food Science and Technology, 11, 152 – 160.
6. Hedden, P. and Sponsel, V. A Century of Gibberellin Research. 2015, Journal Plant Growth Regul, 2015(34): 740-760.
7. Khandaker, M. M., Majrashi, A., Boyce, A. N. The Influence of Gibberellic Acid on the Chlorophyll Fluorescence, Protein Content and PAL Activity of Wax Apple (Syzygium samarangense var. Jambu madu) Fruits. 2015, Australian Journal of Crop Science (AJCS), 9(12): 1221-1227.
8. Korus, A. Level of Vitamin C, Polyphenols, and Antioxidant and Enzymatic Activity in Three Varieties of Kale (Brassica oleracea L. var. acephala) at Different Stages of Maturity. 2011, International Journal of Food Properties, 14(5): 1069 – 1080.
9. M. Kaplan, K. Klimek, E. Jabłonska-Ry´s, A. Sławinska, A. Stój, Effect of Hormonization Treatment on Yield Quantity and Quality, Contents of Biologically Active Compounds, and Antioxidant Activity in ‘Einset Seedless’ Grapevine Fruits and Raisins, 2021, Molecules, 26, 6206.
10. Maharani, A., Suwirmen, Noni, Z. A. Pengaruh Konsentrasi Giberelin (GA_3) terhadap Pertumbuhan Kailan (Brassica oleracea L. Var alboglabra) pada Berbagai Media Tanam dengan Hidroponik Wick System. 2018, Jurnal Biologi Universitas Andalas, 6(2): 63-70.
11. Makmur, E., Hunnazah, M., & Harahap, I. Antibacterial Activity of Ethanol Extract of Kale Leaves (Brassica oleracea var. sabellica) against Escherichia coli and Staphylococcus aureus. 2021, Photon: Jurnal Sain dan Kesehatan, 12(1), 67-77.
12. Matos, F. S., Freitas, I. A. S., Pereira, V. L. G., Pires, W. K. L. Effect of Gibberellin on Growth and Development of Spondias tuberosa Seedlings. Rev. Caatinga, 2020, Mossoró, 33(4): 1124-1130.
13. Mazed, H. E. M. 12. Effect of gibberellic acid on the growth and yield of cabbage (Brassica oleracea var. capitata L.). 2015, International Journal of Applied Research. 01. 16-19.
14. Miceli, A., Moncada, A., Sabatino, L., & Vetrano, F. Effect of Gibberellic Acid on Growth, Yield, and Quality of Leaf Lettuce and Rocket Grown in a Floating System. 2019 Agronomy, 9(7): 1-22.
15. Mutryarny, E and Lidar, S. Respon Tanaman Pakcoy (Brassica rapa L.) Akibat Pemberian Zat Pengatur Tumbuh Hormonik. 2018, Jurnal Ilmiah Pertanian 14(2): 29-34.
16. Netam, J. L., and Sharma, R. Efficacy of Plant Growth Regulators on Growth Characters and Yield Attributes in Brinjal (Solanum melongena L.) Cv. Brinjal 3112. 2014, IOSR Journal of Agriculture and Veterinary Science 7(7): 27-30.
17. Nweze, C. C., Abdulganiyu, M.G., Erhabor, O.G. Comparative analysis of vitamin C in fresh fruit juice of malus domestica, citrus sinensi, ananas comosus and citrullus lanatus by idiometric titration. 2015, International Journal of Science, Environment and Technology 4 (1): 17-22
18. Oktaviani, N. A., Syarifah, Apriliani, I. Pengaruh Tingkat Pemberian ZPT Giberelin (GA3) terhadap Pertumbuhan Tanaman Sawi (Brassica juncea L.) secara Hidroponik pada Sumbu Wick System, 2021, Prosiding Seminar Nasional, Sains dan Teknologi Terapan. 4(1): 244-254.
19. Onggo, D. T. M. and Kusumiyati.. Pengaruh Berbagai Konsentrasi dan Metode Aplikasi Hormon GA3 Terhadap Pertumbuhan dan Hasil Tanaman Brokoli Kultivar Lucky di Lembang. 2017, I J A S, 7(2): 5-9.

20. Ouzounidou, G. Ilias, I., giannakoula, A., Papadopoulou, P. Comparative Study on Effects of Various plant Growth Regulator on Growth, Quality and Physiology of Capsicum annum L. 2010, Pakistan Journal of Botany 42(2): 805-814.
21. Podsedek A. Natural Antioxidant and Antioxidant Capacity of Brassica Vegetables. 2007, Food Science Technology, 40(1): 1-11.
22. Riko, Aini, S. N., Asriani, E. Aplikasi Berbagai Konsentrasi Giberelin (GA3) terhadap Pertumbuhan Tanaman Kailan (Brassica oleracea L.). 2019, Journal Horticultura. 29(2): 181-188.
23. Šamec, D., Urlić, B., Salopek-Sondi, B. Kale (Brassica oleracea var. acephala) as a superfood: Review of the scientific evidence behind the statement. 2019 Crit. Rev. Food Sci. Nutr., 59, 2411–2422.
24. Sardoei, A. S., Shahadadi, F., Vakili, M. A., Gholamshahi, S, Effects of Gibberellic Acid (GA3) on Phenolic Compounds and Antiradical Activity of Marigold (Calendula officinalis), 2014, International Journal of Biosciences, 4(3): 1-8.
25. Singh, S. dan Singh, T. 2021. Effect of Gibberellic Acid on Growth, Yield and Quality Parameters of Chilli (Capsicum annum L.). Journal of Pharmacognosy and Phytochemistry. 8(2): 2021-2023.
26. Soad, M., Lobna S. T., Farahat, M. Vegetative Growth and Chemical Constituents of Croton Plants as Affected by Foliar Application of Benzyl Adenine and Gibberellic Acid. 2010, Journal of American Science, 6(7): 126-130.
27. Treutter, D. Managing Phenol Contents in Crop Plants by Phytochemical Farming and Breeding Visions and Constraints. 2010, International Journal of Molecular Sciences, 11(3): 807–857.
28. Wareing, P.F. and Pillips, I. D. J. The Control of Growth and Differentiation in Plants. Toronto: Pergamon Press. 1978.
29. Wen, Y., Su, S., Ma, L., Wang, X. Effects of gibberellic acid on photosynthesis and endogenous hormones of Camellia oleifera Abel. in 1st and 6th leaves. 2018. Journal of Forest Research, 23(5): 309–317.
30. Wu, Y., Gong, W. and Yang, W, Shade Inhibits Leaf Size by Controlling Cell Proliferation and Enlargement in Soybean. 2017Scientific Reports, 7(1): 1-10.