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Morphoanatomy Characters, Protein and Chlorophyll Contents of *Vanilla planifolia* Jacks. ex Andrews Cuttings to Application of Natural Growth Regulators and Vegetable Elicitors

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Abstract. This study aimed to examine the morphoanatomy characteristics, protein and chlorophyll contents of shoots on vanilla cuttings in response to application of natural growth regulators (NGR) and vegetable elicitors (VE). The study was conducted according to factorial design. The natural growth regulators included shallot extract 300 g/L (P1), banana weevil extract 300 g/L (P2), bamboo shoot extract 300 g/L (P3), coconut water 400 mL/L (P4) and sprouts extract 300 g/L (P5) and vegetable elicitors (E): *Coleus scutellarioides* extract (E1) and *Amaranthus spinosus* extract (E2) were used in the study. The results of the quantitative morphological characters showed that the highest shoot length (46.60 cm) was observed in P1E2, the number of leaves (7.67) in the P1E1, leaf length (8.25 cm) in P1E1, leaf width (2.82 cm) in P5E1 and stem diameter (0.87 cm) in P1E2 treatment, respectively. The quantitative and anatomical characters showed highly varied responses to different natural growth regulators and vegetable elicitors. The maximum stomatal pore length (29.22 µm), stomatal pore width (6.95 µm), stem cortex diameter (1039.02 µm) and leaf mesophyll (1565.97 µm) were recorded in P1E1, P5E1, P1E2 and P4E1, respectively. The highest mean protein and chlorophyll content (82.71 mg/Kg) and (2.69 mg/L) were observed in P1E1 and P1E2, respectively. The application of natural growth regulators and vegetable elicitors affects the morphoanatomy and increases vanilla plants' protein and chlorophyll content.

Keywords: vegetable elicitors, natural growth regulators

Introduction

The *Vanilla planifolia* Jacks. ex Andrews is a group of plants from the Orchidaceae family with creeping growth type and semi-epiphytic life. Vanilla as an industrial plant has a high economic value and is considered a spice export commodity in Indonesia (Maghraby, 2020; Anggraeni *et al*., 2019). Vanilla fruit is widely used in the pharmaceutical, cosmetic, cigarette, food and beverage industries because it contains vanillin, which gives off an aroma (Singletary, 2020; Vijayalakshmi *et al*., 2019). In Indonesia, the centres of vanilla production are the islands of Java, Bali, Sulawesi and Sumatera.

Vanilla fruit is a spice export commodity with high value and can increase the country's foreign exchange (Anggrasari *et al*., 2021). The availability of cuttings and appropriate cultivation techniques determine the export potential of vanilla. Propagation of vanilla plants could be done vegetatively by cuttings (Morwal *et al*., 2015). This propagation technique was an effective and efficient way to meet the need for seeds on a large scale in a short time. Applying natural growth regulators (NGR) and vegetable elicitor (VE) can stimulate root growth of vanilla stem cuttings. Research on NGR has been carried out by several researchers using coconut water (Devitriano and Syarifuddin, 2021; Ariyanti *et al*., 2020), shallots (Rugayah *et al*., 2021; Yanengga and Tuhuteru, 2020) a banana weevil (Belit *et al*., 2021; Putro *et al*., 2021) and bamboo shoots (Rahmawati, 2021). NGR application on cuttings aimed to increase root formation, accelerate root initiation, improve root quality and quantity and constant root growth. Shallot extract contained IAA, gibberellin, vitamins and starch.

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Coconut water contains auxins, vitamins and minerals. Banana hump contains cytokinin compounds and bamboo shoots have gibberellin (Kurniati *et al*., 2017). Sprout extract contained vitamins, amino acids, carbohydrates, proteins and auxin hormones (Warohmah *et al*., 2018). The positive response of plants to NGR application was influenced by several factors, including plant type, plant growth phase, NGR type, concentration and method of NGR application. Elicitors stimulate physiological and morphological responses and induce the production of phytoalexins (Bektas and Eulgem, 2014; Thakur and Sohal, 2013). They activate enzymes associated with secondary metabolism (Cham *et al*., 2021). The natural elicitors of plants are terpenoids, phenolics and alkaloids. Bioactive compounds play a role in plant defense and protection and kill pathogens (Jamiolkowska, 2020). Hassinia *et al*. (2019) found that elicitor methyl jasmonate, salicylic acid and methionine can increase phenolic bioactive compounds and sulfur content compared to control. Research on activating resistance genes using plant extract elicitors was carried out by (Rampe *et al*., 2018) using plants, namely *Coleus scutellarioides* and *Amaranthus spinosus* which is applied to peanut plants. The results showed that the elicitor application could respond to resistance by increasing the protein content by 1.29 mg/Kg and flavonoids by 8.01 mg/Kg compared to the control. The use of NGR in plant propagation by cuttings could increase plant growth and development, including morphoanatomy, protein content and chlorophyll of vanilla plants. In view of above facts, the current study was designed to explore the effect of NGR and VE on growth and development of vanilla cuttings.

Materials and Methods

Experimental design. The study research uses a factorial design. The natural growth regulators included shallot extract 300 g/L (P1), banana weevil extract 300 g/L (P2), bamboo shoot extract 300 g/L (P3), coconut water 400 ml/L (P4), sprouts extract 300 g/L (P5), vegetable elicitors (E): *Coleus scutellarioides* extract (E1) and *Amaranthus spinosus*extract (E2).

Preparation of vanilla stem cuttings. Vanilla cuttings measure 20-30 cm, the 3-6th internodes from the shoots were obtained from Tomohon city. Vanilla stems were cut at an angle of 45° to enlarge the absorption area and facilitate root growth.

Soaking cuttings in NGR solution and planting. Vanilla stem cuttings were soaked according to the treatment for 3 h and then the cuttings were planted in polybag planting media in a shaded area with 50% shading using a shading net (Kartikawati and Rosman, 2018; Rampe *et al*., 2018).

Preparation of elicitor solution and application on vanilla. The leaves of *C. scutellarioides* (E1) and *A. spinosus* (E2) were washed, dried and then cut into 1-2 cm sizes to facilitate grinding, then crushed with a mortar and diluted 1:1 (w/v) with phosphate buffer pH 7.0. The plant extracts were then filtered with sterile gauze. The supernatant was put in a sample bottle with a lid and stored in a refrigerator at 10 °C. The elicitor application on vanilla seedlings was carried out in the afternoon when they were 1 and 2 months old (Rampe *et al*., 2018).

Observation recorded. Observations of morpho-anatomical characters, protein and chlorophyll contents of vanilla shoots were carried out after three months of planting of cuttings. Morphological characters were taken by using a calliper. Observations of anatomical structures were calculated using a microscope connected to an optilab camera with a raster image program. A spectro-meter was used to measure protein and chlorophyll content.

***Protein analysis*.** Protein analysis was done using the Lowry method with the Folin-Ciocalteu reagent (Harborne, 1987). The standard solution of serum Bovin Albumin was put in a phosphate buffer solution having a pH of 7.4 and 1 g of vanilla powder was put into a test tube for protein analysis. After the sample was macerated with 5 mL of ether for 30 min the supernatant was discarded. Then the sample was washed with 50% ethanol and centrifuged several times until the solution was clear. Then 3 mL of 10% TCA was added and allowed to stand for 10 min. The supernatant was centrifuged for 10 min and then the supernatant was discarded and the precipitate was taken and diluted with 3 mL of distilled water. 0.1 mL of supernatant, added 2 mL of Lowry's solution B and then shaken was done. Then 0.25 mL of Lowry A solution was added and vortexed for 2 min. It was then incubated for 30 min and analyzed by the spectrometer at a wavelength of 750 nm.

***Chlorophyll analysis*.** The chlorophyll content was calculated using the Sims and Gamon (2002) method. The 0.25 g of leaf sample was crushed and 1 mL of

acetic reagent (acetone 85% + tris 15%) was added. The finely ground leaves were put into a 2 mL microtube and the mortar was rinsed with acetic acid until the microtube was 2 mL total. After that, centrifuged at 14,000 rpm for 10 sec. 1 mL of supernatant was taken and then put into a test tube and added 3 mL of acetate to the test tube and the lid was vortexed. The spectrometer absorbance at 537 nm, 647 nm and 663 nm. The determination of chlorophyll content in vanilla leaves was calculated based on the following equation:

Chlorophyll a (mg/mL) = (0.01373 x A663) - (0.000897 x A537) - (0.003046 x A647)

Chlorophyll b (mg/mL) = (0.02405 x A647) - (0.004305 x A537) - (0.005507 x A663)

Total chlorophyll = chlorophyll a + chlorophyll b

Results and Discussion

Quantitative morphological characteristics of vanilla cuttings. The data on the average results of quantitative morphological character measurements of vanilla cuttings are shown in Table 1 and Fig. 1. The highest average shoot length (46.60 cm) was observed in P1E2. The number of leaves (7.67) in the P1E1 and leaf length (8.25 cm) in the P1E1 treatment. The highest result was for the leaf width (2.82 cm) in the P5E1 treatment and the stem diameter (0.87 cm) in the P1E2 treatment.

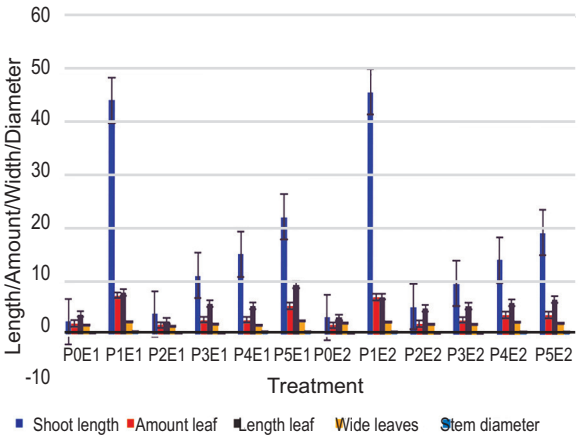

Fig. 1. Quantitative morphological characters of vanilla cuttings.

The results of the analysis of variance were shoot length (sig.0.03) and number of leaves (sig.0.03), leaf length (sig.0.04), leaf width (sig.0.01) and stem diameter (sig.0.01 and 0.15). Least Significant Difference (LSD) test results, shoot length (standard error (SE) = 0.76), the number of leaves (SE = 0.36) and leaf length (SE = 0.56), leaf width (SE = 0.13), stem diameter (SE=0.02).

Table 1. Quantitative morphological characters of vanilla cuttings after application of natural growth regulators and vegetable elicitors

Treatment combination	Parameter (cm)				
	Shoot length ± SE	Amount leaf ± SE	Leaf length ± SE	Leaf width ± SE	Stem diameter ± SE
P0E1	2.70 ± 0.25^{g}	2.33 ± 0.34^{e}	4.09 ± 0.46^{f}	2.02 ± 0.09^{c}	0.57 ± 0.02^{cd}
P1E1	44.97 ± 1.19^{a}	7.67 ± 0.34^{a}	8.25 ± 0.15^{a}	2.71 ± 0.09^{a}	0.79 ± 0.02^{a}
P2E1	4.10 ± 0.36^{g}	2.00 ± 0.00^{e}	2.80 ± 0.37^{h}	1.75 ± `0.28^{d}	0.64 ± 0.02^{c}
P3E1	11.50 ± 0.49^{e}	3.00 ± 0.00^{d}	6.08 ± 0.12^{de}	2.30 ± 0.12^{bc}	0.64 ± 0.01^{c}
P4E1	15.53 ± 0.48^{d}	3.00 ± 000^{d}	5.67 ± 0.45^{e}	2.09 ± 0.18^{c}	0.74 ± 0.01^{b}
P5E1	22.63 ± 0.65^{b}	5.67 ± 0.34^{b}	7.92 ± 0.51^{a}	2.82 ± 0.11^{a}	0.72 ± 0.01^{b}
P0E2	3.51 ± 0.17^{g}	2.00 ± 0.00^{e}	3.48 ± 0.99^{g}	2.38 ± 0.13^{bc}	0.56 ± 0.01^{d}
P1E2	46.60 ± 2.02^{a}	7.33 ± 0.66^{a}	7.43 ± 0.27^{b}	2.70 ± 0.02^{a}	0.87 ± 0.03^{a}
P2E2	5.50 ± 0.21f^{g}	2.33 ± 0.34^{e}	5.29 ± 0.75^{e}	2.17 ± 0.06^{c}	0.64 ± 0.02^{c}
P3E2	9.93 ± 0.24e	3.00 ± 0.00^{d}	5.64 ± 0.36^{e}	2.11 ± 0.45^{c}	0.65 ± 0.02^{c}
P4E2	14.47 ± 0.34^{d}	4.00 ± 0.00^{c}	6.34 ± 0.74^{d}	2.56 ± 0.14^{ab}	0.75 ± 0.01^{ab}
P5E2	19.73 ± 0.26^{c}	4.00 ± 0.58^{c}	6.93 ± 0.88^{c}	2.41 ± 0.11^{bc}	0.71 ± 0.02^{b}

Note: Mean values followed by the same superscript in the same column were not significantly different (P>0.05); SE (standard error).

Observation of growth parameters obtained in treatment P1 gave the highest yield, while leaf width data was highest in treatment P5. Growth regulators were non-nutrient organic compounds (Shambhu *et al*., 2022; Zhenxi *et al*., 2022), which in low concentrations could support, inhibit and change plant physiological processes (Arifin *et al*., 2022). Shallot tubers contain vitamin B1, thiamin, riboflavin and nicotinic acid and contain auxin and rhizokaline growth regulator, which could stimulate root growth. In addition, the red onion that had been crushed would form allithiamin compounds. These compounds could facilitate metabolism in plant tissues (Siburian and Luthfi, 2019). The physiological effects of auxin revealed positive effect on cell elongation, phototropism, geotropism, apical dominance, root growth, parthenocarpy, abscission, callus formation and respiration. Auxin in the NGR solution promotes increased metabolic activity (Arifin *et al*. 2022; Lesmana *et al*., 2018). Auxin encourages plant growth, increasing proliferation and cell elongation (Sezgina and Kahya, 2018).

The application of bamboo shoots positively responded to the morpho-anatomical parameters, protein content, and chlorophyll. Bamboo shoots contain IAA, GA, and cytokinins (Manurung *et al*., 2020). The research results by Hasibuan *et al*. (2020) showed that the application of bamboo shoot extracts significantly increased plant length at 5-10 weeks after planting. Furthermore, according to Mardaleni and Sutriana (2014), the application of bamboo shoot extract of 4.5 ml/L water on green bean plants significantly affected the plant's height, harvest age and the number of pods, while on the dry's weight parameters, 100 seeds the great bamboo shoot extract was in 1.5 ml/L.

PGR sprout extract showed the highest results for leaf width. Bean sprout extract contains auxin, cytokinin and gibberellin acid (Kamson *et al*., 2021). The other components of sprouts are vitamin C, thiamin, riboflavin, niacin, pantothenic acid, vitamin B6, folate, choline, β -carotene, vitamin A, vitamin E (tocopherol) and vitamin K. In addition, sprouts also contain minerals consisting of Ca, Fe, Mg, P, K, Na, Zn, Cu, Mn and Se. The essential amino acids in bean sprouts included: tryptophan, threonine, phenylalanine, methionine, lysine, leucine, isoleucine and valine.

The composition of macronutrients and micronutrients in the sprout extract was balanced. So, the application of vanilla cuttings showed the highest response for leaf width. Furthermore, the application of coconut water revealed the second-highest response for growth parameters. Coconut water as a natural growth regulator was cheaper and easier to obtain. Coconut water contains the hormones auxin and cytokinin. Coconut water had a high enough potassium content (upto 17%), vitamins and minerals to qualify. The results regarding quantitative morphological parameter measurements showed that the NGR treatment of shallot extracts gave the highest results. Shallots contain auxin phytohormones that have the potential as growth hormones that play a role in stimulating cell division and elongation, which as a whole stimulate plant growth and development.

Qualitative anatomical characteristics. Observation of the qualitative anatomical characteristics of vanilla, shoots included the structure of stomata, stem cortex and leaf mesophyll. The results of longitudinal slices (40× Magnification) of the lower epidermis and the stomata shape of the vanilla leaves are shown in Fig. 2.

Based on the results of longitudinal slices of the lower epidermis, it was observed that the leaf epidermal tissue was composed of cells with an elongated round shape, without or with narrow intercellular spaces. The epidermis is the outermost layer that provides physical protection against moisture loss and physical damage (Crang *et al*., 2018). According to Sumardi and Pudjarinto (1994), in most seed plants, the epidermis consists of a single layer of cells with parenchyma tissue inside. Based on the ontogeny of the epidermis, it was derived from the protoderm meristematic tissue (Fahn, 1982).

Stomata were a modification of the leaf epidermis. The stomata shape observed in the vanilla leaf epidermis was the kidney shape. Stomata consist of holes (pores) surrounded by two guard cells. Epidermal cells adjacent to the guard cells often showed differences in form or arrangement with other epidermal cells and were called "nearby cells." Plant physiological processes could be affected by slits in the stomata pores. The type of stomata in vanilla leaves was parasitic. The long shaft of the guard cell was parallel to the neighbouring cells.

The results of transverse slices of the vanilla shoot at 4X magnification using an optilab connected microscope are shown in Fig. 3. It was observed that there were ergastic objects on the stems of vanilla plants. In in-plant cells, there were many non-living cell components, which were usually found in vacuoles, cell plasma and plastids, known as ergastic materials. Non-protoplasmic components consisted of organic and inorganic

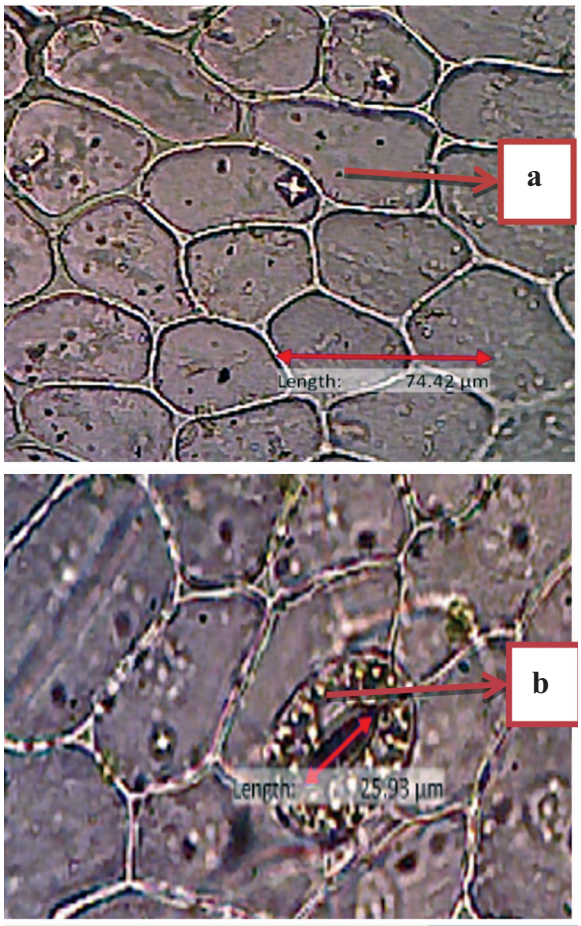

Fig. 2. Cell shape of the epidermis (**a**) and stomata (**b**) of vanilla buds.

Fig. 2. Cell shape of the epidermis (a) and stomata (b) of vanilla buds.

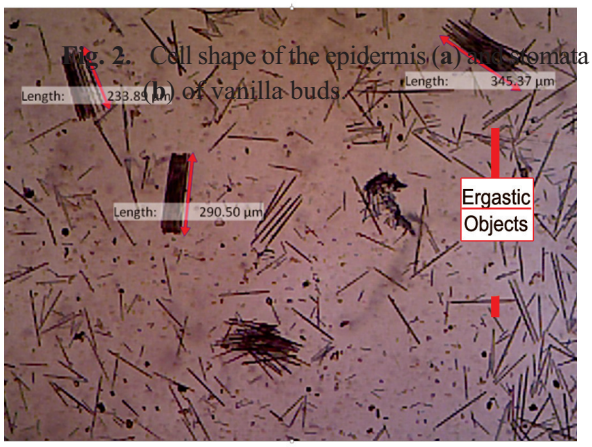

Fig. 3. Ergastic objects on vanilla budding stems.

substances that were solid or liquid. The cell wall is the non-protoplasmic part that lies outside the plasma.

Non-protoplasmic components in a solid plasma consist of crystals, namely Ca-oxalate crystals, grit crystals, and aleurone grains. In plants, Ca-oxalate crystals could be prisms, rods, cylindrical, star-shaped or druses (Ekeke *et al*., 2019; Fahn, 1982). Through precipitation, Ca-oxalate crystals were present in the cells of various plants: in cortical cells, phloem parenchyma cells and xylem parenchyma cells. These crystals are present in vacuoles and plasma or fill the entire cell space. The form of Ca-oxalate crystals in vanilla plants, namely needle-shaped crystals or broomsticks which was called rafida.

Vanilla plants are included in the Liliopsida group, with a book stem and *Phyllotaxis folia* Sparse with leaf formula. The cross-section of the stem showed that it was composed of three regions, namely the epidermis, cortex and stele. The xylem and phloem transport bundles are present in the cortex and stele without cambium (Fig. 4). The absence of cambium in vanilla plants caused no secondary thickening growth. The cross-section of vanilla leaves showed that the leaves were composed of the upper epidermis, mesophyll and lower epidermis. Without chloroplasts, the mesophyll in vanilla plants does not differentiate into palisade parenchyma and spongy parenchyma.

The average data for measuring the quantitative anatomical characteristics of vanilla cuttings are shown in Table 2 and Fig. 5. Observation of vanilla cuttings with the highest means of stomatal pore length (29.22 µm) were recorded in the P1E1. The most increased stomatal pore width in P5E1 was 6.95 µm. The average diameter of the highest stem cortex (1039.02 µm) in the P1E2. The highest average mesophyll thickness (1565.97µm) was in the P4E1 treatment.

The results of the analysis of variance were stomatal pore length (sig.0.92), stomatal pore width (sig.0.91), cortex diameter (sig.0.80) and mesophyll thickness (sig.0.93). LSD test results, stomatal pore length (SE = 1.12), stomatal pore width (SE = 0.36).

Internal and external factors influence the anatomical development of stomata, cortex and mesophyll. Application of NGR and VE on vanilla cuttings show different anatomical responses. NGR contains hormones, namely IAA and gibberellin in shallot extract (Alimudin *et al*., 2017) and auxins in coconut water (Mayura *et al*.,

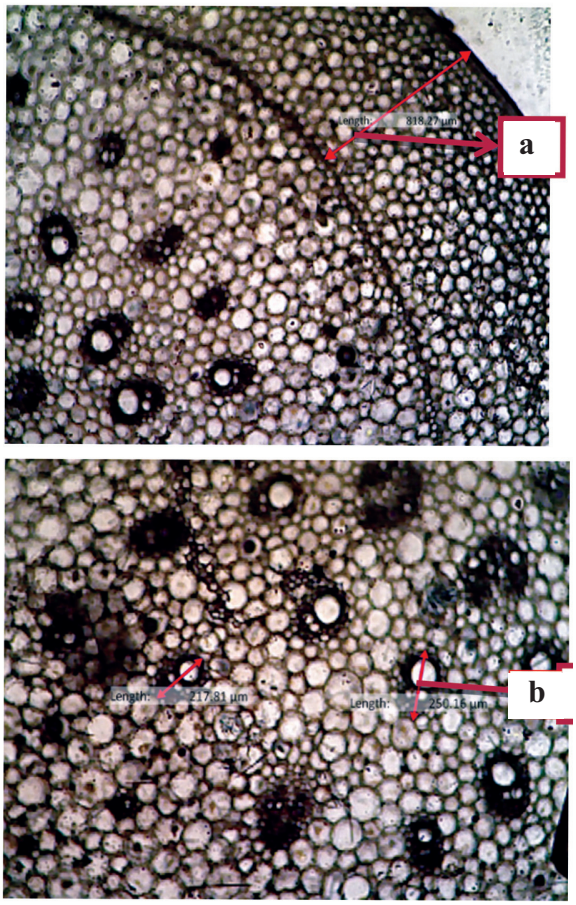

Fig. 4. Transverse slice of vanilla cutting stem (4x magnification) (**a**)Vanilla plant stem cortex (**b**) Vanilla stem xylem and phloem carrier bundle.

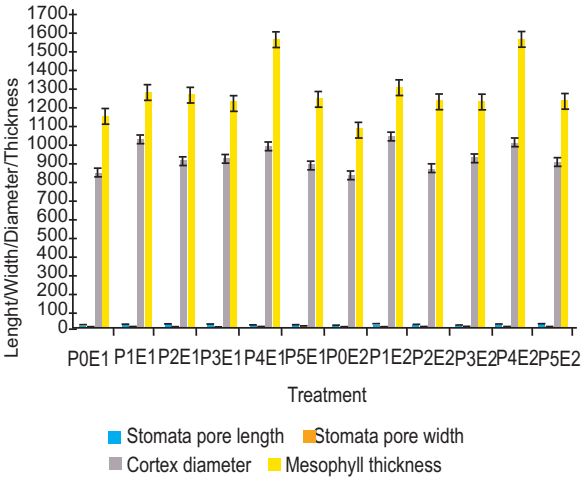

Fig. 5. Quantitative anatomical characters of vanilla cuttings.

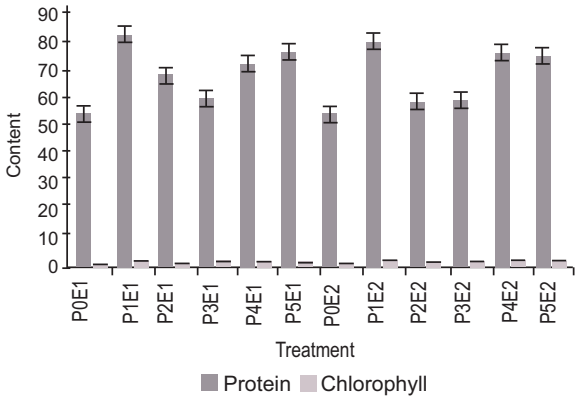

Fig. 6. Content of protein and chlorophyll vanilla cuttings.

2016), cytokinins in a banana hump, gibberellin in bamboo shoots and auxin in sprout extract. NGR is a bioregulator in cell reproduction, cell elongation and differentiation in plant tissues (Sajjad *et al*., 2017). The length and width of the stomata are related to the hormone-induced ontogeny of the stomata. VE can induce biochemical activity, electron transport, phospholipid activity, protein and gene activation (Walters *et al*., 2013).

Protein and chlorophyll content. A spectrometer analysis of protein and chlorophyll content is shown in Table 3 and Fig. 6. The results obtained show that the application of NGR can increase the protein and chlorophyll content of vanilla cutting shoots. The highest mean protein content (82.71 mg/Kg) was observed in the P1E1 and chlorophyll (2.69 mg/L) in the P1E2 treatment. The analysis of variance shows that that there was a significant difference between treatment for protein and chlorophyll content. The results of the LSD test of protein content (SE = 1.29) for Z4-Z5 treatments were not significant. The chlorophyll content (SE = 26.28), Z1-Z4, Z2-Z3, Z2-Z5 and Z3-Z5 were insignificant.

Proteins are macromolecules with diverse structures, built from 20 amino acids to form polypeptide polymers. One end of the polypeptide chain had a free amino group and the opposite end had a carboxyl group. As bio-stimulants, hormone precursors, signalling factors of different physiological progressions and regulators of nitrogen uptake (Khan *et al*., 2019).

Table 2. Quantitative anatomical characteristics of vanilla cuttings to application of natural growth regulatqrs and vegetable elicitors

Treatment combination	Parameter (µm)			
	Stomata pore length ± SE	Stomata pore width ± SE	Cortex diameter ± SE	Mesophyll thickness ± SE
P0E1	20.56 ± 1.01^{c}	6.09 ± 0.27^{b}	843.41 ± 3.12^{cd}	1147.23 ± 28.59^{c}
P1E1	29.22 ± 1.40^{a}	6.75 ± 0.18^{a}	1027.63 ± 30.50^{a}	1278.38 ± 18.43^{b}
P2E1	23.40 ± 1.28^{b}	5.85 ± 0.49^{b}	908.89 ± 20.38^{c}	1265.77 ± 81.76^{b}
P3E1	23.41 ± 0.80^{b}	6.90 ± 0.10^{a}	920.23 ± 22.82^{bc}	1219.96 ± 13.95^{b}
P4E1	23.91 ± 1.43^{b}	6.23 ± 0.25^{a}	985.86 ± 34.20^{a}	1565.97 ± 53.30^{a}
P5E1	23.17 ± 0.80^{bc}	6.95 ± 0.48^{a}	889.42 ± 56.74^{cd}	1237.79 ± 58.36^{b}
P0E2	9.80 ± 0.24c	5.72 ± 0.27^{b}	832.04 ± 2.91d	1083.08 ± 12.45^{de}
P1E2	28.48 ± 1.58^{a}	6.94 ± 0.47^{a}	1039.02 ± 14.49^{a}	1303.56 ± 28.16^{b}
P2E2	21.80 ± 0.35^{c}	5.85 ± 0.50^{b}	862.32 ± 20.08^{cd}	1232.96 ± 57.93^{b}
P3E2	23.48 ± 1.56^{b}	6.38 ± 0.28^{a}	919.22 ± 4.87c	1226.01 ± 23.74^{b}
P4E2	22.49 ± 1.40^{c}	5.86 ± 0.49^{b}	1008.71 ± 31.42^{a}	560.49 ± 22.45^{a}
P5E2	23.71 ± 0.39^{b}	6.89 ± 0.27^{a}	904.19 ± 20.37^{b}	1229.61 ± 46.19^{b}

Note: Mean values followed by the same superscript in the same column were not significantly different (P>0.05); SE (Standard Error).

Table 3. Protein and chlorophyll content of vanilla cuttings

Treatment combination	Protein (mg/Kg) ± SE	Chlorophyll (mg/L) ± SE
P0E1	54.28± 1.32^{f}	0.92 ± 0.03^{g}
P1E1	82.71 ± 1.89^{a}	2.52 ± 0.02^{b}
P2E1	68.02 ± 1.34^{d}	1.69 ± 0.05^{e}
P3E1	59.73 ± 0.99^{e}	1.93 ± 0.29^{d}
P4E1	72.25 ± 1.00^{c}	2.23 ± 0.07^{c}
P5E1	76.47 ± 0.91^{b}	1.86 ± 0.05^{d}
`P0E2	54.25 ± 2.12^{f}	1.16 ± 0.04^{f}
P1E2	80.07 ± 1.26^{a}	2.69 ± 0.04^{a}
P2E2	58.43 ± 0.49^{e}	1.56 ± 0.05^{f}
P3E2	59.17 ± 1.51^{e}	1.72 ± 0.04^{e}
P4E2	76.18 ± 1.00^{b}	2.50 ± 0.04^{b}
P5E2	75.21 ± 0.83^{b}	1.93 ± 0.32^{d}

Note: Mean values followed by the same superscript in the same column were not significantly different (P>0.05); SE (standard error).

Auxin could increase protein synthesis and affect the synthesis of nucleic acids. Giving auxin to tissue would support the synthesis of new RNA and protein formation. Auxin would liberate DNA from histone proteins. The mRNA helped form new enzymes, which increased the plasticity and widening of the cell wall. Auxin is vital for regulating plant growth, development and adaptation to a fluctuating environment (He *et al*., 2021; Khadr *et al*., 2020). Proteins are nitrogen-containing organic compounds that are composed of amino acids. Protein content in plants is determined by the availability of nitrogen in the growing medium. Plants absorb nitrogen in the form of NO^{-3} and NH^{+4}. Plant's nitrogen source results in the decomposition of organic matter and fertilizers (Krounbi *et al*., 2021; Muratore, 2021).

The response of plant growth can be observed from the ability of plants in photosynthesis and the formation of primary and secondary organic compounds as tolerance resistance. Environmental factors such as light intensity, aeration, humidity, soil pH, soil type and soil nutrient content determine the ability of plants to produce primary metabolites. Nitrogen is an essential nutrient for plants and a significant component of proteins, nucleic acids, hormones and vitamins.

Chlorophyll is the primary pigment in chloroplasts, which plays a role in photochemical reactions, absorbs light and transfers excitation energy to the reaction centre. Chlorophyll a and b are the primary pigments in the thylakoid membrane. Application of PGR could increase vanilla chlorophyll content, improving photosynthetic reactions and photosynthetic products.

Chlorophyll plays a role in plants through photochemical reactions and photophosphorylation to produce ATP and NADPH. Furthermore, ATP and NADPH were used to reduce CO_2 to form carbohydrate compounds (Oktavia *et al*., 2021; Fornari *et al*., 2020). PGR application increases metabolic parameters, namely protein, chlorophyll and NR activity (Khan, 2015).

Conclusion

The applied natural growth regulator and vegetable elicitor affected the morpho-anatomical character and increased the vanilla cuttings protein and chlorophyll content. However, further study needs to be performed by applying other plant growth regulators and vegetable elicitors to assess their impact on morphological and physiological chracters of vanilla cuttings.

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