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Dynamics of Nutrient Concentrations, Endogenous Hormones, Photosynthetic Capacity, and Phenological Changes in Black Orchid (*Coelogyne pandurata* Lindl.) from the Vegetative to Generative Phase

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Abstract

The black orchid (*Coelogyne pandurata* Lindl.) is one of the native Indonesian orchids from Borneo island. This study analyzed the dynamics of pigments, photosynthetic capacity, endogenous hormones, nutrient concentrations, and phenology across the vegetative to generative stages of the black orchid. The plant materials are one-year-old plants after splitting from the mother plants. Endogenous hormones, nutrient and pigment concentrations, and leaf photosynthetic capacity were measured during vegetative and generative phases. Chlorophyll, anthocyanins, and carotenoids were analyzed using UV-VIS spectrophotometry, Nitrogen (N) was analyzed by the Kjeldahl method, phosphorus (P) and potassium (K) by the Bray 1 method and Flame Photometry, and organic carbon by the Walkley-Black method with titration. The hormone levels were analyzed using HPLC, and photosynthetic capacity was determined using a Li-Cor 6800 system. Phenological changes in leaves, bulbs, and flowers were recorded. Results showed total chlorophyll increased from 1.96 to 2.36 $ng.g^{-1}$ from the vegetative to the generative stage while carotene slightly declined. Leaf nitrogen rose from 1.54% to 1.70%, bulb nitrogen decreased from 0.67% to 0.45%, whereas the C/N ratio increased from 65.24 to 85.36 from the vegetative to the generative phase. Flower nitrogen was 1.50%, and phosphorus was 0.17%. IAA in leaves decreased from 25.12 to 21.37 $ng.g^{-1}$ dry weight post-flowering, while gibberellin in bulbs increased from 12.28 to 12.96 $ng.g^{-1}$ dry weight. Zeatin in bulbs declined from 10.52 to 8.81 $ng.g^{-1}$ dry weight. Photosynthesis peaked at 2.73 $\mu mol.m^{-2}.s^{-1}$ in early generative stages and then declined. Photosynthetic photon flux density and stomatal conductance decreased, reducing water and CO_2 exchange efficiency, while net assimilation and transpiration rates showed no significant changes. These physiological adjustments, including increased chlorophyll levels, changes in nitrogen allocation, and fluctuations in hormone concentrations, reflect the plant's adaptive strategies to meet higher energy demands during reproductive growth, ensuring efficient resource distribution for flowering.

Keywords: auxin, carotene reduction, chlorophyll content, gibberellin, nitrogen concentration, phenology

Introduction

The tropical region is a habitat for thousands of orchid species, including the black orchid (*Coelogyne pandurata* Lindl.), which grows in the tropical forests of Indonesia (Heriansyah, et al., 2025) an orchid native to Indonesia, has medicinal properties and potential for lowland cultivation. This study assessed the phenolic and flavonoid contents and antioxidant activity of the leaves, bulbs, and flowers in the vegetative and generative phases of *C. pandurata*. *Coelogyne pandurata* is distributed along the islands of Sumatra and Borneo, Indonesia (Hartati and Darsana, 2017). Due to its unique and distinctive flower shape and color, it has enormous potential to become an export

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commodity. However, its development is constrained by insufficient information on the factors that affect flowering.

Changes in photosynthetic capacity during plant growth stages are crucial for monitoring factors that change at different growth stages. These changes included the net assimilation rate (NAR), transpiration rate, stomatal conductance, and total chlorophyll, carotene, and anthocyanin content. These factors are likely to affect plant metabolism during the flowering phase. Previous research has reported changes in photosynthesis, carbon dioxide, and chlorophyll in *Corallorhiza trifida* orchids. (Cameron et al*.,* 2009). Zhang et al. (2011) reported a decrease in photosynthesis in the orchid *Paphiopedilum armeniacum* and reported differences in photosynthetic capacity between *Cypripedium* and *Paphiopedilum* orchids*.* Zhang et al. (2021) reported photosynthetic performance in *Cypripedium* spp., but reliable information is needed regarding the changes in photosynthetic capacity at different growth stages of *C. pandurata*.

Exogenous and endogenous nutrients, such as total nitrogen, phosphorus, potassium, and C-organic concentration, influence changes in plant phenology. The concentration of nutrients in plants is involved in plant metabolism, so it plays a role in the growth of organs at the cellular level. Plant nutrient concentration changes are determinants of plant growth and development (Yousefi et al., 2019). Studies on changes in total N concentration in *Paphiopedilum armeniacum* reported by Zong-min et al. (2012) 105, 210, and 420 $mg.L^{-1}$ showed that the total N concentration in leaves increased when entering the adult phase, with a value of 0.70 to 1.23% in mature leaves. Furthermore, the study of N concentration in *Odontioda* sp. leaves reported by Kubota et al*.* (2009) showed that the total N in the leaves of flowering plants was 1.86%, which was not significantly different from the total N in non-flowering plants. Studies on N, P, and K concentration changes in *Dendrobium nobile* Lindl. reported by Ichinose et al. (2018) In the generative phase, there was an increase in N, P, and K accumulation in the leaves, bulbs, and roots from 30 days to 360 days of age. N accumulation in leaves was 116.40 mg per plant, in bulb 163.35 mg, in roots 59.68 per plant. P accumulation in leaves was 37.18 mg per plant, in bulb 58.99 per plant, in root 22.66 per plant, and K accumulation in leaves was 244.87 per plant, in bulb 401.80 per plant, in root 54.40 per plant. Despite this, detailed information on the changes in N, P, K, and C-organic concentrations in the leaves and bulbs of *C. pandurata* from vegetative to generative phases is lacking. This study aims to elucidate how these phenological and nutrient dynamics and physiological and environmental factors, including endogenous plant hormones, influence flowering. Endogenous hormone content stimulates cell differentiation, thereby forming a floral embryo. Each plant species has a different endogenous hormone content in each plant part. Therefore, it is necessary to understand the changes in endogenous hormone contents in different plant parts and growth stages. Previous research on changes in endogenous hormone content has been reported by Campos and Kerbauy, (2004) in *Dendrobium*, a terrestrial orchid *Cypripedium japonicum* Thunb (Yan et al., 2017), *Laelia anceps* (Tejeda et al*.,* 2022). Endogenous hormone changes in *C. pandurata* are unknown, whereas photosynthetic factors influence its flowering.

The development of *C.pandurata* orchids for commercial purposes has experienced obstacles in flowering induction and yet-to-be-known phenological characterization distinguishing between vegetative and generative phases. Previous research on phenology in orchids has been conducted by Herrera et al. (2020) on the phenology of *Anacamptis morio subspecies. Champagneuxii*, and by Lori et al. (2018) on Platanthera praeclara Sheviak and Bowles. Research on the characterization of *C. pandurata* has been carried out by Hartati and Darsana, (2017), nonetheless, detailed information regarding the phenological transitions from vegetative to generative phases in *C. pandurata* is lacking

This study aimed to determine the changes in photosynthetic capacity, chlorophyll, anthocyanin, carotene, nutrient concentrations, endogenous hormones, and phenological characterization at different growth stages in *C. pandurata*. This information is essential for optimizing black orchid production by providing insights into key physiological and biochemical changes that influence growth, development, and reproductive success. Understanding these factors can aid in developing targeted cultivation strategies, improving nutrient management, and enhancing stress tolerance to maximize yield and quality.

Material and Methods

Bulbs, leaves, and flowers of *C. pandurata* were collected from the Orchid House collection at Leuwikopo Experimental Station of IPB University, Ciampea, Bogor Regency, West Java, Indonesia, at 6°33’50.3”S 106°43’29.3”E and 188 m of altitude. The orchids were initially sourced from West Borneo, Indonesia. The samples were plants aged one year after splitting. Leaf and bulb samples

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before the flowering phase were collected between September and November 2022, and those after the flowering stage were collected between November and December 2022. Plants were grown in 15 cm diameter perforated clay pots with charcoal as the growing medium, ensuring uniform coverage of the pot's surface.

Plants were maintained under greenhouse conditions from the vegetative stage until the generative stage. Maximum and minimum temperatures were 32°C and 20°C, respectively, with an average temperature of 26°C. The daytime PPFD ranged from 189 to 81 $\mu mol.m^{-2}.s^{-1}$, maximum and minimum relative humidity (RH) were 90 % and 40%, respectively, photosynthetic photon flux (FFF) was 120 $\mu mol.m^{-2}.s^{-1}$, and the photoperiod was 12/12 h per day.

Three plants from the vegetative, early generative, and generative phases were selected for analysis. These plants were prepared and analyzed for endogenous hormones, chlorophyll, anthocyanins, carotenoids, and photosynthetic capacity. Each plant was divided into leaves and pseudobulbs for further analysis. Each plant organ was fragmented, and half of its fresh weight was used to determine the total chlorophyll, anthocyanin, and carotenoid contents. The other half was used to analyze endogenous hormones. The materials were stored at -70°C in a freezer (Thermo Scientific, Revco Elite Series; Waltham, MA, USA) until further analysis. Photosynthetic capacity was analyzed in fresh leaves under greenhouse conditions.

Anthocyanin, chlorophyll, and total carotenoid contents were extracted and determined following the method described by Zhu et al. (2018). Fresh leaf samples (2.0 g) were added to 30 mL of extraction solution (0.1 $mol.L^{-1}$ HCl, 95% ethanol = 1:1). The samples were extracted in a water bath at 60°C for 1h, centrifuged at 5000 rpm at 4°C for 15 min, and the supernatant was collected. Two 1 mL aliquots of anthocyanin were collected, added to 2 mL of 1 $mol.L^{-1}$ KCl buffer at pH 1.0 and 2 mL of 1 $mol.L^{-1}$ sodium acetate buffer at pH 4.0, and shaken well. The absorbance values at 520 and 700 nm were determined. Three independent technical replicates were used for all the experiments. Anthocyanin content was determined using the following equations:

$$A = \frac{(\mathrm{A520} - \mathrm{A700})\mathrm{pH\ 1:0} - (\mathrm{A520} - \mathrm{A700})\mathrm{pH\ 4:5}}{\mathrm{C\ (mg.g{-}1)} = \mathrm{A \times V \times n \times M}} \over 2a\varepsilon \times \mathrm{m}$$

Where

A : the difference of the absorbance value at pH 1.0 and pH 4.5 under 520 nm and 700 nm
V : the total volume of extract (mL)
N : dilution time
M : the molecular mass of cyanidin–3–glucoside chloride (449.2)
E : the molar absorptivity of cy–3–glu (26,900)
M : sample weight (g).

Chlorophyll and carotenoid contents were determined as follows. Fresh leaf samples (0.5 g) were rapidly ground to homogeneity in 2–3 mL of 95% ethanol. Then, 10 mL of 95% ethanol was added, and the sample was ground to pulp before being left aside for 3–5 min. The extract was filtered through a brown volumetric bottle and diluted to 15 mL with 95% ethanol. Absorbance values were measured at 665, 649, and 470 nm, with 95% ethanol used as a blank. Three independent technical replicates were used for each experiment. The following equations were used to determine the chlorophyll and carotenoid content:

$$Cchl = \mathrm{Ca} + \mathrm{Cb}$$

Chlorophyll a content ($mg.g^{-1}$ FW) = 13:95A665 nm - 6:88A649 nm
Chlorophyll b content ($mg.g^{-1}$ FW) = 24:96A649 nm - 7:32A665 nm
Total chlorophyll content ($mg.g^{-1}$ FW) = C ($mg\ g^{-1}$) - (Cchl x V x n)/m
Carotenoid content ($mg.g^{-1}$ FW) = (1000 × A470 nm - 1.82 × Chl a - 85.02 × Chl b) / 198

$$A = \frac{(\mathrm{A520} - \mathrm{A700})\mathrm{pH\ 1:0} - (\mathrm{A520} - \mathrm{A700})\mathrm{pH\ 4:5}}{\mathrm{C\ (mg.g{-}1)} = \mathrm{A \times V \times n \times M}} \over 2a\varepsilon \times \mathrm{m}$$

Where
Ca : chlorophyll a
Cb : chlorophyll b
V : the total volume of extract (mL)
N : dilution time (minute)
M : sample weight (g)

The nitrogen analysis was carried out on leaves, bulbs, and flowers at the vegetative and generative phases. The leaves and bulbs analyzed are the first ones next to the flowering bulb. The Kjeldahl method was used, and the measurement was performed using titration. The total P extraction method used the P-Bray 1 method, and the measurement was performed using a Spectrophotometer (UV VIS type UV-1800). The total K analysis extraction method used the Bray-1 method, and the measurement was performed using a Flame Photometer. The total C-organic analysis extraction method used the Walkley and Black method, and the measurements used the titration method.

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A sample that had been previously frozen was placed in a lyophilizer (Labconco) for 24 hours. For the extraction of hormones, the method proposed by Pan et al. (2010) was followed. Endogenous hormone analysis and quantification were performed using a diode array detector according to Ricker (2000). One hundred mg of each sample was weighed into 2.0 mL conical-bottom plastic tubes in triplicate, followed by 500 µL of extraction solution. After shaking the tubes at 100 rpm for 30 min at 4°C, 1 mL of HPLC-grade methylene chloride was added and stirred for another 30 min. The tubes were centrifuged at 13,000 rpm for 5 minutes. The organic phase (900 µL) was transferred to an amber vial and dried under a stream of nitrogen gas until the volume reached 100 µL. Subsequently, 500 µL HPLC-grade methanol was added. A volume of 100 µL was injected into the liquid chromatograph. High-performance liquid chromatography (HPLC) determined the endogenous hormones listed below. The liquid chromatograph (Agilent Technologies, model 1100) was equipped with an automatic injector (model 1200) and a Model 1100 Diode Array Detector. The column used was an Rx/SB-C8 Rapid Res (4.6 × 75 mm). The mobile phase consisted of solvent A (80%): 0.1% trifluoroacetic acid and solvent B (20%): 0.1% trifluoroacetic acid in acetonitrile. The flow was 2 mL.min^{-1}, the temperature was 60°C, the injection volume was 100 µL, and the detector was set at 254 nm (Tejeda-Sartorius et al., 2022b).

To ensure accuracy in hormone profiling, endogenous cytokinins (CKs), including zeatin (ZEA) and trans-zeatin (T-ZEA), indoleacetic acid (IAA), and gibberellins (GA), were analyzed using high-performance liquid chromatography (HPLC) coupled with a UV detector, following the method described by Linskens and Jackson (1987). Hormone extraction and quantification were performed at Laboratorium Starlab Analitik Indonesia in Bogor, Indonesia. A minimum of three replicates was used to ensure reproducibility. Each hormone was identified and quantified against a standard curve using growth regulator standards obtained from Sigma-Aldrich (Saint Louis, MO, USA). The method adhered to the guidelines outlined by Linskens and Jackson (1987), emphasizing the importance of sample purification to remove interfering substances such as proteins, carbohydrates, pigments, and lipids from plant extracts.

In-vivo measurements used a portable gas-exchange system (Li-Cor 6800, Li-Cor, Lincoln, Neb, USA), which allows measurements of the exchange of water and CO_2 between leaves and air and simultaneous chlorophyll fluorescence measurements. Single leaves were enclosed in a 6 cm^2 gas-exchange cuvette (for the scale-like leaves of *C. pandurata*, a round-shaped 200 cm^3 conifer cuvette, Li-Cor 6800) and sampled under growing conditions of temperature, light, humidity, and CO_2 concentration. The temperatures in the study area ranged from 20.1°C to 33.4°C, with light intensity of 100–400 mmol.m^{-2}.s^{-1} and relative humidity of 40–60% (BMKG, 2022).

Photosynthesis, transpiration, stomatal conductance, and intercellular CO_2 concentration (Ci) were calculated from gas exchange measurements following the formulations outlined in Caemmerer and Farquhar (1981). Fluorescence measurements were performed to estimate the maximal quantum yield in dark-adapted leaves, as indicated by the ratio between the variable and maximal 254 D. fluorescence (Fv/Fm), and quantum yield in illuminated leaves, as indicated by the parameter DF/Fm' (the ratio between maximal steady-state fluorescence and maximal fluorescence). The parameter DF/Fm' was then used to calculate the driving photosynthesis and photorespiration linear electron transport (Jf), as shown by Loreto et al. (1994). Details of the nomenclature and connotation of the fluorescence parameter are given in Olaf and Jan, (1990). All gas exchange measurements were performed between 10:00 and 14:00 h.

Phenological analysis was conducted on selected plants based on their vegetative and generative phases. Measurements of plant parts were performed with nine replications. The data were then analyzed to determine changes in plant phenology, particularly in leaves, bulbs, and flowers.

The mean ± standard error of the mean (SEM) was determined for three replicates. The ExpDes package in R was used to perform ANOVA followed by the Scott-Knott test and the correlation analysis. Statistical significance was set at $P < 0.05$.

Results and Discussion

Total Chlorophyll, Anthocyanin, and Carotene Concentration at Different Growth Stages

Table 1 demonstrated increased chlorophyll a, b, and total chlorophyll in the leaves. This increase occurs during the vegetative phase and is correlated with a higher photosynthetic capacity. The augmentation of total chlorophyll facilitates increased PPFD, water vapor uptake, and CO_2 assimilation. This boost in photosynthetic capacity is critical for preparing biomass for the flowering phase, which demands adequate energy, water, and CO_2 and is accompanied by increased leaf chlorophyll content.

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The total chlorophyll content, including chlorophyll-a and chlorophyll-b, exhibited slight variations between the vegetative and generative phases. Chlorophyll-a content increased from 1.21 ± 0.17 mg.g^{-1} during the vegetative phase to 1.30 ± 0.24 mg.g^{-1} in the generative phase. Similarly, chlorophyll-b content rose from 0.76 ± 0.10 mg.g^{-1} to 0.82 ± 0.17 mg.g^{-1}. Consequently, the total chlorophyll (a + b) content was higher in the generative phase (2.36 ± 0.42 mg.g^{-1}) compared to the vegetative phase (1.96 ± 0.27 mg.g^{-1}), indicating an overall increase in chlorophyll accumulation as the plant matured. The results showed that the total chlorophyll, chlorophyll-a content, and chlorophyll-b increased when entering the flowering phase. The increase in chlorophyll content is important as the plants require energy and high assimilates when entering the flowering phase. Similar results were reported in *Cymbidium macrorhizon* orchids (Suetsugu et al*.,* 2018).

Anthocyanin content remained relatively stable across both phases, with values of 0.06 ± 0.00 mg per 100 g fresh weight during the vegetative phase and 0.06 ± 0.03 mg per 100 g in the generative phase, suggesting minimal changes in anthocyanin accumulation over time. The total content of anthocyanins and carotene did not change from vegetative to generative. The changes did not occur significantly in the leaves; no analysis was conducted on the pseudo bulbs. These results are consistent with significant changes in anthocyanin and carotene content in the flower organs of *Rhyncholaeliocattleya* Beauty Girl 'KOVA' (Li et al., 2020) and *Cymbidium* (Wang et al., 2014) but not in the leaves and bulbs.

Total N, P, K, and C-Organic

The increase in photosynthetic Content in *C. pandurata* organs capacity aligns with the rise in nitrogen levels in orchid plant organs. Nitrogen content was highest in leaves, with values of 1.54% during the vegetative phase and 1.70% during the generative phase, showing a slight increase as the plant matured. In contrast, bulbs' nitrogen levels were significantly lower, decreasing from 0.67% in the vegetative phase to 0.45% in the generative phase. The highest nitrogen concentration was observed in flowers during the generative phase (1.50%), indicating a substantial nitrogen allocation towards reproductive organs. The highest nitrogen content is found in the leaves. Nitrogen in the leaves increases upon entering the flowering phase, whereas nitrogen in the bulbs decreases. This reduction in bulb nitrogen content is a physiological change in the plant to enhance carbon absorption in preparation for flowering (Zhang et al., 2022). Nitrogen is essential as a signaling molecule for various photosynthetic processes, thus requiring high levels in the leaves during the vegetative phase. However, upon entering the generative phase, the nitrogen concentration in the bulbs is transferred to the leaves, resulting in a decrease in the bulb, which acts as a sink (Inkham et al., 2022).

Table 1. Changes in total chlorophyll, anthocyanin, and carotene content from the vegetative stage to the generative stage of *C. pandurata*

Pigments	Unit	Vegetative phase	Generative phase
Total chlorophyll-a	mg.g^{-1} of fresh weight	1.21 ± 0.17	1.30 ± 0.24
Total chlorophyll-b	mg.g^{-1} of fresh weight	0.76 ± 0.10	0.82 ± 0.17
Total chlorophyll-a and b	mg.g^{-1} of fresh weight	1.96 ± 0.27	2.36 ± 0.42*
Anthocyanin	mg.100 g of fresh weight	0.06 ± 0.00	0.06 ± 0.03*
Carotene	mg.g^{-1} of fresh weight	0.46 ± 0.04	0.44 ± 0.07

Notes: each value is presented as mean ± standard error (SE); *significant at $p < 0.05$ (t-student test); n=9.

Table 2. Changes in the total N, P, K, and organic C in black orchid plant *C. pandurata organs*

Plant organ	Phase	N (%)	P (%)	K (%)	Organic C (%)	C/N ratio
Leaf	Vegetative	1.54 ± 0.17a	0.11 ± 0.01b	1.22 ± 0.08	43.90 ± 0.86	28.85 ± 2.10 b
Leaf	Generative	1.70 ± 0.15a	0.11 ± 0.01b	1.30 ± 0.10	42.97 ± 1.30	25.40 ± 1.25 b
Bulb	Vegetative	0.67 ± 0.19b	0.10 ± 0.02b	1.52 ± 0.62	40.83 ± 3.70	65.24 ± 12.90 a
Bulb	Generative	0.45 ± 0.02b	0.10 ± 0.15b	1.31 ± 0.35	38.46 ± 3.70	85.36 ± 2.50 a
Flower	Generative	1.50 ± 0.27a	0.17 ± 0.00a	1.82 ± 0.26	35.00 ± 8.60	24.12 ± 4.54 b

Notes: each value is presented as mean ± standard error (SE); values followed by different lowercase letters (a-b) in each column showed significant differences (Tukey, $p \leq 0.05$) n = 9.

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Changes in total N concentration in plant organs have been widely studied during changes from the vegetative to the generative phase. Studies on *Phalaenopsis Sogo* Yukidian 'V3' orchids (Susilo and Chang, 2014) including long periods without fertilization. Significant nutrient storage is thought to account for this characteristic; however, the use of stored nutrients in *Phalaenopsis* has not been fully studied. We used 15 N-labeled Johnson's solution to trace the use of stored nitrogen (N concluded that orchid plants require high concentrations of N when entering the flowering phase. The best nitrogen treatment was 14.3 mM, which supported the standard flowering performance without delaying spiking or reducing flower count (Susilo and Chang, 2014). This level ensured sufficient nitrogen for vegetative growth and flowering, maintaining a balance between both stages. In contrast, high nitrogen (28.6 mM) delayed flowering while low nitrogen (1.4 mM) or no nitrogen induced earlier spiking but with a reduced flower count in the miniature cultivar. The standard cultivar remained unaffected by these variations (Susilo and Chang, 2014). A study by Zhang et al. (2022) on *P. aurita* orchids showed an increase in total N concentration in the leaves and a decrease in total N concentration in the bulbs upon transition to the flowering phase. Studies on *Dendrobium* orchids showed an increase in total N in the leaves of plants entering the flowering phase (Karoojee et al., 2021). The results of previous studies (Lin et al., 2019) These results are consistent with this study's results, which show an increase in total leaf N concentration when entering the flowering phase and a decrease in total N in the bulbs.

The concentrations of leaf and bulb phosphorus (P) do not differ significantly (Table 2). Phosphorus content remained relatively stable across different plant growth phases, ranging from 0.10% to 0.17%. The highest phosphorus level (0.17%) was found in flowers during the generative phase, while other plant parts had comparable levels (0.10%–0.11%), suggesting a more significant phosphorus demand in reproductive structures. During early vegetative growth, P concentration is lower in the leaves and higher in the bulbs compared to nitrogen. This indicates that the plant absorbs less P during the vegetative phase, subsequently accumulating P in the bulbs, which require sufficient energy for the onset of flowering. In the following stages, P is transferred to the flower-forming shoots. Phosphorus is an essential component of adenosine triphosphate (ATP), a molecule that stores and transfers energy within cells (Bonora et al., 2012). In the photosynthesis process, solar energy is used to convert adenosine diphosphate (ADP) into ATP through light reactions in chloroplasts. The formed ATP is then utilized in the dark reactions (Calvin cycle) for sugar synthesis from carbon dioxide (Hader, 2022). Phosphorus is also an integral part of the structure of deoxyribonucleic acid (DNA) and ribonucleic acid (RNA), which are crucial for protein synthesis and cell replication, essential processes for the growth and repair of photosynthetic cells in leaves (Odoom and Ofosu, 2024). Additionally, phosphorus is a component of phospholipids that compose cell and organelle membranes, including chloroplasts, which are the main sites of photosynthesis. These membranes are vital for maintaining an optimal internal environment for photosynthetic reactions (Sharma et al., 2020). Many photosynthetic enzymes require phosphorus for activation and function, including ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCO), the key enzyme in the Calvin cycle that requires phosphate. In the light reactions of photosynthesis, NADP+ is reduced to NADPH through the electron transport chain in the thylakoid membrane (Hajong et al., 2013). NADPH then provides electrons and protons for the dark reactions in the Calvin cycle, with phosphorus being a critical component of NADP+ (Zhang et al., 2018). Phosphorus's role in photosynthesis is by activating key enzymes such as RuBisCO and facilitating NADPH formation in the electron transport chain (Khan et al., 2023). Optimal phosphorus availability enhances carbon assimilation efficiency in the Calvin cycle, directly influencing plant growth, chlorophyll content, and endogenous hormone concentrations (Odoom and Ofosu, 2024).

The phosphorus concentration in the leaves and bulb of *C. pandurata* was 0.10 and 0.11%, respectively, which is different from the total P concentration in leaves and bulbs of 7.61 and 6.54 ppm in *Laelia autumnalis* (Emeterio et al., 2021). The differences occur due to different plant species. Leaf potassium concentration in *C. pandurata* increased when entering the generative phase but decreased in the bulb when entering the generative phase. The results of this study are consistent with previous research on *Dendrobium* spp., which showed that entering the generative phase, plants experienced an increase in potassium demand in plant organs, reaching 0.02 to 0.03% (De, 2020). Changes in total C-organic concentration in *C. pandurata* plants decreased in bulb and leaf plants when entering the generative phase. The results of this study are consistent with research conducted by Tsai and Chang (2022). *Phalaenopsis* Sogo Lotte 'F2510' and P. Sogo Yukidian 'V3' decreased their C-organic concentration when entering the generative phase.

Potassium levels varied between plant parts, with the highest flower concentration during the generative phase (1.82%) and bulbs in the vegetative phase

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(1.52%). Leaves contained slightly lower potassium levels, increasing from 1.22% (vegetative) to 1.30% (generative). In bulbs, potassium levels declined from 1.52% (vegetative) to 1.31% (generative), possibly due to nutrient remobilization. Potassium (K) concentration in the plant does not show significant differences. However, leaf K concentration increases when entering the flowering phase and decreases in the bulbs. High leaf potassium concentration is related to its role in stomatal regulation, affecting stomatal conductance, water absorption, and carbon dioxide (PPFD). The reduced potassium (K) concentration in the bulbs suggests that a significant amount of potassium is necessary to regulate osmotic pressure and facilitate the transfer of nitrogen to the leaves and phosphorus to the bulbs. Potassium in plants plays a crucial role in stomatal conductance. Potassium ions move in and out of the guard cells of the stomata, causing changes in osmotic pressure (Eburneo et al., 2017)providing data on the anatomical differentiation that aids the acclimatization process of this species. Plants from in vitro seeding were used; 5 protocorms of approximately 0.5 cm were inoculated into vials with a capacity of 500 mL containing 100 mL of alternative culture medium plus potassium silicate (0.0, 0.5; 1.0 $mL.L^{-1}$. When potassium ions enter the guard cells, water also enters due to osmosis, causing the guard cells to swell and open the stomata. Potassium regulates stomatal conductance by influencing the movement of potassium ions into and out of the guard cells, affecting osmotic pressure. In this study, applying potassium silicate led to significant changes in stomatal characteristics. The stomatal density decreased when 1 $mL.L^{-1}$ K_2SiO_4 was used, and the stomata exhibited smaller polar and equatorial diameters with values of 0.0 and 0.5 $mL.L^{-1}$. Additionally, the guard cells became more elliptical with 0.5 $mL.L^{-1}$ and 1 $mL.L^{-1}$ K_2SiO_4 under artificial light conditions (TAE), enhancing the plant's ability to regulate water loss efficiently. (Biswas et al., 2021. Conversely, when potassium ions exit, water also exits, causing the guard cells to shrink and close the stomata. Furthermore, potassium ions assist in the activation of enzymes such as RuBisCO (ribulose-1,5-bisphosphate carboxylase/oxygenase), which is a key enzyme in carbon fixation (Zotz and Winkler, 2013).

Leaf organic carbon content was the highest during the vegetative phase (43.90%) but slightly decreased in the generative phase (42.97%. Table 2). Bulbs gradually reduced carbon content, from 40.83% in the vegetative phase to 38.46% in the generative phase. The lowest organic carbon content was found in flowers during the generative phase (35.00%), suggesting higher metabolic activity and carbon utilization for reproductive development. Nutrient concentrations in plant organs are closely related to organic carbon absorption. The concentration of organic carbon (C-organic) does not show significant differences in the leaf and bulb organs. However, its concentration decreases when entering the flowering phase. The concentration increase during the vegetative phase is related to the enhanced photosynthetic capacity requiring high carbon levels. Its concentration continues to rise until the early generative phase and decreases upon entering the complete generative phase.

The concentration of C-organic in plant organs is closely associated with nitrogen. The C/N ratio varied significantly, with the highest values observed in bulbs (65.24 in the vegetative phase and 85.36 in the generative phase), indicating a high carbon accumulation relative to nitrogen. Leaves had a moderate C/N ratio, decreasing from 28.85 (vegetative) to 25.40 (generative). The lowest C/N ratio was found in flowers during the generative phase (24.12), reflecting a higher nitrogen concentration in reproductive structures. The carbon-to-nitrogen ratio (C/N) can indicate physiological changes as the plant enters the flowering phase. The C/N ratio in the bulbs increases because, physiologically, the accumulation of P in the bulbs reduces N concentration, which is then transferred by K to the leaves. Upon entering the generative phase, the plant increases carbon absorption in the bulb as a sink, leading to changes in the plant apex, which shifts the growth direction from vegetative shoots to flowering.

Photosynthesis occurs intensively during the vegetative phase and involves the use of macro and microelements, with nitrogen (N), phosphorus (P), and sulfur (S) contributing approximately 30%, 25%, and 15%, respectively. Microelements such as zinc (Zn) and iron (Fe) play key roles, with zinc enhancing chlorophyll synthesis by up to 40% and iron supporting electron transport chains, thereby improving photosynthetic efficiency by approximately 20% (Kumar et al., 2021)phosphorus (P. Nitrogen is one of the crucial elements in photosynthesis, as it is a primary component of chlorophyll, the pigment that captures solar energy. Nitrogen deficiency significantly reduces chlorophyll content, typically by up to 30–50%, which impacts plants' photosynthetic efficiency (Simkin et al., 2022). Lower chlorophyll levels can result in a 20–40% decrease in light absorption capacity, leading to reduced energy capture for photosynthesis. This is crucial as chlorophyll, along with carotenoids, is responsible for light harvesting and protecting the plant from photodamage, which is necessary for maintaining optimal photosynthetic function (Simkin et al., 2022). Without sufficient nitrogen, plants cannot produce chlorophyll optimally,

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leading to decreased photosynthesis efficiency. Additionally, nitrogen is a major component of amino acids that form proteins. These proteins include enzymes that catalyze photosynthesis reactions, both in the light reactions and the dark reactions (Calvin cycle) (Zhou et al., 2023). Nitrogen-containing enzymes such as ATP Synthase catalyze the formation of ATP from ADP and inorganic phosphate using energy from the proton gradient generated by electron transport. NADP+ Reductase plays a role in the light reactions by converting NADP+ to NADPH, which is required in the Calvin cycle for the reduction of CO_2 to sugar (Yin et al., 2015)diazotrophs were grown in the presence of various concentrations of nitogen (N.

The C/N ratio, which indicates the balance between carbon and nitrogen in plant tissues, differs significantly across various plant organs and growth stages. In the vegetative leaf phase, the C/N ratio was 28.85, decreasing slightly to 25.40 in the generative leaf phase. In the bulb, the C/N ratio was much higher, reaching 65.24 in the vegetative phase and 85.36 in the generative phase. The flower had a C/N ratio of 24.12, indicating its distinctive nitrogen requirements for flowering. These ratios highlight the importance of nitrogen and carbon in different developmental phases and plant parts. Orchid plants experience a decrease in the C/N ratio upon entering the flowering phase in the bulb, characterized by a reduction in nitrogen uptake and an increase in carbon uptake. This shift aims to redistribute photosynthetic products to form and redirect growth toward flowering (Minasiewicz et al., 2023). The decrease in the C/N ratio is also associated with the enzyme Sucrose Synthase (SuSy), which catalyzes the formation of sucrose essential for transporting sugars to developing floral organs (Tsai and Chang, 2022).

Changes in the C/N ratio in this study showed a decrease in the leaves by 13.01 (Table 2), but an increase of 28.33 in the bulb when entering the generative phase, and total C/N in all parts of the plant decreased by 1.54. The results differed from an increase in the C/N ratio of 44.49 in the bud when entering the flowering phase and an increase of 15.6 for all parts of the *Phalaenopsis* orchid plant. This difference in C/N ratio occurs due to differences between *C. pandurata* and *Phalaenopsis (*Lin et al., 2019).

This study is the first reported change in the phenology of *C. pandurata* in the leaves and bulbs, which increased in size. There was an increase in total N concentration in the leaves and a decrease in the bulb entering the generative phase. The total P concentration did not change in the leaves and bulb when entering the flowering phase. Total K concentration increased in the leaves and decreased in the bulb when entering the generative phase. When entering the generative phase, the total C-organic concentration decreased in bulb and plant leaves. The C/N ratio was found to decrease in the leaves, whereas the C/N ratio was shown to increase in the bulbs once the plants had reached their optimal vegetative growth.

Endogenous Hormone Levels

Changes in growth direction are also marked by alterations in auxin content within plant organs (Figure 1A). The natural auxin in plants (IAA) shows the highest concentrations in leaves and bulbs during the vegetative phase. This hormone concentration decreases in leaves and bulbs when entering the flowering phase. IAA synthesis at the growth points is then transferred to the leaves and bulbs to regulate cell growth and division. IAA plays a role in stomatal conductance regulation in leaves, as evidenced by the high concentration of IAA in leaves during the vegetative phase. It also enhances stomatal conductance, water absorption, and CO_2 uptake. Upon entering the flowering phase, IAA is transferred mainly to flower organs to distribute the hormonal signals required for flower initiation.

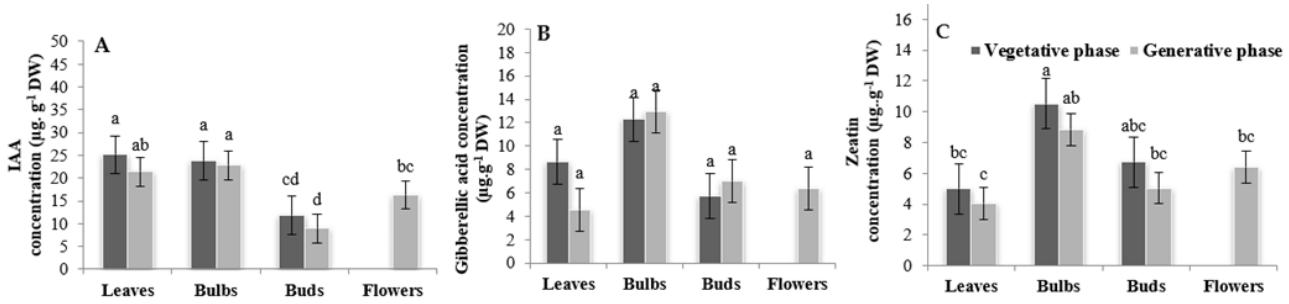

Figure 1. Changes in the content of endogenous indole acetic acid (IAA)(A), gibberellic acid (GA), (B), and zeatin (C) at different *C. pandurata* organs and growth stages. Bars (standard deviations) with different letters indicate significant statistical differences (Tukey, $p \leq 0.05$, n = 3). DW = dry weight.

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The decreasing IAA concentration in leaves and bulbs contrasts with the gibberellin concentration in plant organs. Statistically, gibberellin concentrations in plant organs do not show significant differences; however, its concentration pattern differs from IAA, which tends to decrease upon entering the flowering phase. Gibberellin concentration increases in the bulbs during the flowering phase. This increase aims to receive hormonal signals related to flowering. Gibberellin also helps break dormancy in bulbs, which serve as nutrient storage.

In contrast to GA, zeatin, a cytokinin hormone, exhibits a concentration pattern similar to IAA, as it decreases when entering the flowering phase. The highest concentration of zeatin is found in the bulbs, indicating that zeatin physiologically synergizes with GA during the flowering phase. Zeatin increases the sensitivity of plant tissues to GA, enhancing GA's effect in promoting flowering. Additionally, the high concentration of zeatin in bulbs is associated with its role in regulating flowering genes.

The plant hormone IAA plays a role in several metabolic activities of orchids, particularly related to the induction of flowering (Ferreira et al., 2006). IAA aids in initiating flowering by modulating the expression of genes associated with flower initiation (Ahmad et al., 2021). The optimal level of IAA in meristematic tissues can stimulate the transition from the vegetative phase to the generative (flowering) phase (Huang et al., 2021). Several genes are involved in the induction of flowering in orchids (Li et al., 2021).

The endogenous hormone auxin decreases in the leaf and bulb organs upon entering the flowering phase (Figure 1A). In orchids, the decrease in auxin upon entering the flowering phase is part of a natural process known as flower differentiation. Auxin, which is responsible for cell growth and tissue elongation, experiences a decrease in concentration owing to a shift in the growth focus from vegetative growth (such as leaves and stems) to flower formation (Doorn† and Ketsa, 2005). During the flowering phase, plants must allocate resources for flower formation and reproduction, thus shifting the focus from vegetative to generative growth. The decrease in auxin triggers cell differentiation processes that lead to the formation of flower structures, such as petals and sepals. Conversely, other hormones, such as cytokinins and gibberellins, may become more dominant in facilitating flower formation and fruit development (Wan et al., 2024). The results of this study differ from those of Campos and Kerbauy (2004), who reported that leaf and bud's IAA content increased in *Dendrobium "*Second Love" when entering the flowering phase. The results of this study also differ from those of *Arundina graminifolia* orchids, which reported an increase in IAA content in the leaves and bulbs entering the flowering phase (Ahmad et al., 2021).

Gibberellin A3 (GA_3) is a phytohormone that influences the flowering process by modifying various physiological and molecular mechanisms (Zhang et al*.,* 2023). GA_3 enhances flowering by promoting floral meristem identity and accelerating the floral transition. This process involves activating specific transcription factors and the upregulation of genes responsible for flower development (Singh and Roychoudhury, 2022). In orchids, GA_3 induces the expression of key genes essential for the initiation of flowering (He et al*.,* 2020). Furthermore, GA_3 influences flower stalk elongation and enhances overall flower architecture by stimulating cell division and elongation in floral tissues (Shah et al., 2023). This is particularly beneficial in ornamental orchids, where flower appearance is a crucial commercial attribute.

Analysis of endogenous hormones GA showed a different pattern to auxin and cytokinins. There was an increase in the GA content in the bulb when entering the flowering phase (Figure 1 B). The gibberellin levels increase upon entering the flowering phase in orchids, which is part of the complex hormonal regulation involved in the transition from the vegetative to the generative phases. Gibberellin, a plant hormone, regulates stem growth, seed germination, and flowering. In orchids, the increase in gibberellin may contribute to several aspects of flowering, such as stimulation of stem growth, which may be necessary to support flower growth and development. Gibberellin has been shown to trigger flowering in some plant species, and an increase in gibberellin concentration may signal the initiation of the flowering process in orchids. Gibberellin can influence various aspects of flower development, including flower formation, flower opening, and differentiation of flower organs, such as stamens and pistils. The significance of gibberellin in orchid flowering indicates that this hormone plays a key role in regulating the plant's life cycle and ensuring a smooth transition from vegetative to reproductive phases. However, the role of gibberellin in regulating orchid flowering is still a subject of ongoing research, and further studies are needed to understand better the hormonal interactions involved in this process (Yin et al., 2022). Changes in endogenous hormone content in plant parts would determine the flowering pathway (Wang et al., 2019). The increase in endogenous GA content entering the flowering phase indicates that *C. pandurata* orchids go through the GA flowering pathway. Matsumoto

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(Matsumoto, 2006) reported that increases in the GA content induce flowering in *Miltoniopsis hybrid* orchids. Similar results were reported by Cardoso et al. (2010). Five GA_3 concentrations (0, 125, 250, 500 and 1,000 $mg.L^{-1}$ in *Brassocattleya* and *Cattleya* hybrid orchids.

Zeatin plays a crucial role in orchid flowering through various physiological mechanisms (Gajdošová et al., 2011). Cytokinins are involved in various processes of growth and development, including cell division, cell enlargement, and cell differentiation (Li et al., 2021). In orchids, zeatin influences flowering by promoting the transition from vegetative to reproductive phases. Zeatin helps induce and maintain apical meristem activity, the central hub for flower growth and development. This is achieved through the regulation of gene expression involved in flower development, as well as interactions with other hormones such as auxin and gibberellin. Zeatin also plays a role in enhancing plant resistance to unfavorable environmental conditions, such as drought stress, by increasing chlorophyll content and improving photosynthetic activity (Blanchard and Runkle, 2008).

Changes in cytokinin content in the leaves and bulb organs decreased after the flowering phase. This decrease aligned with endogenous auxin, but differed from GA in bulb organs (Figure 1 B). The decrease in cytokinin levels after entering the flowering phase in orchids is part of the hormonal regulation associated with the transition from vegetative to reproductive growth. Cytokinins play roles in cell division, shoot growth, and delay senescence (Werner et al., 2001). During the flowering phase, the plant reallocates resources from vegetative growth to flower development and reproduction. This shift in resource allocation may result in a decrease in cytokinin levels, as the plant prioritizes flower formation over other growth processes. Additionally, cytokinin levels may decrease to allow for a proper balance of hormonal signals necessary for flower development. Although cytokinins promote shoot growth and cell division, excessive levels of cytokinins may inhibit the differentiation of floral organs and interfere with flowering initiation (Ahmad et al., 2021). Therefore, the decrease in cytokinin levels in orchids after entering the flowering phase likely facilitates the transition to reproductive growth and ensures the proper development of flowers. This hormonal regulation ensures that the plant can effectively allocate resources to produce healthy and viable flowers (Ahmad et al., 2022). The results of this study contrasted with those of Blanchard and Runkle (2008), who reported that endogenous cytokinin content increased when *Doritaenopsis* and *Phalaenopsis* orchids entered the flowering phase. The difference between the two plant responses is due to plant type and species differences. Likewise, Blanchard and Runkle (2008) reported that endogenous cytokinin content increased when *Phalaenopsis* orchids entered the flowering phase.

The correlation between endogenous hormones IAA and GA_3 was significant and positive, indicating that IAA has a mutually supportive influence on the metabolism of black orchids. These results are consistent with those of previous studies by Asyraf et al. (2021), who reported that IAA and GA positively affect the number of flowers and resupination in a hybrid *Dendrobium Burmese* Ruby, Bee Lian 1989 × *Dendrobium* Mae-klong, Semachai 1975. The correlation between endogenous hormones IAA and zeatin also showed a significant positive correlation, as reported by Netlak et al. (2022) in *Dendrobium* spp. and *Laelia anceps* (Tejeda et al., 2022).

NAR value does not show significant changes at different growth stages (Table 3). The leaf PPFD values showed significant differences, with the highest value in the early vegetative stage. The Transpiration rate value does not show a significant difference at different stages. Stomatal conductance to water vapor value showed differences between the vegetative and early generative phases; the value of total conductance to CO_2 and total conductance to water vapor changed when the plant entered the generative phase and decreased, and the value of total conductance to water vapor (Table 3).

The increase in photosynthetic photon flux density occurs during the vegetative growth phase and continues to rise until the early flowering stage. The plants' photosynthetic capacity varies across different growth phases. During the vegetative phase, the net assimilation rate was 2.35 $\mu mol.m^{-2}.s^{-1}$, while in the early generative phase, it increased to 2.73 $\mu mol.m^{-2}.s^{-1}$, but decreased to 1.85 $\mu mol.m^{-2}.s^{-1}$ in the generative phase. PPFD absorbed by the leaf was highest in the early generative phase (82.12 $\mu mol.m^{-2}.s^{-1}$) compared to the vegetative (71.39 $\mu mol\ m^{-2}\ s^{-1}$) and generative phases (56.76 $\mu mol.m^{-2}.s^{-1}$). The transpiration rate was slightly positive in the vegetative (0.02 $mmol.m^{-2}.s^{-1}$) and early generative phases (0.3 $mmol.m^{-2}.s^{-1}$), while it drastically decreased to -2.4999 $mol.m^{-2}.s^{-1}$), in the generative phase. Stomatal conductance to water vapor and CO_2 was highest in the vegetative and early generative phases (11.3 and 11.5 $mmol.m^{-2}.s^{-1}$, respectively), dropping sharply in the generative phase (1.4 $mmol.m^{-2}.s^{-1}$). Total conductance to water vapor followed a similar pattern: higher in the vegetative and early generative phases (11.3 and 11.5 $mmol.m^{-2}.s^{-1}$), and decreasing in the generative phase (1.4 mmol.

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$m^{-2}.s^{-1}$). These data indicate significant physiological changes in photosynthesis, transpiration, and gas exchange as the plant transitions through its growth stages.

Plant photosynthesis generally occurs in the leaves but can also occur in other chlorophyll-containing organs. (Henry et al., 2020). Plants experience increased light absorption through their leaves (Hogewoning et al., 2021), followed by stomatal conductance, which serves as the pathway for water vapor and CO_2 exchange. The products of photosynthesis assimilate and are then transferred to the plant's sink organs to meet energy and nutrient requirements during the early flowering phase (Feng et al., 2021).

The photosynthetic capacity of plants can be determined by various factors, including the net assimilation rate, PPFD absorbed by the leaf, transpiration rate, stomatal conductance to water vapor, total conductance to CO_2, and total conductance to water vapor. The photosynthetic capacity of plants can be determined by various factors, including the net assimilation rate, which decreased by 21.16%, PPFD absorbed by the leaf, which decreased by 20.58%, transpiration rate, which increased dramatically by 1,249,950%, stomatal conductance to water vapor, which decreased by 87.38%, total conductance to CO_2, which decreased by 87.38%, and total conductance to water vapor, which decreased by 87.67%. The results of this study show that the net assimilation rate of plants has no significant difference between the vegetative stage and the generative stage; this result is different from (Wolfe et al., 2023) and is important for many crops. Structural reorganization of chromosomes and repatterning of gene expression are frequently observed in allopolyploids, with physiological and ecological consequences. Recurrent origins from different parental populations are widespread among polyploids, resulting in an array of lineages that provide excellent models to uncover mechanisms of adaptation to divergent environments in early phases of polyploid evolution. We integrate here transcriptomic and ecophysiological comparative studies to show that the net assimilation rate in the orchid *Dactylorhiza majalis* changed at different growth stages and environments, with variations observed in chlorophyll content (42.9), light energy flux (LEF: 50.1), and photosynthetically active radiation (PAR: 244.7). Significant species effects were found in parameters such as NPQt (0.34, p = 1.5e-02) and PhiNPQ (0.08, p = 2.9e-03), indicating physiological adaptability. The difference in results is due to differences in orchid species and plant environments. The results of the PPFD absorbed by the leaves showed significant differences in the growth stage of *C. pandurata*, with the highest absorption found in the early generative stage (Tabel 3) . This result differs from Ceusters et al. (2019), who reported that the highest PPFD absorbed by leaves occurred at the vegetative stage in *Phalaenopsis* orchids. Differences in plant species, morphology, and environment can cause these differences.

The transpiration rate value in this study showed insignificant results between the stages. Physiologically, plants produce relatively the same amount of assimilates in each phase; however, their utilization differs at each stage. This finding contrasts with the results reported by Jindamol et al. (2019), who reported significant changes in different stages and environments of *Dendrobium Sonia* and 'Earsakul' orchid. Different genera and plant environments caused differences in the results. In the present study, the stomatal conductance to water vapor in this study differed at each stage, with the highest value in the early generative stage. This result differs from that of Onipchenko et al. (2023), who reported that the value of stomatal conductance to water vapor decreased

Table 3. Change in photosynthetic capacity from vegetative to generative phase of *C. pandurata*

Photosynthetic capacity	Phase		
	Vegetative phase	Early generative	Generative phase
Net assimilation rate ($\mu mol.m^{-2}.s^{-1}$)	2.3504 ± 0.41	2.7368 ± 0.38	1.8507 ± 0.24
Leaf PPFD ($\mu mol.m^{-2}.s^{-1}$)	71.3940 ± 5.48 ab	82.1197 ± 3.56 a	56.7570 ± 3.02 b
Transpiration rate ($mmol.m^{-2}.s^{-1}$)	0.2 ± 0.00	0.3 ± 0.00	-2499.9 ± 2.50
Stomatal conductance to water vapor ($mmol.m^{-2}.s^{-1}$)	18.2 ± 0.00 a	18.5 ±0.00 a	2.3 ± 0.00 b
Total conductance to CO_2 ($mmol.m^{-2}.s^{-1}$)	18.1 ± 0.00 a	18.4 ± 0.00 a	2.3 ± 0.00 b
Total conductance to water vapor ($mmol.m^{-2}.s^{-1}$)	11.3 ± 0.00 a	11.5 ± 0.00 a	1.4 ± 0.00 b

Notes: Different letters in each column indicate statistical differences (Tukey, $p \leq 0.05$; n = 3).

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during flowering in some epiphytic orchid plants. Differences in species, habitat, and other factors may have contributed to these results. The total conductance to CO_2 and total conductance to water vapor values showed a significant difference between plant growth stages; the highest value was shown in the early generative stage. This result differs from (Kobayashi et al., 2021), who reported that the total conductance to CO_2 and total conductance to water vapor decreased when entering the generative phase in *Cymbidium macrorhizon* orchids. Transpiration and stomatal conductance differ between growth phases due to changes in physiological needs. During the vegetative phase, both are typically higher to support rapid leaf expansion and efficient nutrient transport. In the generative phase, transpiration and conductance may decrease as the plant prioritizes energy and resources for reproductive development, with reduced stomatal opening to minimize water loss. Measuring these parameters is crucial for understanding water use efficiency, plant stress responses, and resource allocation, helping optimize irrigation and management practices to enhance crop productivity.

Plant Phenological Changes

Changes in photosynthetic capacity, total chlorophyll, anthocyanin content, nutrient, and phytohormone alterations in plants are followed by phenological changes. These phenological changes are driven by the rate of water and CO_2 absorption through the roots and stomata in the leaves. During the vegetative phase, the hormone auxin (IAA) stimulates the formation and elongation of young leaves and mesophyll cell differentiation and regulates stomatal conductance. The role of auxin (IAA) in promoting intensive plant transitions from the vegetative to the generative phase is significant. Additionally, the interaction between auxin (IAA) and cytokinin (Zeatin) optimizes phenological changes during the vegetative phase until reaching optimal nutrient availability. Meanwhile, the hormone gibberellin receives exogenous signals that activate flowering genes, causing a shift in growth direction. Growth then focuses on the formation of flower organs, resulting in insignificant phenological changes in leaves and bulbs. The plants undergo intensive phenological changes during the vegetative phase until nutrient requirements are met, and hormone accumulation occurs in the sink (bulb). Once the plant reaches a maximum vegetative phase, phenological changes focus on the flower organs, namely the stalk, sepals, petals, labellum, and column.

Significant physiological changes occur in various plant parts during the transition from the vegetative to the generative phase. Leaf length increases from 26.91 cm to 33.57 cm (+24.7%), and width from 5.03 cm to 6.49 cm (+29.0%), indicating enhanced photosynthetic capacity. Bulb size also expands, increasing length from 7.54 cm to 8.04 cm (+6.6%) and width from 4.60 cm to 5.42 cm (+17.8%), suggesting nutrient accumulation for reproductive development. In the generative phase, floral structures emerge, with lateral sepals measuring 4.50 cm × 1.21 cm, dorsal sepals 5.01 cm × 1.82 cm, petals 4.81 cm × 1.01 cm, the labellum 3.71 cm × 2.12 cm, and the column 2.01 cm × 0.51 cm. These morphological changes reflect increased metabolic activity, supporting carbohydrate production, nutrient storage, and reproductive organ formation. Physiological changes such as nutrient availability, growth hormones, and photosynthetic capacity significantly influence the phenology of orchids. Phenology encompasses various phases in the plant life cycle, such as flowering time, duration, and fruit and seed formation. The nutrients available to the plant significantly affect orchid phenology. Deficiencies in essential nutrients such as nitrogen, phosphorus, and potassium can hinder growth and delay flowering time. Conversely, adequate and balanced nutrient availability can accelerate the transition to the reproductive phase and extend the flowering duration. Optimum fertilization can

Table 4. Changes in plant phenology from the vegetative to the generative phase

Part of plant	Vegetative phase		Generative phase in *C. pandurata*	
	Length (cm)	Width (cm)	Length(cm)	Width (cm)
Leaves	26.91 ± 0.35	5.03 ± 0.01	33.57 ± 0.51	6.49 ± 0.30
Bulb	7.54 ± 0.05	4.60 ± 0.10	8.04 ± 0.02	5.42 ± 0.01
Sepal Lateral	-	-	4.50 ±0.00	1.21 ± 0.00
Sepal Dorsal	-	-	5.01 ± 0.01	1.82 ± 0.01
Petal	-	-	4.81 ± 0.01	1.01 ± 0.00
Labellum	-	-	3.71 ± 0.01	2.12 ± 0.01
Column	-	-	2.01 ± 0.01	0.51 ± 0.01

Notes: Each value is presented as mean ± standard error (SE), n=9.

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enhance the frequency and quality of flowering in orchids (Cong et al., 2016). Growth hormones such as gibberellin (GA), cytokinins, and auxins play a crucial role in regulating the phenology of orchids. Gibberellin (GA_3) can accelerate the transition from the vegetative phase to the reproductive phase by stimulating the expression of genes related to flowering. Cytokinins, such as zeatin, enhance cell division and differentiation and are essential for flower formation. Auxins aid in the elongation of flower stalks and regulating flower growth direction. The harmonious interaction between these hormones is vital to ensuring that the flowering process occurs at the optimal time and in the most effective manner.

Many studies have been conducted on the phenological changes of plants under various conditions, including on terrestrial orchids *Dienia physis* (J. Koenig) Seidenf., *Disperis zeylanica* Trimen., *Epipogium roseum* (D. Don) Lindl., *Eulophia spectabilis* (Dennst.) Suresh., *Geodorum densiflorum* (Lam.) Schltr., *Habenaria* spp, *Liparis* spp, *Malaxis versicolor* (Lindl.) Abeyw., *Nervilia* spp, *Pecteilis gigantea* (Sm.) Raf. *Peristylus plantagineus* (Lindl.), *P. spiralis* A. Rich., *Satyrium nepalense* D. Don, *Zeuxine longilabris* (Lindl.) Trimen showed changes in the phenology of plants' stems, leaves, and flowers (Hegde and Krishnaswamy, 2021). Furthermore, research on phenological changes in *Vanda tesselata, V. testasis,* and *V. nana* showed changes in the length of roots, leaves, and flowers at different temperatures conducted by Sahu and Chaudhary (2021). Compared with previous research, the results of this study are similar.

Relationship of Plant Growth Hormones to Photosynthetic Capacity

The correlation between growth hormones and photosynthetic capacity is shown in Figure 3. IAA and GA exhibit a positive correlation, physiologically demonstrating that IAA supports optimizing exogenous signal absorption and cell elongation by GA. GA, in turn, supports the role of auxin in stimulating the growth of young leaves, mesophyll cell differentiation, and controlling stomatal opening. Additionally, IAA and zeatin have a positive correlation. This means that physiologically, IAA supports the role of Zeatin in cell division, lateral bud formation, and photosynthetic activity. Similarly, with optimal concentrations, Zeatin synergistically enhances the role of IAA in plant growth. A strong positive correlation is observed between GA and zeatin. GA will receive exogenous signals in the optimal vegetative phase, then transmitted throughout the plant to activate flowering genes. In this process, the presence of zeatin in plant cells promotes the reception of exogenous signals by GA, and zeatin also facilitates GA in cell permeability efforts, leading to cell elongation.

The correlation between GA and IAA exhibits a negative relationship during the vegetative phase but turns positive during the generative phase. Physiologically, GA inhibits the formation of IAA in plant tissues during the vegetative phase; however, GA supports the formation of IAA during the flowering phase. Furthermore, the relationship between GA and zeatin in plant tissues shows a positive correlation in vegetative and generative phases. This implies that GA promotes the formation of zeatin in tissues (Anuar et al., 2017)The achievement of the achievement of a particular job is a form of a performance. Thus, work performance is the stage of work achievement by an individual in an organization. Some of the cases that have occurred at Adi Soermarmo Boyolali International Airport, one of which is negligence by Ground Handling officers, namely, the lack of awareness of Ground Handling officers in using PPE (personal protective equipment. Meanwhile, the correlation between IAA and zeatin during the

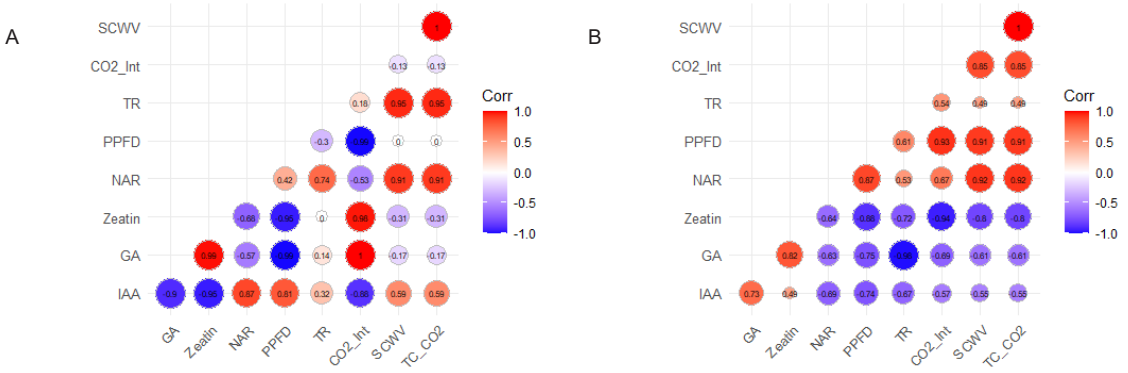

Figure 3. Pearson's correlation visualization in the vegetative (A) and generative (B) of *C. pandurata*. Correlation between endogenous hormones IAA and GA, net assimilation rate (NAR), PPFD absorbed by the leaf, transpiration rate (TR), stomatal conductance to water vapor (SCWV), total conductance to CO_2 (TC_CO_2), CO_2 interceptions (CO_2_INT).

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vegetative phase is negative but shifts to positive upon entering the flowering phase. This indicates that the presence of IAA in plant tissues during the vegetative phase inhibits the formation of zeatin physiologically. In contrast, during the generative phase, IAA supports the formation of zeatin in plant tissues.

The correlation between IAA and photosynthetic capacity parameters shows a positive correlation during the vegetative phase, except for CO_2 interception (Figure 3). In contrast, all photosynthetic capacity parameters negatively correlate during the generative phase. This indicates that during the vegetative phase, the hormone IAA supports the photosynthesis rate, leading to an increase in all parameters of water absorption, CO_2, light interception, net assimilation rate (NAR), and stomatal conductance in the presence of IAA in plant tissues, except for CO_2 interception. However, upon entering the flowering phase, IAA suppresses the photosynthesis rate, thereby reducing all photosynthetic rate parameters in the presence of the hormone IAA.

The correlation between GA and photosynthetic capacity parameters during the vegetative phase is negative, except for CO_2 interception (Figure 3). During the generative phase, all parameters exhibit a negative correlation. This indicates that physiologically, the presence of GA in plant tissues during the vegetative phase inhibits the photosynthesis rate, except for CO_2 interception, and inhibits all photosynthetic rate parameters during the generative phase, as GA stimulates the formation of flower organs. Furthermore, the correlation between Zeatin and photosynthetic capacity parameters shows the same response as the correlation between GA and photosynthetic parameters.

The hormones IAA and GA show a positive correlation (Figure 3); IAA supports GA's absorption of exogenous signals and cell elongation, and GA supports IAA in stimulating the growth of young leaves, mesophyll cell differentiation, and the regulation of stomatal opening (Khalid and Aftab, 2020). IAA and zeatin also positively correlate, supporting cell division, lateral bud formation, and photosynthetic activity. At optimal concentrations, zeatin synergizes with IAA to optimize plant growth (Yan et al., 2017).

IAA shows a strong negative correlation with NAR and PPFD, while its correlation with transpiration rate, stomatal conductance, CO_2 interception, and total CO_2 conductance is weaker (Figure 3). IAA degrades when exposed to sunlight, affecting stomatal opening and inhibiting the absorption of PPFD, CO_2, and water (Kępczyńska and Orłowska, 2021).

GA and zeatin exhibit a weak negative correlation with NAR and PPFD but a positive correlation with stomatal conductance, CO_2 interception, and transpiration rate, indicating an integrative role in CO_2 absorption, stomatal opening, and water management (Duursma et al., 2013). The relationship between photosynthesis variables shows a positive correlation, reflecting synergy in the photosynthesis process where light absorption, stomatal conductance, and transpiration contribute to carbohydrate production as net assimilates (Kumudini and Patil, 2019).

The analysis of Pearson correlation coefficients indicates that the relationship between the growth hormones IAA, GA, and zeatin is mutually supportive in inducing flowering, suggesting that these three hormones are essential in flowering. This finding is consistent with the report by Song et al. (2023)which is used in ethnic food and traditional medicine in China. Unfortunately, the molecular mechanism related to the nutritional quality and regulation of floral organ development has yet to be elucidated in Huaihua. To understand the molecular mechanism of the different developmental stages of Huaihua, this study evaluated the transcriptome analyses of five different developmental periods from Huaihua. A total of 84,699 unigenes were reassembled from approximate 50 million high-quality clean reads. The results showed that the phenylpropanoid biosynthesis, plant hormone signal transduction, starch and sucrose metabolism, and fatty acid elongation process pathways were strongly induced at different developmental stage genes in Huaihua. During this study, 394 differentially expressed genes (DEGs showed that both auxin and cytokinin are required to induce flowering in *Sophora japonica* L. Alwan et al. (2023) reported a synergistic relationship between gibberellin and cytokinin in inducing flowering in *Dianthus chinensis*. Conversely, growth hormones exhibit a negative relationship with photosynthetic capacity factors. This is because the formation of growth hormones is influenced by various factors, including light, which can inhibit the formation of growth hormones, thereby reducing their concentration in plant organs. This is consistent with the report by Suzuki and Kerbauy (Suzuki and Kerbauy, 2006), which showed that auxin and cytokinin synergistically enhance shoot growth in *Catasetum fimbriatum* (Orchidaceae) under low photosynthetic conditions and light intensity. GA does not have a positive synergy with light in *Dendrobium taurulium* J. J Smit (Budiasih et al., 2020).

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Conclusions

This study first observed and reported changes in physiological parameters during flowering: chlorophyll-a and chlorophyll-b increased, anthocyanins were unchanged, and carotene decreased. Net assimilation and transpiration rates were stable, whereas PPFD absorption, stomatal conductance, and total conductance to CO_2 and water vapor increased. Phosphorus levels were stable, K increased in leaves but decreased in bulbs, and C-organic levels decreased. The C/N ratio decreased in leaves but increased in bulbs. Auxin and zeatin levels decreased, while gibberellin decreased in leaves but increased in bulbs. Phenological changes included larger leaves and bulbs. These findings are crucial for designing treatments to induce flowering in *C. pandurata*.

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