Application of multivariate and univariate data analysis to evaluate the response of chili (*Capsicum annuum* L.) to various plant growth regulators

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Abstract. Chili peppers (*Capsicum annuum* L.) are a strategic horticultural commodity in Indonesia that greatly influences consumption patterns. This commodity also affects market dynamics. One effective approach to increasing productivity is through the application of plant growth regulators (PGRs). This study evaluated the effects of two types of PGR: Atonik, which contains nitrophenol compounds (sodium para-nitrophenolate, sodium ortho-nitrophenolate, and sodium 5-nitroguaiaolate), and Agrogibb, which contains gibberellic acid (GA_3), as well as a combination of the two. This experiment used TM999 curly red chili peppers. This experiment was designed to assess growth parameters and crop yield. Data analysis was performed using univariate and multivariate approaches. Multivariate analysis (PCA) showed a clear separation between treatments. Univariate analysis (ANOVA and Tukey's post hoc test) confirmed that the 60 mg/L Agrogibb treatment provided a significant increase in growth in several parameters observed. The results of the univariate and multivariate tests reinforced each other, showing that Agrogibb had a better and more dominant effect on chili plants. Correlation analysis revealed a strong positive relationship between vegetative traits, such as plant height, number of leaves, and branch development. These parameters can serve as reliable predictors in determining chili crop yield performance. This approach demonstrates the potential of statistical integration in optimizing agricultural productivity and supporting chili production development in Indonesia.

1 Introduction

Chili peppers (*Capsicum annuum* L.) are one of Indonesia's strategic horticultural commodities. Chili peppers are important not only for their contribution to food security and household economies, but also for their powerful role in shaping the national culinary identity. Chili consumption in Indonesia has been increasing every year. Fluctuations in chili production are highly sensitive to market dynamics and vulnerable to triggering food commodity inflation [1]. Enhancing productivity is essential to fulfill the ever-increasing demand.

One of the most effective agricultural approaches to improving chili growth and yield is the application of plant growth regulators (PGRs). PGRs play a crucial role in regulating various plant physiological processes, including cell division, cell elongation, flowering, and fruit development [2, 3]. In the context of chili cultivation, the use of plant growth regulators has been proven to improve the growth and yield of chili plants. The use of PGRs also has a significant impact on plant height, number of leaves, branches, flowers, and fruits [4].

Atonic, a plant growth regulator (PGR) based on nitrophenolate compounds, including sodium para-nitrophenolate, sodium ortho-nitrophenolate, and sodium 5-nitroguaiaolate, has been widely used on a variety of crops. Atonic has been demonstrated to enhance vegetative growth, nutrient uptake, and plant resistance to abiotic stress [5]. Agrogibb, which consists of gibberellic acid (GA_3), is known to play a role in stem elongation, fruit size increase, and regulation of endogenous hormone balance [4, 6]. The combination of these two PGRs has the potential to create a synergistic effect on the growth and productivity of curly red chili peppers.

This study was conducted to evaluate the response of chili plants to Atonik, Agrogibb, and a combination of both treatments. The response was assessed by measuring growth parameters and crop yield. Data analysis was performed using univariate and multivariate data analysis (MVDA) approaches. Multivariate approaches such as principal component analysis (PCA) and partial least squares (PLS) allow for an integrated assessment of the effects of PGR on various growth variables simultaneously. This provides a deeper understanding of complex physiological responses [7]. The integrated use of univariate and multivariate analysis offers a more holistic perspective in evaluating the effects of treatment. This enabled a comprehensive interpretation of the growth dynamics of chili peppers.

This experiment underscores the important role of statistics and multivariate analysis in optimizing agricultural production, particularly in developing more efficient and sustainable chili production systems in Indonesia. By understanding the physiological response of plants through a combination of analytical techniques, this study is intended to provide a scientific basis for the application of PGR in increasing chili productivity on a field scale.

2 Materials and methods

The experiment was conducted using a completely randomized design (CRD) with one treatment factor (plant growth regulators (PGRs)). This study used TM999 curly red chili variety. TM999 is a variety of curly red chili pepper that is widely cultivated by farmers in Indonesia. The treatments consisted of four levels, namely Z0 (control), Z1 (Atonik 2 mL/L), Z2 (Agrogibb 60 mg/L), and Z3 (Atonik 1 mL/L + Agrogibb 30 mg/L). Each treatment was repeated four times to obtain representative data for statistical analysis. PGR application was carried out at 40, 50, and 60 days after planting (DAP). PGR was applied by spraying using a hand sprayer, directed evenly over the entire plant. Spraying was carried out until the surface of the leaves and stems were thoroughly wet so that hormone absorption can take place optimally.

The parameters observed in this study included various aspects of vegetative and generative growth of plants, namely plant height at 5 to 14 weeks after planting (WAP), number of dichotomous branches at 5 to 14 WAP, and number of leaves at 5 to 14 WAP. In addition, the time of 50% flowering, fresh biomass weight, dry biomass weight, cumulative number of fruits per plant, longest fruit length, and fruit weight per plant were also observed.

Agronomic data were analyzed using Analysis of Variance (ANOVA) to determine the effect of treatment on individual parameters. If ANOVA revealed significant differences, Tukey's post hoc test at the 5% level was performed to distinguish between treatments that were significantly different. In addition, Pearson's correlation analysis was employed to evaluate the relationship between growth variables and yield. To support a more comprehensive physiological interpretation, multivariate analysis was performed using MetaboAnalyst 6.0. Principal Component Analysis (PCA) was then conducted to identify separation patterns between treatments. PCA grouped samples based on their response to PGR application.

3 Result and discussion

PCA showed clear variation between treatments Z0 (control), Z1 (Atonik 2 mL/L), Z2 (Agrogibb 60 mg/L), and Z3 (Atonik 1 mL/L + Agrogibb 30 mg/L) when all growth and yield parameters were analyzed simultaneously. The first principal component (PC1) explained most of the total diversity, namely 86.9%, while the second principal component (PC2) contributed 9.6% and described a smaller additional variation. Each treatment formed a separate cluster which indicated strong multivariate differentiation. Treatments Z2 and Z3 appeared close to each other which indicated a similar response pattern. Treatments Z2 and Z3 appeared to be better than Z0 and Z1 which were in opposite positions. Z2 and Z3 were located on the positive side of PC1 which described higher growth and yield performance. Z0 and Z1 were on the negative side of PC. It suggested lower response values (**Fig. 1.**).

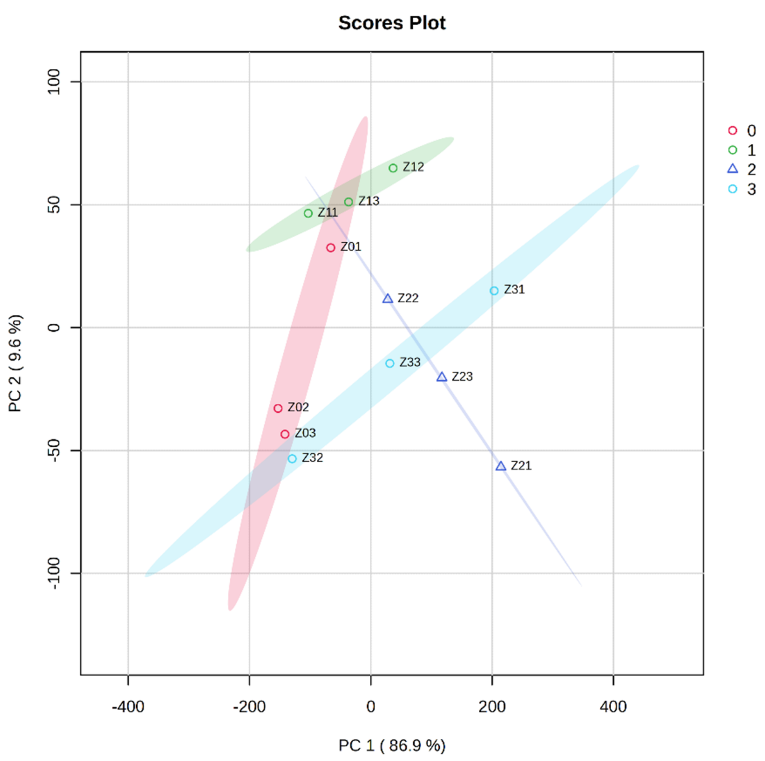

Fig. 1. Score plot of multivariate analysis results based on 36 parameters covering aspects of vegetative and generative growth. PERMANOVA analysis yielded F = 2.95; R^2 = 0.52522; and p-value = 0.079 (based on 999 permutations).

The first principal component (PC1) explained 86.9% of the total variation. This PCA was strongly associated with yield traits, including fruit weight per plant, cumulative fruit number per plant, and fresh biomass. Meanwhile, the second principal component (PC2) contributed 9.6% of the variation. The second PCA was mainly represented vegetative growth traits, particularly the number of leaves at 9–14 weeks after planting (WAP). Number of leaves reflects differences in canopy vigor between treatments. Based on their position on the PC1 axis, treatments Z2 and Z3 were on the positive side. It indicated higher yield performance. Treatments Z0 and Z1 tended to be on the negative side of PC1 which indicated lower yields but relatively stronger vegetative growth (**Fig. 2.**).

The close positions of Z0 and Z1 indicated that plants in the control treatment and single Atonik treatment showed similar responses. On the other side, Z2 and Z3 were in the same group which indicated that both single Agrogibb and the Atonik + Agrogibb combination produced higher and more similar plant growth patterns and yields. This implied that Agrogibb has a more dominant effect on improving plant performance than single Atonik.

Atonik contains three active phenolic compounds, namely sodium para-nitrophenol, sodium ortho-nitrophenol, and sodium 5-nitroguaiacol, which function as plant metabolism activators. The combination of these three compounds stimulates root formation, increases growth vigor, and helps plants recover more quickly from stress. The effectiveness of Atonik in increasing crop yields has been consistently proven over many years on various important crops around the world. This synthetic bio stimulant from Japan contains 0.3% sodium para-nitrophenolate, 0.2% sodium ortho-nitrophenolate, and 0.1% sodium 5-nitroguaiacolate with water as the solvent [8]. Agrogibb 40 SL is a growth regulator containing 40 g/L of gibberellic acid (GA_3) as its main active ingredient. Giberellic acid (GA_3) is a plant hormone that functions as a growth regulator, stimulating cell division and elongation, breaking dormancy, and accelerating germination. GA_3 is also known to increase shoot growth, lengthen stem internodes, determine sex expression in some plants, and help stimulate flowering [9,10]. This hormone can be applied through various methods such as seed soaking, dipping, adding to the growing medium, or leaf spraying [11].

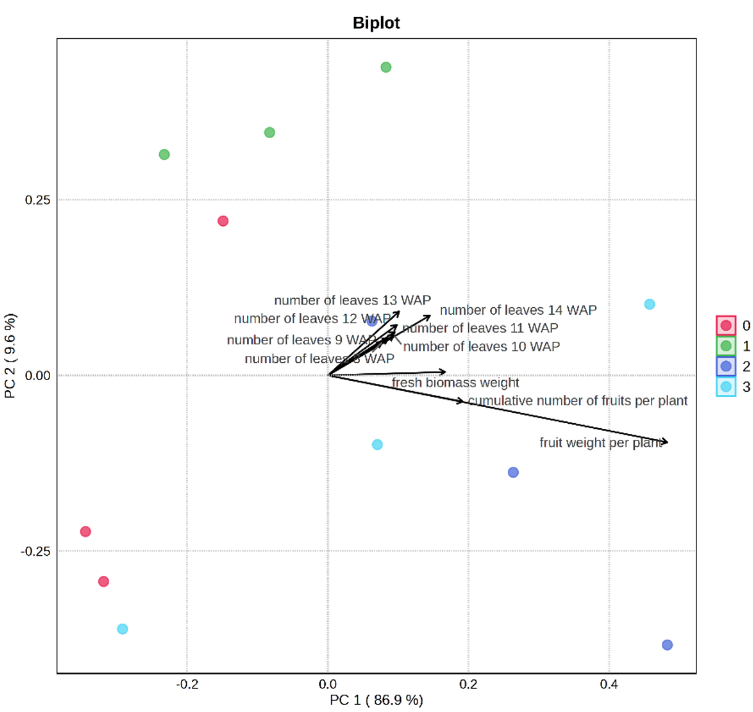

Fig. 2. Biplot of multivariate analysis results based on 36 parameters covering aspects of vegetative and generative growth.

Based on the results of ANOVA at the 5% level (**Table 1.**), 13 growth and yield parameters showed significant differences between treatments. These parameters included plant height at 8 to 14 MST, number of dichotomous branches at 10 to 14 MST, and longest fruit length. Further testing using Tukey's HSD showed that treatment Z2 (Agrogibb 60 mg/L) provided the most stable and significant response at various stages of growth. These findings indicate that this concentration of GA_3 was the most effective treatment in supporting the growth and development of chili plants. The results of this study are in line with other findings that indicate that the application of gibberellic acid (GA_3) does play a role in fruit development [12].

Table 1. ANOVA result from yield and growth parameters.

No	Parameters	F-value	p.value	−log10 (p-value)	FDR	Tukey's HSD
1	Plant height 8 WAP	11.859	0.002581	2.5882	0.010325	2-0; 3-0
2	Plant height 9 WAP	55.116	1.10E-05	4.9604	7.89E-05	1-0; 2-0; 3-0; 2-1; 3-1
3	Plant height 10 WAP	71.072	4.15E-06	5.3822	3.73E-05	1-0; 2-0; 3-0; 2-1; 3-1
4	Plant height 11 WAP	95.518	1.32E-06	5.878	2.38E-05	1-0; 2-0; 3-0; 2-1; 3-1; 3-2
5	Plant height 12 WAP	102.11	1.02E-06	5.9905	2.38E-05	1-0; 2-0; 3-0; 2-1; 3-1; 3-2
6	Plant height 13 WAP	82.498	2.34E-06	5.6316	2.80E-05	1-0; 2-0; 3-0; 2-1; 3-1; 3-2
7	Plant height 14 WAP	37.644	4.59E-05	4.3385	0.00027518	1-0; 2-0; 3-0; 2-1; 3-1
8	Number of dichotomous branches 10 WAP	12.633	0.002107	2.6764	0.0094799	2-0; 2-1; 3-2
9	Number of Dichotomous Branches 11 WAP	10.216	0.004127	2.3844	0.014856	2-0; 2-1; 3-2
10	Number of dichotomous branches 12 WAP	12.92	0.001959	2.7081	0.0094799	2-0; 2-1; 3-2

No	Parameters	F-value	p.value	−log10 (p-value)	FDR	Tukey's HSD
11	Number of dichotomous branches 13 WAP	9.8566	0.004608	2.3365	0.015079	2-0
12	Number of dichotomous branches 14 WAP	9.0016	0.006067	2.217	0.018202	2-0
13	Length of the longest fruit	6.2849	0.016907	1.7719	0.046818	3-2

*WAP: Week After Planting

The results of univariate and multivariate analyses reinforce each other, as seen from the consistency of the clustering pattern (**Fig. 1.** and **Fig. 2.**) with the ANOVA test results (**Table 1.**). This indicates that the differences between treatments are consistent both at the single parameter level and in the overall plant response pattern. In univariate analysis (ANOVA), each parameter is tested individually so that small but consistent differences between treatments can be seen as statistically significant. In contrast, multivariate analyses such as PCA focus on the overall variance structure and the relationships between all variables simultaneously. Parameters that show significant differences univariately do not necessarily contribute greatly to the main axis of multivariate variation, especially if these parameters are correlated with other properties or only represent a small proportion of the variance. Thus, a variable can be significant in ANOVA but not dominant in PCA, meaning that the variable is important individually, but is not the main driver of the collective pattern formed from the overall parameters.

To see the relationship between parameters and determine the indicator parameters, a correlation analysis was performed. The correlation analysis results showed that the cumulative number of fruits per plant and fruit weight per plant had a very strong relationship ($p < 0.001$), indicating a consistent yield pattern across all treatments. Plant height at 7–14 MST and number of leaves at 5–14 MST also correlated positively with yield, suggesting that vegetative growth vigor contributes to increased productivity. In addition, the number of dichotomous branches at 8–14 MST had a significant relationship with yield, confirming its important role during the mid to late growth phase. Both fresh and dry biomass weight were positively related to yield, confirming that high vegetative biomass supports fruit formation and development. Overall, vegetative growth parameters, particularly plant height, number of leaves, and branch development were reliable indicators for predicting the yield potential of chili plants (**Table 2. and Table 3.)**.

Table 2. *p*-values showing the significance of correlations between yield and growth parameters.

	Cumulative number of fruits per plant	Fruit weight per plant	Plant height (7–14 WAP)	Number of leaves (5–14 WAP)	Number of dichotomous branches (8–14 WAP)	Fresh biomass weight	Dry biomass weight
Cumulative number of fruits per plant	1	6.63E-11	0.000055	0.0023	0.001	0.000086	0.000033
Fruit weight per plant	6.63E-11	1	0.000049	0.00055	0.00056	0.000066	0.000027
Plant height (7–14 WAP)	0.000055	0.000049	1	0.0012	0.00089	0.0075	0.006
Number of leaves (5–14 WAP)	0.0023	0.00055	0.0012	1	0.0021	0.009	0.007
Number of dichotomous branches (8–14 WAP)	0.001	0.00056	0.00089	0.0021	1	0.0045	0.0036
Fresh biomass weight	0.000086	0.000066	0.0075	0.009	0.0045	1	0.0028
Dry biomass weight	0.000033	0.000027	0.006	0.007	0.0036	0.0028	1

Table 3. Correlation coefficients between yield and growth parameters.

	Cumulative number of fruits per plant	Fruit weight per plant	Plant height (7–14 WAP)	Number of leaves (5–14 WAP)	Number of dichotomous branches (8–14 WAP)	Fresh biomass weight	Dry biomass weight
Cumulative number of fruits per plant	1.000	0.844	0.586	0.597	0.719	0.811	0.863
Fruit weight per plant	0.904	1.000	0.854	0.718	0.859	0.847	0.813

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	Cumulative number of fruits per plant	Fruit weight per plant	Plant height (7–14 WAP)	Number of leaves (5–14 WAP)	Number of dichotomous branches (8–14 WAP)	Fresh biomass weight	Dry biomass weight
Plant height (7–14 WAP)	1.000	0.994	1.000	0.727	0.893	0.895	0.913
Number of leaves (5–14 WAP)	0.994	1.000	0.803	1.000	0.876	0.900	0.917
Number of dichotomous branches (8–14 WAP)	0.789	0.818	0.692	0.914	1.000	0.869	0.846
Fresh biomass weight	0.895	0.900	0.768	0.804	0.774	1.000	0.989
Dry biomass weight	0.913	0.917	0.758	0.763	0.782	0.989	1.000

Correlation testing is an important statistical analysis method in horticultural research that measures the strength and direction of the relationship between two or more variables of plant growth, production, or quality [13]. Through correlation analysis, researchers can understand whether changes in one parameter (e.g., plant height or number of leaves) are significantly related to changes in other parameters (such as crop yield or fruit quality). This understanding helps in determining key parameter indicators that have a major impact on the productivity and quality of horticultural crops. This study shows that plant height is an important parameter in growth and yield. This finding is in line with previous study which states that plant height can be used as one of the selection criteria for large red chili plants [14]. Taller plants have a greater chance of capturing sunlight than shorter plants. As a result, it allows photosynthesis to occur more efficiently and supporting increased yields [15].

4 Conclusion

Multivariate analysis (PCA) indicated a clear separation between treatments. These results suggested distinct overall responses to the various types of growth regulators. Univariate analysis (ANOVA and Tukey's post hoc test) confirmed that the Agrogibb 60 mg/L (Z2) treatment provided the most stable and significant improvements in growth across several parameters. The results of the univariate and multivariate tests reinforced each other. Both analyses demonstrated that Agrogibb had a better and dominant effect on chili plants. Correlation analysis revealed a strong positive relationship between vegetative traits, such as plant height, number of leaves, and branch development. These traits can serve as reliable predictors in determining chili crop yield performance. Overall, this integrated analytical framework provides a comprehensive understanding of chili plant response to PGR application through the disclosure of growth patterns, significant effects, and key traits associated with crop yield.

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