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Role of Gibberellic Acid (GA_3) in enhancing growth and yield of hydroponically grown lettuce (*Lactuca sativa* L.)

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Abstract

Gibberellin (GA_3) is a growth hormone that is essential in plant physiological processes. At present, gibberellins are employed in commerce to enhance the physical traits and yield of various vegetable, horticultural, ornamental, and medicinal crops. This research aimed to study the effects of gibberellin application on the morphological characteristics and yield of lettuce plants. The study was arranged in a Completely Randomized Design (CRD) with six treatments of gibberellins concentration with a hydroponic system, namely: G0 = control, G1 = 20 ppm, G2 = 40 ppm, G3 = 60 ppm, G4 = 80 ppm, and G5 = 100 ppm, and each treatment consisted of four replications. This research was conducted in May–July 2023 at the Kemuning Greenhouse, Belu Regency, East Nusa Tenggara. GA_3s were sprayed twice at 2 weeks after sowing and 1 week after transplanting. The results showed that the highest GA_3 concentration (100 ppm) had a negative impact on plant morphology and yield, producing the smallest number of leaves, too long stem, pale green leaves, and the lowest fresh weight. Meanwhile, GA_3 concentration of 40 ppm resulted in the highest number of leaves. However, this increase is not directly proportional to the fresh weight of the leaves. GA_3 concentration of 40 ppm resulted in the best and most efficient yield of leaves and fresh weight, making it highly recommended.

INTRODUCTION

Currently, the need for healthy vegetables is increasing, creating an impetus to improve plant productivity efficiently and environmentally friendly. Consumer awareness of a healthy lifestyle with high vegetable intake can reduce the risk of various diseases. The impact of this phenomenon is that Indonesia imported around 1 million tons of vegetables in 2022. This figure has increased by around 3.3% compared to 2021, and became the largest vegetable import in the last five years (BPS 2022). Addressing the increasing demand for vegetables necessitates improving crop yields through environmentally sustainable, cost-effective, and simple methods. These objectives can be accomplished through a variety of approaches including genetic enhancement, inventive farming techniques, grafting, beneficial microorganisms for growth promotion, and growth regulators (Miceli et al., 2016).

GA_3s are growth regulators whose roles are to stimulate stem growth, affect flowering initiation, stimulate germination, and stimulate the formation and growth of fruit development (Salisbury and Ross, 1992). Giving GA_3s at different concentration levels can affect the response shown by plants. Numerous studies have concentrated on utilizing this plant hormone to enhance both the yield and quality of various crop

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species (Bultynck et al., 2004; Dayan et al., 2012; Frasetya et al., 2018; Gelmesa et al., 2010; Khan M.M.A et al., 2006; Miceli et al., 2019; Pal et al., 2016; Riko et al., 2020; Setiawan et al., 2014; Triani et al., 2020; Vetrano et al., 2020). Gibberellic acid (GA_3) plays a crucial role in several metabolic pathways that affect leaf emergence, including the synthesis and breakdown of chlorophyll, the translocation of assimilates, and the metabolism and distribution of nitrogen.

As mentioned earlier, these impacts can vary significantly depending on the species, growth phases, concentration and application methods, and farming practices. One of the vegetables that may produce a positive response to gibberellic acid application is lettuce. Lettuce contains a wealth of essential dietary minerals crucial for human health, including iron (Fe), zinc (Zn), calcium (Ca), phosphorus (P), magnesium (Mg), manganese (Mn), and potassium (K). It also contains bioactive compounds that support overall well-being (Kim et al., 2016). Despite this nutritional richness, limited human clinical studies have explored the role of lettuce consumption in disease prevention. Studies examining the potential nutrients in lettuce linked to decreased colorectal cancer risk found no association with calcium, vitamin E, and folate, but did find a notable correlation with β-carotene. The beneficial health effects of lettuce are largely attributed to carotenoids and other plant-based compounds like phenolic compounds (Lopez et al., 2014).

The use of GA_3 as a growth regulator for lettuce is one of the approaches drawing attention in related research. Currently, the existing research findings regarding the impacts of GA_3 applications on leafy vegetables cultivated in hydroponic systems are limited. The quality of lettuce is closely related to the appearance of the leaves and their nutritional value (vitamins A, C, E, manganese, phosphorus, sodium, nitrate, antioxidants, etc.). Therefore, this research sought to evaluate the influence of incorporating gibberellic acid (GA_3) into the mineral nutrient solution on the growth, yield, and quality of leaf lettuce in a hydroponic system. The study is distinctive in its concentration on leafy vegetables, particularly investigating how the addition of GA_3 to the nutrient solution affects the growth and quality of hydroponically grown leaf lettuce.

MATERIALS AND METHODS

Location and material

The location was conducted at the Kemuning Green House, Belu Regency, East Nusa Tenggara Province at an altitude of 300 meters above sea level. Research was conducted in May–July 2023. The materials used consisted of curly lettuce seeds (var. Caipira), GA_3s from Indo Biotech Agro, rockwool, water and plant nutrients. While the tools used were 2.5" PVC pipe, pump, nutrient storage tank, EC meter, netpot, pH meter, seedling container, 5 mm diameter hose, knee (L PVC pipe) measuring 1", 1.5" PVC pipe. (PVC pipe cover), 1 in. pipe, pipe glue, analytical balance, oven, label paper, socket outlet, stationery, drill, solder and hacksaw.

The research was arranged in a Completely Randomized Design (CRD). The experiment involved varying concentrations of GA_3 across six levels, consisting of G0 (control), G1 (20 ppm), G2 (40 ppm), G3 (60 ppm), G4 (80 ppm), and G5 (100 ppm). Each treatment was replicated four times, resulting in a total of 24 experimental units. Each experimental unit comprised two plant samples, yielding a total of 48 experimental units.

Lettuce cultivation

The experiment began with carrying out hydroponic cultivation including: a) seeding; The seeds were sown on rockwool media slightly submerged in water. Planting holes were made with a distance of 2.5 cm, and each planting hole consisted of one lettuce seed. After three days the seedling appeared, then the nursery was moved to a location exposed to sunlight; b) transplanting; After the plants were 14 days old after sowing, the seedlings were transferred to netpots and transferred to a hydroponic installation with the NFT system, and the first GA_3 application was carried out; c) providing nutrition; Provision of nutrients to plants was given twice. Half a dose of AB Mix solution diluted with water was given during the initial growth, when the first leaves appeared to avoid stunting. The second nutrient solution was given when transplanting and put in the net pot. The nutrient solution was prepared by dissolving stock A, stock B, and water in a ratio of 5 ml : 5 ml : 1 liter of water to obtain EC < 1000 μS/cm at early plant development. Furthermore, the EC solution was controlled every week according to plant needs. The concentration of AB Mix used in this study was 1000–1200 ppm; d) GA_3 application; GA_3 hormone was diluted at a concentration of 0 ppm, 20 ppm, 40 ppm, 60 ppm, 80 ppm, 100 ppm and dissolved with water to a volume of 1000 ml. GA_3s were sprayed twice at 2 weeks after sowing and 1 week after transplanting; e) harvesting; Harvesting was done at 55 days after sowing.

Statistical and principal component analysis

The experimental setup included four replications for each concentration of GA_3, where each leafy vegetable was randomly assigned to one of four distinct experimental setups. A one-way ANOVA test was conducted to assess the impact of GA_3 concentrations on each vegetable growth variable. Variations in means were evaluated using Tukey's multiple range test with a significance level of 5%. Principal component analysis (PCA) was employed to investigate the correlation between different concentrations of GA_3 and the agronomic and qualitative characteristics of leaf lettuce. The input matrix for this analysis comprised growth variables such as plant height, root length, total fresh weight of the plant, total dry weight of the plant, stem diameter, fresh weight of leaves, and the number of leaves. According to Wildani et al. (2021), when dealing with complex research variables or a large number of treatments, performing PCA enables the interpretation of varied datasets through graphical representations like plots. The ideal number of principal components (PCs) was identified based on retaining components with eigenvalues exceeding 1.0. This was followed by plotting these PCs to analyze correlations among the variables in the dataset. In particular, the original variables were mapped onto the space defined by the first and second PCs to identify correlations among them. The PCA was performed using Orange 2023 version 3-3.35.0-Miniconda-x86_64 (Demsar et al., 2013).

RESULTS AND DISCUSSION

During the growth period, observations were made four times from the first to the fourth weeks after transplantation for plant height and number of leaves. At harvest time, observations were made on the number of leaves, fresh weight, dry weight, stem length, root length and whole leaf weight. At the time of transplantation (2 weeks after sowing), the first spraying of GA_3 was also carried out, as well as the first observation of the height and number of plants so that the difference in growth after the application of GA_3 would be measurable. The pH of the water ranged from 6–6.5. The pH measurement of the water was carried out once every three days.

Morpho–physiological characteristics and yield of lettuce

Giving GA_3 had a significant effect on the height of lettuce plants. The highest plant height were found in the pants treated with 100 ppm GA_3 (14.75 cm), followed by those treated with 80 ppm (12.75 cm), 60 ppm (11.62 cm), 440 ppm (11.25 cm), 20 ppm (12.06 cm) and 0 ppm (11.00 cm). The second observation on plants was carried out 2 weeks after transplanting (WAT). After 2 weeks of spraying GA_3, the impact on plant height growth was much better. The highest plant height were found in the pants treated with GA_3 100 ppm (22.88 cm), followed by thise treated with 80 ppm (19.88), 60 ppm (18.81 cm), 40 ppm (13.00), 20 ppm (13.81 cm) and 0 ppm (10.12 cm). The increase in plant height at the age of 1 WAT to 2 WAT at 100 ppm GA_3 was 8.13 cm. Further observations on plants were made 3 WAT. All plants experienced an increase in plant height, but the highest plant height was found on the plants treated with GA_3 100 ppm (31.75 cm), followed by those treated with 80 ppm (27.25 cm), 60 ppm (24.62 cm), 40 ppm (16.75 cm), 20 ppm (17.38cm), and 0 ppm (14. 12cm). Then the last observation for plant height was made at the age of 4 WAT, in which the highest plant height was foubnd in the plants treated with 100 ppm GA_3 (33.62cm) but not significantly different from those treated with 80 ppm (31.00 cm) and 60 ppm (32.00cm) (Table 1).

As a leafy vegetable, the number of lettuce leaves is the most important variable in this study. Spraying gibberellins on lettuce leaves had a very significant effect on the number of lettuce leaves. The highest number of leaves at 2 WAT was found in the plants treated with GA_3 60 ppm (10.25 leaves); at this concentration, there were 5.25 leaves more than the control (6 leaves). When the plants were 2 WAT, the second and last spraying of GA_3 was also carried out. At 3 WAT, the highest number of leaves was found in the plants treated with 80 ppm (16.25 strands) at this concentration the number of leaves was 6 more than the control (9.50). The number of

Table 1. Morphological variables of leaf lettuce plants treated with different concentrations of gibberellic acid (GA3)

GA3 (ppm)	Plants height (cm)				Number of leaf			
	1 WAT	2 WAT	3 WAT	4 WAT	1 WAT	2 WAT	3 WAT	4 WAT
0	11.00±0.00 c	10.12±0.00 c	14.12±0.48 c	16.00±0.41 c	4.12±0.00 c	6.00±0.00 c	9.50±0.25 c	15.00±0.00 d
20	12.06±0.00 bc	13.81±0.00 bc	17.38±0.31 c	23.31±0.43 b	5.12±0.00 ab	9.00±0.00 c	13.00±0.48 b	21.88±0.00 bc
40	11.25±0.00 bc	13.00±0.00 c	16.75±0.65 c	24.06±1.41 b	5.25±0.00 a	9.00±0.00 c	13.00±0.50 b	25.75±0.00 ab
60	11.62±0.00 bc	18.81±0.00 ab	24.62±0.25 b	32.00±0.80 a	5.00±0.00 ab	10.25±0.00 a	15.62±0.00 a	26.75±0.65 a
80	12.75±0.00 b	19.88±0.00 a	27.25±1.19 ab	31.00±0.59 a	4.88±0.00 abc	10.12±0.00 a	16.25±0.25 a	24.38±0.25 ab
100	14.75±0.00 a	22.88±0.00 a	31.75±1.19 a	33.62±5.95 a	4.38±0.00 bc	9.75±0.00 ab	15.62±0.48 a	20.88±0.50 c

Remarks: Data within a column followed by the same letters are not significantly different at $p \leq 0.05$ according to Tukey's test; ±standard deviation; WAT: Week after Transplanting.

Table 2. Yield and morphological variables of lettuce plants treated with different concentrations of gibberellic acid (GA3)

	GA3 (ppm)					
	0	20	40	60	80	100
Whole leaf weight (g)	80.62±0.29 a	62.12±1.38 a	62.12±0.71 a	49.88±1.49 b	46.25±0.63 b	42.75±1.38 b
Number of leaves harvested	22.25±9.54 c	28.00±15.33 b	34.00±5.26 a	32.25±6.79 a	31.38±5.69 a	29.25±8.46 b
Stem length (cm)	5.19±0.37 e	18.00±1.58 d	21.81±2.59 c	27.88±4.87 c	36.38±3.01 b	47.50±4.95 a
Fresh weight (g)	112.25±9.94 a	88.38±6.21 b	102.62±6.62 a	86.38±10.78 b	84.75±11.90 b	84.12±9.11 b
Root length (cm)	31.50±1.29 a	28.25±0.96 b	22.88±2.01 c	25.38±1.11 d	16.62±0.48 e	11.25±1.26 f
Dry weight (g)	7.73±1.01	5.78±1.49	7.82±1.62	7.02±0.63	7.30±0.41	6.87±1.60
Leaf weight percentage (%)	71.78±4.43 a	70.41±15.05 a	60.16±2.73 ab	57.53±1.08 ab	54.60±2.75 b	51.14±4.61 b

Remarks: Data within a row followed by the same letters are not significantly different at $p \leq 0.05$ according to Tukey's test; ±standard deviation; Variables without letter notation are not affected by treatments.

leaves increased at 4 WAT, in which the plants treated with 60 ppm (26.75) experienced an increase of 11.13 strands from the previous week and 11.75 more than the control. Leaf weight of lettuce plants was also counted. Gibberellin application did not significantly affect leaf weight. Meanwhile, the highest plant fresh weight was found in control plants (112.25 g), followed plants treated with by 20 ppm (62.12 g) and 40 ppm (62.12 g). Giving GA_3 did not have a significant effect on root length where control plants (31.50 cm) had longer roots than the other treatments. The percentage of leaf composition of all plant parts in the control was also the highest, namely 71.78%. The higher the gibberellin given, the lower the leaf composition in lettuce (Table 2). On the other hand, 100 ppm gibberellins had a significant effect on plant length (47 cm) compared to control (5.19 cm) (Table 2).

Several experiments have investigated the impact of applying gibberellic acid (GA_3) at varying concentrations on multiple crop species (Dayan et al., 2012; Gelmesa et al., 2010; Khan et al., 2006; Maharani et al., 2018; Miceli et al., 2019; Pal et al., 2016; Pertiwi et al., 2014; Riko et al., 2020; Suherman, 2017; Triani et al., 2020). These studies reveal that the hormonal requirements and plant responses can differ significantly across species and growth stages (Miceli et al., 2019). Direct application of gibberellins to the planting medium, such as mineral nutrient solutions, allows absorption through the roots. However, gibberellins are commonly applied via foliar spraying because these hormones are naturally synthesized in young leaves and subsequently transported throughout the plant both acropetally and basipetally (Dayan et al., 2012).

According to Dayan et al. (2012), GA and Auxin are each essential for cambial activity (meristematic layer). Endogenous IAA and GA hormones are available in the plant itself with their respective quantities. With the addition of exogenous GA_3, there will be more GA_3 hormone layers than IAA, in which according to Digby et al. (1966), the number of IAA and GA layers determines whether xylem or phloem tissue is produced; a high IAA:GA ratio favors xylem formation, while a low IAA:GA ratio supports phloem production.

This means that application of exogenous GA_3 on the leaves will be more effective than on the roots because it will be immediately transported by the phloem. In this experiment, the feasibility of supplying GA_3 to leaf lettuce plants with a hydroponic system was tested and their effects on growth and yield were evaluated.

Lettuce plant height is directly proportional to the increase in GA_3 concentration. At each observation, the plant height at a concentration of 100 ppm GA_3 increased rapidly so that the stems became curved and not sturdy with a pale green color on the leaves of the lettuce plant (Figure 1 and 2). It is known that GA_3 treatment promotes cell division and cell enlargement, which can lead to stem elongation (Miceli et al., 2019; Riko et al., 2020). GA_3 promotes cytogenesis and cell elongation in stems and also plays an important role in plant growth and development. GA_3 is able to stimulate stem elongation by inducing the formation of amylase enzymes, which hydrolyze starch, thereby increasing sugar levels and osmotic pressure of cell fluids, then water enters the cells and elongation occurs, thus increasing stem length and diameter (Setiawan et al., 2014).

Below concentration of 100 ppm, the presence of GA_3 was effective as a plant growth promoter and yield enhancer, especially at 40 ppm. During the growth period, the number of lettuce leaves showed

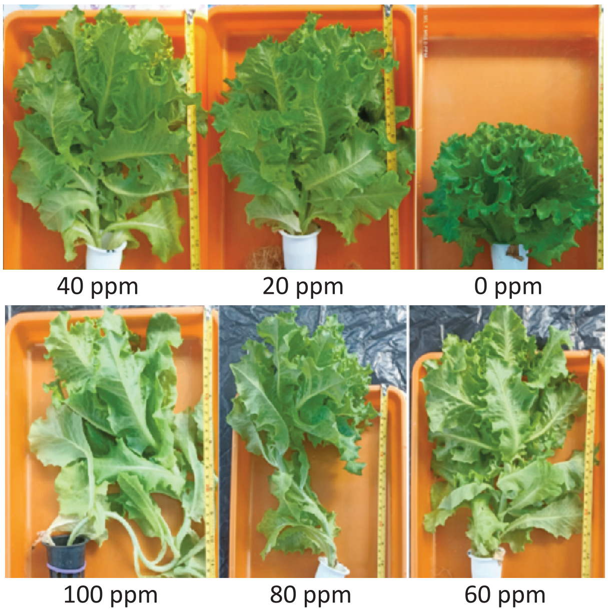

Figure 1. Plants of steam and leaf lettuce on GA3s various contretation

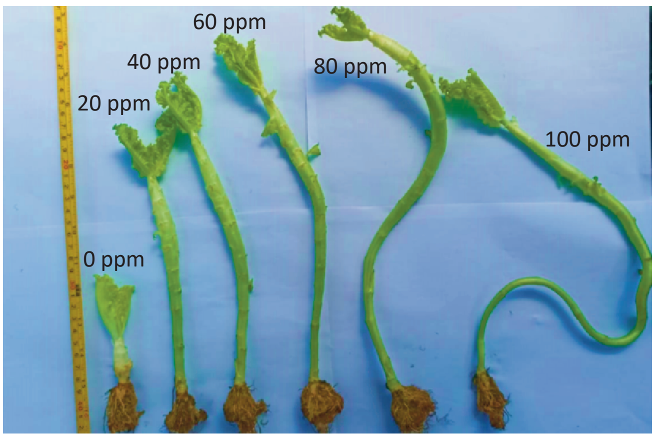

Figure 2. Stem lenght of lettuce treated with different concentrations of gibberellic acid (GA3)

different responses at various concentrations of GA_3. GA_3 at the concentrations of 60 and 80 ppm always managed to result in the highest number of leaves, but at harvest, the highest number of leaves was at 40 ppm, which was not significantly different from those at 60 and 80 ppm compared to the control (34, 32.25, 31.38 and 22.25, respectively). Spraying gibberellins on plant leaves is effective in increasing the number of leaves because when the stomata open, gibberellins enter the stomata and make gibberellin absorbed more quickly (Frasetya et al., 2018; Pertiwi et al., 2014).

The application of gibberellins did not affect leaf fresh weight and total fresh weight. Control plants had the highest leaf weight and total fresh weight of 80.62 and 112.25, respectively. Leaf fresh weight and total fresh weight were not directly proportional to the number of leaves; this was due to the fact that the leaf area of lettuce was even larger in the control. Unfortunately, in this study, leaf area measurements had not been carried out. Based on the observation on plant height, it can be seen that the higher the concentration of gibberellins used, the narrower and paler the leaf color will be. According to its function, gibberellin is a hormone that promotes cell division and cell enlargement, which can cause stem elongation, but at inappropriate or even higher concentrations can cause too fast cell division (Miceli et al., 2019; Ogasawara et al., 2001).

Rahayu et al. (2018) conducted experiments on the application of GA_3 (control, 5 ppm, 10 ppm, 15 ppm and 20 ppm) on the New Grand Rapids variety of lettuce, showing the number of leaves in the control (7.18 strands) and not significantly different from the other treatments. The application of GA_3 10-6 M (0.004 ppm) resulted in the highest number of leaves (9.1 pieces) compared to the control (8.0) on lettuce plants var. Crispa (Miceli et al., 2019). Even at a higher concentration of GA_3, the number of lettuce leaves in this study–which employed the same curly lettuce (var. Caipira)–was 34.00 strands (40 ppm), significantly more than in previous study.

According to Sugiura et al. (2016), species with high endogenous GA levels are characterized by a higher leaf : root or shoot : root ratio compared to genotypes that have low endogenous GA levels. Likewise, the addition of exogenous GA_3 can change the morphological properties of plants and encourage the allocation of biomass to leaves. It has been proven that GA is involved in the allocation of biomass to leaves; plants with the addition of exogenous GA have morphology and physiology similar to plants that have high endogenous GA_3 (Bultynck et al., 2004).

Control plants showed the highest values of leaf weight and total fresh weight because the basic nutrient composition (AB Mix) was already in the optimum condition required by the plant, so that without the addition of GA_3, the leaf weight remained high but was outnumbered by giving 40 ppm GA_3. The use of stock A, stock B, and water in this study was in a ratio of 5 ml : 5 ml : 1 liter of water, with EC < 1000 µS/cm and pH at 6–6.5.

Foliar application of gibberellins has gained widespread adoption due to their natural synthesis in young leaves, which are then transported throughout the plant both upwards and downwards. Vegetables treated with GA_3, especially leafy ones, show accelerated stem growth. In lettuce, this results in pale green, narrow leaves, while rocket plants exhibit rapid flower development. Research indicates that GA_3 treatments stimulate cell division and enlargement, promoting stem elongation (Miceli et al., 2019). Exogenous gibberellins can alter the rosette structure of plants or regulate flowering in rosette species by rapidly enlarging differentiated tissues. GA are involved in cell division and stem elongation, and they can induce flowering when they reach the shoot apex (Miceli et al., 2019).

Plants treated with GA_3 also demonstrate increased activity of carbonic anhydrase (CA), a critical enzyme for photosynthetic CO_2 fixation that facilitates CO_2 hydration and is closely associated with chloroplasts (Maciel et al., 2022). This ensures ample CO_2 supply at the fixation site, leading to higher net photosynthetic rates and increased accumulation of dry mass. Yuan and Xu (2001) observed that applying GA_3 to broad bean leaves not only increased the net photosynthetic rate but also enhanced stomatal conductance while reducing intercellular CO_2 partial pressure. The beneficial effects of GA_3 supplementation are especially useful for densely cultivated leafy vegetables in hydroponic floating systems. This approach helps mitigate problems such as self-shading and restricted air circulation within the canopy, which can otherwise impair light interception and CO_2 availability.

Principal Components Analysis

The Principal Component Analysis (PCA) results identify two principal components (PCs) with

Table 3. Correlation of variables to the factors of the principal components analysis (PCA) based on factor loadings

Variables	PC1	PC2
Stem length	-0.959	-0.195
Root length	0.855	0.436
Whole fresh weight	0.579	-0.472
Dry weight	0.268	-0.771
Plants height	-0.917	-0.056
Whole leaf weight	0.889	-0.172
Number of leaves	0.588	0.071

Remarks: Values in bold within the same factor indicate the variable with the largest correlation.

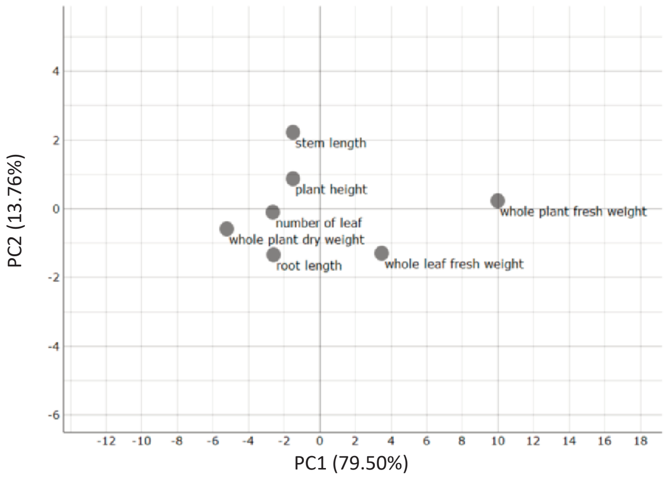

Figure 3. Plot of loadings of morpho–physiological and quality characteristics of lettuce plants).

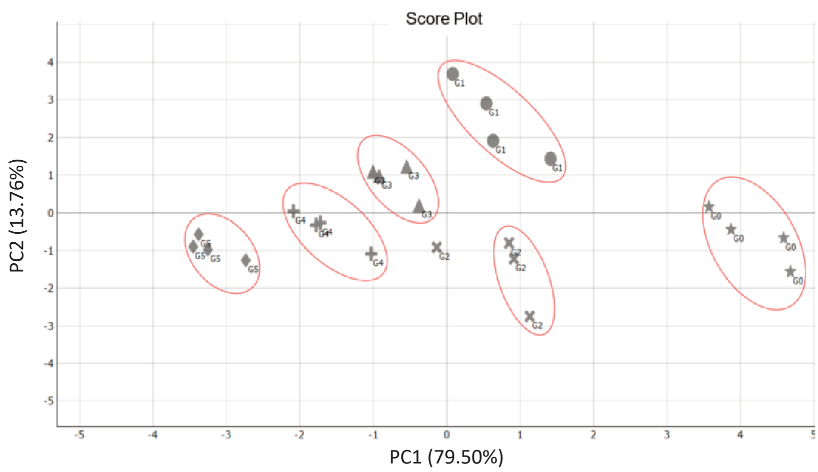

Figure 4. Scores (trials) formed by the two principal components from the PCA analysis. G0, G1, G2, G3, G4 dan G5: GA3 concentration: 0 ppm, 20 ppm, 40 ppm, 60 ppm, 80 ppm, and 100 ppm, respectively

eigenvalues exceeding 1, as presented in Table 3. These PCs account for 79.50% and 13.76% of the total variance, respectively. Consequently, the seven initial variables can be effectively represented as a linear combination of these two PCs, capturing 93.26% of the overall variance. The first principal component (PC1) is primarily associated with plant height, root length, total plant fresh weight, whole leaf fresh weight, and the number of leaves. In contrast, the second principal component (PC2) is predominantly related to plant dry weight, as shown in Table 3.

The relationship between the two PCs can be seen in the biplot (Figure 3). The separation of the various GA_3 concentrations sprayed on lettuce leaves can be seen in the score plot (Figure 4), where the six groups can be significantly distinguished. The response of lettuce plants was different when given various concentrations of GA_3. Treatment scores g1 and g3 are on the positive part of the PC2 axis, while the rest are on the negative PC2. G1 and G2 are positively related to PC1, while G3, G4 and G5 are negatively related to PC1. On the other hand, the G0 score has the highest PC1 value, this is because giving GA_3 has a negative relationship to the whole leaf weight (g), fresh weight (g) and root length (cm) variables. Conversely, an increase concentration of GA_3 is positively related to stem length, number of leaves, and plant length.

Principal Component Analysis (PCA) revealed that administering various doses of gibberellic acid (GA_3) significantly influenced the morphology of lettuce, with differing impacts on various morphological variables. Moreover, the plant's response to GA_3 treatment was found to be contingent on the plant species and GA_3 concentrations, suggesting that hormone requirements and responses are species-specific. These findings underscore the necessity for more comprehensive research on GA_3 concentration, particularly in leafy vegetables, and suggest the potential for exploring lower GA_3 concentration. According to Micel et al. (2019), the observed traits contributed variably, resulting in their distribution away from the PCA diagram's central point. Nonetheless, clusters of similar observations differed, likely due to the use of distinct genotypes.

CONCLUSIONS

GA_3 application in hydroponically grown lettuce significantly influences growth and yield. Excessive concentration (100 ppm) negatively impacts morphology and yield, while 40 ppm GA_3 proves effective in promoting growth, resulting in a larger number of leaves.

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