PISSN : 2615-2207 Agrosainstek, 9 (2) 2025: 75-84 EISSN : 2579-843X

AGROSAINSTEK

Jurnal Ilmu dan Teknologi Pertanian

Website jurnal : http://agrosainstek.ubb.ac.id

Research Article

Morphophysiological and Vanillin Quality Evaluation of Vanilla Plants (*Vanilla planifolia* Andr.) under Water Stress

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Received: December 07, 2024 /Received in revised : October 26, 2025/ Accepted: October 29, 2025

ABSTRACT

Vanilla (Vanilla planifolia Andr.) is a globally popular flavoring cultivated in tropical regions such as Madagascar, Indonesia, and Mexico. Due to global climate change, particularly the El Niño phenomenon, droughts have become more frequent, impacting water availability and quality for vanilla plants. This study addresses these challenges by examining the plant's responses to drought at a vanilla plantation in Yogyakarta, Indonesia, over two years. A completely randomized design was used, testing five water stress levels (100%, 50%, 25%, 150%, and 200% field capacity). Variables measured included relative water content, physiological activity, chlorophyll, proline content, leaf total acid, photosynthesis efficiency, and morphological traits. Data analysis was performed using ANOVA and Tukey's HSD test. Vanilla plants exhibited significant physiological and morphological changes in response to varying water conditions. Severe drought (25% water stress) led to reduced relative water content, chlorophyll levels, and CO_2 assimilation, alongside increased proline accumulation. Moderate drought (50% water stress) had a lesser impact. Under field capacity (100%) and excess water (150% and 200%), plants maintained higher relative water content and chlorophyll levels, efficient CO_2 assimilation, and optimal morphological traits. The presence of proline under excess water suggests a dual stress response to drought and waterlogging. Beans from severely drought-stressed plants showed a significant decrease in vanillin content and weight. Identifying and developing vanilla varieties with greater tolerance to water scarcity is essential to ensure sustainable production in the face of climate change.

Keywords: CAM; Drought stress memory; Epiphyte; Quantum yield; Vanilin.

1. Introduction

Vanilla (*Vanilla planifolia* Andr.) is one of the most popular flavoring agents in the world. It is known for its distinct taste and aroma in food products. As a key export commodity, the largest vanilla producers are Madagascar, Indonesia, India, and Mexico. Their products sold to major markets in Europe and America (Tran et al., 2024). This plant is an epiphytic orchid that climbs on trees and shrubs. Its natural habitat is in tropical regions with humid and warm forests (Havkin-Frenkel & Belanger, 2018). The part of the plant utilized is the fruit, which is fermented and dried. The fruit is then extracted to produce vanillin, a compound with high commercial value (Baqueiro-Peña & Guerrero-Beltrán, 2017).

Droughts are becoming increasingly frequent around the world due to global climate change, which affects rainfall patterns, temperature, and hydrological cycles (Naumann et al., 2018). The El Niño phenomenon has worsened this situation. According to Supari et al. (2018) El Niño causes disruptions in rainfall distribution in tropical regions. In tropical vanilla-producing countries such as Madagascar, Indonesia, and India, prolonged droughts lead to a reduction in groundwater availability. This situation is further aggravated by the fact that proper irrigation practices are not widely applied in vanilla cultivation (Adiputra, 2018). Meanwhile, water is crucial for the growth of vanilla plants. As an epiphyte that thrives in humid environments, vanilla is highly susceptible to the impacts of

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DOI: https://doi.org/10.33019/agrosainstek.v9i2.882

drought (De Lima & Moreira, 2022). This is because vanilla plants contain high amounts of water in their tissues (Martínez-Santos et al., 2021). Such disturbances pose a significant risk to both the yield and quality of vanilla beans. Over time, this can disrupt the global supply chain and affect the livelihoods of farmers in vanilla-producing countries.

Drought exerts significant physiological stress on vanilla plants. Reduced water availability disrupts water transport through the xylem (Botomanga et al., 2024). Moreover, low turgor pressure leads to stomatal closure as an adaptive mechanism to reduce transpiration (Martínez-Santos et al., 2021). However, this also restricts gas exchange and CO_2 uptake, resulting in a decline in transpiration and photosynthesis. It directly impacts biomass accumulation. Vanilla (*Vanilla planifolia* Andr.) is an epiphytic orchid that climbs on trees and shrubs and performs Crassulacean Acid Metabolism (CAM) photosynthesis, allowing it to fix CO_2 primarily at night to reduce water loss under dry conditions. During drought stress, CAM plants such as vanilla struggle to efficiently utilize absorbed light energy. This leads to an increased production of reactive oxygen species (ROS) (Barreda-Castillo et al., 2023). These free radicals cause damage to cell membranes through lipid peroxidation and interfere with protein synthesis by damaging mRNA molecules (You & Chan, 2015). The increased activity of lipoxygenase enzymes during drought accelerates the oxidation of certain unsaturated fatty acids in cell membranes. To counteract this, vanilla plants enhance the activity of antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), and peroxidase (POD) (Guo et al., 2018). However, if drought stress persists or intensifies, the plant's biochemical protective capacity may be exceeded, leading to permanent cellular damage.

Biochemically, vanilla plants respond to drought by increasing the production of osmoprotectants such as proline, soluble sugars, and polyamines (González-Arnao et al., 2018). Barreda-Castillo et al. (2023) stated that osmoprotectants, especially proline, help maintain osmotic pressure within cells. Proline assists plants in surviving drought by reducing osmotic pressure in plant tissues, thus enabling leaves to maintain their structure despite significant water loss. Additionally, proline serves as a nitrogen and carbon reserve for plant cells (Ghosh et al., 2022). This compound protects cell membranes and proteins from drought-induced damage and helps maintain cellular energy balance by replenishing NADP+ supplies.

Morphologically, drought causes several changes in vanilla plants such as leaf drop, reduced leaf size, and stunted growth (Buss et al., 2024). Under drought conditions, the roots of the plants spread further to seek additional water sources (Barreda-Castillo et al., 2023). However, this is often insufficient to address severe water shortages (Botomanga et al., 2024). Consequently, vanilla beans produced under drought conditions tend to be smaller, and their quality deteriorates. This reduction in quality affects the production of vanillin. Previous studies have mainly focused on in vitro drought simulations (Barreda-Castillo et al., 2023; Martínez-Santos et al., 2021), while field-based evaluations of physiological and biochemical responses under natural water regimes remain limited. This study distinguishes itself by combining morphophysiological measurements with vanillin quality assessment in a two-year field experiment in Indonesia. This study aims to specifically examine the impact of drought on vanilla plants to support adaptation efforts in Indonesia.

2. Materials and Methods

Time and Location

The study was conducted over two years from January 2022 to February 2024 at a vanilla plantation in Dusun Sendat, Gerbosari, Samigaluh, Yogyakarta (7° 39' 50" S & 110° 10' 36" E), at an altitude of 542 meters above sea level. The research took place in a greenhouse at the center for the management of herbal simplicia, located on a gently sloping, terraced land. The average temperature inside the greenhouse was 28.3 °C, with an average relative humidity of 68% (Primary Data).

Research Preparations

The study was designed using a completely randomized design (CRD) with a single factor of water stress levels and ten replications per experimental unit. Vanilla planting was carried out using certified cuttings of the Vania-2 variety from BPSI TROA. Healthy cuttings, measuring 100 cm in length and 1.5 ± 0.5 cm in diameter, were used. Each cutting was planted in a 50 cm × 50 cm polybag. The growing medium used was a mixture of soil, cocopeat, rice husk charcoal, and manure in a 2:1:1:1 ratio. A 200 g sample of the growing medium was taken during potting, placed in plastic bags, labeled, and brought to the laboratory for field capacity determination. The soil sample was then dried in an oven at 105 °C for 24 hours, weighed, and the average weight was calculated to determine the total moisture content of the soil at planting (FAO, 2023). Next, soil saturation was calculated by adding distilled water to 100 g of dry soil until a saturated paste was formed. The amount of water

used to reach saturation was measured and averaged to determine the percentage of saturation. Field capacity was calculated using the following formula (FAO, 2021):

$$Field\ Capacity\ (\%) = \frac{Saturation\ Percentage\ (\%)}{2}$$

Each pot was filled with 10 kg of growing medium, and the moisture content was adjusted according to the treatments (100%, 50%, 25%, 150%, and 200% field capacity). The field capacity value of the soil in the pots was monitored by weighing the pots with a digital scale. Irrigation was performed when the pot weight indicated that the moisture content had dropped below the treatment threshold. Moisture levels were adjusted consistently based on pot weighing every 2-3 days using a digital scale. The volume of water added was calculated based on the amount needed to reach the desired moisture level for each treatment.

- Field Capacity 100% (Control): The pots were maintained at moisture levels equivalent to field capacity.
- 50% and 25% Field Capacity (Drought): The soil moisture was reduced to 50% or 25% of field capacity before irrigation was applied.
- Excess Water (150% and 200%): Water was added to reach 1.5x or 2x the field capacity (Saturation).

Relative Water Content Measurement

The relative water content (RWC) was measured before the water stress treatment was applied, utilizing Barrs & Weatherley's (1962) method. For each replication, leaves were collected from the middle of the canopy (approximately the 7th node) in the morning at 07:00. The leaves were then weighed to record their fresh weight. The leaves were submerged in water-filled plastic bags for 8 hours to achieve full turgidity. After draining, the leaves were air-dried and weighed to determine the turgid weight. The leaves were then placed in an oven at 80°C for 4 days until the weight stabilized. This final weight was recorded as the dry weight of the leaves. The RWC was then calculated using the following formula:

$$RWC\ (\%) = \frac{(Fresh\ Weight - Dry\ Weight)}{(Turgid\ Weight - Dry\ Weight)} \times 100$$

Physiological Activity Measurement

The selected plants were measured for gas exchange every hour for a 24-hour cycle using an infrared gas analyzer (Li-6400 XT, Li-COR, Lincoln, Nebraska). The gas flow rate was set at 500 µmol s-1, and the temperature was adjusted to match the average greenhouse temperature of 28.3°C. Data on total CO2 uptake were recorded during the daytime (06:00–18:00) and nighttime (18:00–06:00) cycles, using intercellular carbon data. During the 24-hour cycle, the total CO_2 was calculated by summing the daytime and nighttime cycles. Additional data collected from the analyzer included transpiration rate during the daytime, assimilation rate during both the day and night, and stomatal conductance during the day.

Chlorophyll and Proline Assay

For chlorophyll analysis, vanilla leaf samples were selected from the seventh branch. The chosen leaves were not too old and were healthy. A 1-gram sample of the leaf was weighed and finely ground. To the extract, 20 mL of 80% acetone solution was added and mixed thoroughly. The solution was then filtered using Whatman No. 80 filter paper and collected in a test tube. A 2 mL aliquot of the filtrate was taken and its absorbance measured using a spectrophotometer at wavelengths of 645 nm and 663 nm. The absorbance results were recorded and entered into the formula (Arnon, 1949):

$$Chl = ((0{,}0127 \times A663 - 0{,}00269 \times A645) \times 20) + ((0{,}0229 \times A645 - 0{,}00468 \times A663) \times 20)$$

Proline analysis was determined following Bates et al. (1973). It was conducted using the same leaf sample of 0.5 g, which was ground using a mortar and then mixed with 10 mL of 3% sulfosalicylic acid. The solution was filtered through Whatman No. 40 filter paper. A 2 mL aliquot of the filtrate was transferred to a test tube and mixed with 2 mL of ninhydrin acid and 2 mL of glacial acetic acid (100%). The mixture was then reacted at 100°C for 1 hour. The reaction was stopped by placing the test tube in ice for 15–20 minutes. Afterward, 4 mL of toluene was added to the test tube, and the solution was mixed using a Vortex mixer for 20 seconds. The mixture was left undisturbed until the toluene phase separated from the sample solution phase. The toluene phase was then measured for absorbance at 520 nm, with toluene used as a blank. The proline content was calculated using the following equation:

$$Pro = \left(64{,}3649 \times A520 + (-5{,}2987)\right) \times 0{,}347$$

Leaf Total Acid

The acid content in the leaves is calculated as the total assimilation products over 24 hours by the vanilla plants, with sampling conducted at the end of the day cycle (18:00) and at the end of the night

cycle (06:00) according to Silvera et al. (2005) method. The difference in acid content between the two cycles represents the amount of assimilation products formed during the night cycle. For each replication, two leaf disc samples are taken from the 7th internode with a diameter of 1.2 cm. The leaf discs are weighed for their fresh weight, crushed with a mortar, and dissolved in 50 mL of distilled water. The sample is then filtered using Whatman No. 80 filter paper. The resulting filtrate, 20 mL, is then added with 2 drops of phenolphthalein (PP) indicator and titrated with 0.1 N NaOH until a color change occurs. The volume of NaOH used in the titration is recorded and inserted into the formula to calculate the acid content, as follows:

$$Total\ Acid\ (\%) = \frac{Vol\ NaOH\ \times 0{,}1\ \times 90}{Sample\ Weight\ \times 1000} \times 100$$

Photosynthesis Efficiency Calculation

The photosynthetic efficiency is determined by measuring chlorophyll fluorescence using the OS-30p fluorometer (Opti Sciences). Fluorescence is observed on selected plants used for total CO_2 measurement. The measurements are conducted at 07:00 by wrapping the leaves in dark plastic for 20 minutes. After that, the fluorescence is directly measured with a light intensity of 6000 μmol m^{-2} s^{-1} for 2 seconds.

Morphological Characters and Vanillin Content

The morphological variables observed include the increase in the number of internodes, internode length, number of leaves, and dry weight of fruit. These variables are measured starting from when the plants are 18 months old. When the plants begin to flower, pollination is done manually, and a maximum of 10 fruits is maintained per plant. The extraction process is performed using a Soxhlet apparatus with 200 mL of 99.9% ethanol for 16 hours. The resulting extract is transferred to a 250 mL volumetric flask. The Soxhlet apparatus is rinsed several times with a small amount of 99.9% ethanol, and the rinses are added to the volumetric flask. The volume of the solution is then adjusted to the mark by adding ethanol and mixed until homogeneous (ISO 5565-1982). The vanillin content is analyzed using HPLC with a C18 reverse-phase column, using a mixture of methanol and acidified water (10:90 ratio) as the solvent. Detection is performed at a wavelength of 280 nm. The HPLC used is a Shimadzu model with a flow rate of about 4 mL/min, while UV spectrophotometric analysis is done using a Hitachi device. The standard solution for HPLC is prepared with concentrations of 0.5, 1.0, 2.0, 3.0, and 4.0 ppm in 99.9% ethanol and stored at -2°C until analysis. Meanwhile, the standard solution for spectrophotometric analysis is prepared with concentrations of 1.0, 2.0, 3.0, 4.0, and 5.0 ppm in 99.9% ethanol. The vanillin content is calculated using the following formula:

$$Vanillin(\%) = \frac{CSS\ \times IVS}{Sample\ Weight} \times 100$$

note: CSS = Concentration of Standard Solution;
IVS = Initial Volume of Solution

Data Analysis

The data obtained from each variable were tested for normality and homoscedasticity using the Shapiro-Wilk test and Bartlett's test. The effect of drought on all variables was analyzed using analysis of variance (ANOVA) and a range test. Differences between treatments were further analyzed with Tukey's Honestly Significant Difference (HSD) test. Statistical analysis was performed using R software version 4.4.2 and RStudio with the agricolae package).

3. Results

Physiological Characters

Relative water content (RWC) and chlorophyll content (Chl) were notably reduced under water-limiting conditions compared to field capacity and excess water regimes (Table 1). Severe water stress (25% water) resulted in significantly lower RWC and Chl values. Moderate drought (50% water) also negatively affected these parameters, though the decline was less pronounced. In contrast, under field capacity (100%) and excess water (150% and 200%), RWC and Chl remained consistently high. Proline content (Pro) showed significant accumulation in response to drought. The highest levels were observed under severe water stress. In moderate drought, Pro accumulation persisted but at slightly reduced levels compared to severe stress. Under field capacity and excess water conditions, Pro levels were markedly lower. Water use patterns also varied with water availability. Transpiration rate (E) demonstrated a sharp decline with decreasing water supply. Interestingly, transpiration was almost completely inhibited at field capacity. Slight increases were observed under excess water conditions. Stomatal conductance (gsw) was minimal under all water regimes but displayed no significant difference.

CO_2 Assimilation

Vanilla plants showed significant variations in CO_2 assimilation under different water regimes (Table 2). Nighttime assimilation (An) was lowest under severe drought and increased significantly under moderate drought, with no significant difference under field capacity and excess water conditions. Daytime assimilation (Ad) remained at 0.00 μmol m^{-2} s^{-1} across all treatments. This phenomenon shows no active CO_2 fixation during daylight hours. Daytime CO_2 accumulation (CO_2d) and nighttime CO_2 accumulation (CO_2n) were negative under severe drought but increased significantly with improved water availability. Field capacity showed the highest CO2d (9.54 μmol m^{-2} s^{-1}) and CO2n (25.68 μmol m^{-2} s^{-1}), with slightly lower but still positive values under excess water. Total CO_2 accumulation (TCO_2) followed a similar trend, being significantly lower under severe drought and moderate drought compared to field capacity and excess water conditions. Maximum quantum yield (Fv/Fm) was significantly reduced under severe and moderate drought compared to field capacity and excess water.

Morphology Characters and Quality of Bean

Severe drought (25%) resulted in the lowest growth parameters, including Δ Node (3.05), node length (0.92 cm), and Δ Leaf (3.58), which were significantly lower than all other treatments (Table 3). Excess water conditions (150% and 200%) maintained similar growth parameters to field capacity and moderate drought (50%) with no significant differences observed. Bean quality showed significant variation among treatments. Severe drought produced the lowest fresh weight per 10 fruits (W10F) and vanillin content, which were significantly lower than all other treatments.

Table 1. Physiological characteristics of vanilla under different water regimes.

Water Stress (%)	RWC	Chl	Pro	E	gsw
25	46.23 c	1.16 c	3.19 b	0.283 b	0.00095 a
50	60.59 b	1.69 b	2.87 b	0.015 a	0.00042 a
100	87.15 a	2.89 a	0.68 a	0.000 a	0.00000 a
150	92.03 a	2.69 a	0.97 a	0.131 b	0.00000 a
200	92.81 a	2.62 a	2.75 b	0.175 b	0.00000 a

Note: Means followed by different letters are significantly different (Tukey HSD Test, α = 0.05).

Table 2. CO_2 Assimilation of vanilla under different water regimes.

Water Stress (%)	An	Ad	CO2d	CO2n	TCO2	Fv/Fm	TA
25	2.84 b	0.00 a	-3.58 b	-7.46 d	-11.04 d	0.63 b	0.05 b
50	4.48 a	0.00 a	-1.21 b	3.01 c	1.8 c	0.67 b	0.13 a
100	5.17 a	0.00 a	9.54 a	25.68 a	35.22 a	0.83 a	0.19 a
150	4.32 a	0.00 a	7.13 a	18.91 b	26.04 b	0.81 a	0.17 a
200	4.76 a	0.00 a	5.42 ab	15.32 b	20.74 b	0.80 a	0.13 a

Note: Means followed by different letters are significantly different (Tukey HSD Test, α = 0.05).

Table 3. Morphological characteristics and quality of vanilla bean under different water regimes.

Water Stress (%)	Δ Node	Node Length	Δ Leaf	W10F	Vanillin
25	3.05 b	0.92 b	3.58 b	21.8 b	0.83 b
50	11.28 a	2.47 a	11.24 a	32.10 a	1.65 a
100	11.14 a	3.18 a	13.49 a	35.19 a	1.72 a
150	10.90 a	2.88 a	14.92 a	33.12 a	1.65 a
200	10.88 a	2.95 a	12.22 a	32.98 a	1.58 a

Note: Means followed by different letters are significantly different (Tukey HSD Test, α = 0.05).

4. Discussion

Under varying water regimes, vanilla plants exhibited interconnected physiological responses that highlight their strategies for coping with water availability. Severe drought (25% water stress) significantly decreased RWC. Meanwhile, under moderate stress, RWC values were higher compared to both field capacity and excess water treatments. This decrease indicates impaired water uptake and reduced leaf hydration. According to Fleta-Soriano et al. (2015) CAM plants exhibit an adaptive mechanism known as drought stress memory. This mechanism allows the plant to "remember" or retain its previous responses to drought conditions, enabling it to react more effectively when similar stress occurs in the future.

Low water availability (severe & moderate water stress) disrupted chlorophyll content (Chl), likely due to oxidative stress. This stress led to the degradation of photosynthetic pigments (Guo et al., 2016). As a result, the plants' capacity for photosynthesis was diminished. In response to this stress, plants under severe and moderate drought significantly increased proline content (Pro). Proline acts as an osmoprotectant, stabilizing cellular structures under dehydration. At the molecular level, proline accumulation under drought conditions is primarily regulated by the upregulation of the *Δ^1-pyrroline-5-carboxylate synthase* (*P5CS*) gene, which catalyzes the rate-limiting step in proline biosynthesis. Conversely, the catabolic pathway mediated by *proline dehydrogenase* (*ProDH*) is often downregulated to prevent proline degradation during stress periods (Szabados & Savouré, 2010). This coordinated regulation between *P5CS* and *ProDH* maintains intracellular proline homeostasis. The regulation later supports osmotic adjustment, membrane protection, and reactive oxygen species (ROS) scavenging under water-deficit conditions. It also helps maintain osmotic balance, which allows the plant to cope with water stress (Chaudhuri et al., 2017). A similar observation was noted in the saturated treatment (200% water application), which resulted in elevated proline levels. This finding indicates that proline also accumulates under waterlogging stress. These results are consistent with the findings of Barickman et al. (2019) who reported an increase in proline content in cucumber plants subjected to waterlogging stress.

Interestingly, the transpiration rate (E) peaked under severe drought but declined sharply under moderate drought and field capacity. This suggests that plants initially attempt to maximize water use efficiency by maintaining some transpiration, likely to sustain nutrient transport. However, as conditions improve (moderate drought to field capacity), transpiration stabilizes. This reflects the plants' optimization of water use to balance hydration and gas exchange (Leverett & Borland, 2023; Yu & D'Odorico, 2015). The phenomenon of transpiration in vanilla plants is driven by the stomatal opening and closing mechanism, which can be represented by the variable stomatal conductance (Males & Griffiths, 2017). In CAM plants, transpiration is minimized during the daytime due to stomatal closure. During the night, when stomata are open, CAM plants such as vanilla fix atmospheric CO_2 through the activity of phosphoenolpyruvate carboxylase (PEPC), converting it into oxaloacetate and subsequently malate, which is stored in vacuoles as malic acid (Qiu et al., 2023; Winter & Smith, 2022). This nocturnal fixation enables CO_2 uptake while minimizing water loss. During the daytime, stomata close to prevent transpiration, and the stored malate is decarboxylated to release CO_2 internally, which is then refixed by ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) in the Calvin cycle. Therefore, the consistently low stomatal conductance observed across treatments reflects the CAM-specific water-use optimization strategy, where CO_2 assimilation relies more on internal recycling than on external gas exchange during daylight hours.

As shown in Table 1, stomatal conductance (gsw) remained minimal across all treatments without significant differences, although the highest value was observed under severe drought conditions. This behavior indicates a tightly regulated stomatal closure mechanism to limit water loss, particularly under drought conditions. Under optimal (field capacity) and excess water conditions, gsw values near zero suggest a different limitation. This mechanism is potentially due to water saturation reducing root oxygen availability and metabolic activity, which restricts gas exchange (Pereira et al., 2021).

The CO_2 assimilation patterns of vanilla plants under different water regimes reflect how water availability influences key physiological processes (Table 2). Under severe drought (25% water stress), An, CO2d, CO2n, TCO_2, Fv/Fm, and TA were significantly lower compared to other treatments. This reduction can be attributed to limited stomatal opening and reduced photosynthetic activity caused by water deficit. Furthermore, severe drought caused a substantial negative CO_2 balance due to higher respiratory CO_2 release (CO2n). These findings indicate that stress-induced metabolic adjustments increased respiratory demands

(Savchenko & Tikhonov, 2021). In moderate drought (50% water stress), An and TCO_2 improved significantly compared to severe drought. This implies that the plants partially recovered their ability to photosynthesize. However, CO2n remained lower than field capacity and excess water, meaning that carbon uptake was still limited. While the plants showed some recovery, the carbon balance remained positive but modest. This highlights that moderate drought continues to impact the plants' ability to absorb carbon, likely due to restricted stomatal conductance and stress-related metabolic constraints.

During drought conditions, CAM plants reduce CO_2 uptake. This reduction lowers their capacity to synthesize carbohydrates (Vitale et al., 2020). At the same time, drought disrupts the balance of the photosynthetic process. When plants attempt to continue photosynthesis despite limited CO_2 availability, they may produce excessive reactive oxygen species (ROS) as byproducts of oxidative stress. The overproduction of ROS arises from imbalances in photosynthesis, particularly during the light-dependent reactions (Pospíšil, 2016). In such situations, oxygen may accumulate due to insufficient CO_2 availability. ROS are highly damaging to plants as they harm cellular structures, including membranes, proteins, and DNA, which further aggravates plant stress (Abreu et al., 2018).

At field capacity (100%), vanilla plants achieved the highest TCO_2, primarily driven by a significant increase in both daytime (CO2d) and nighttime (CO2n) CO_2 assimilation, as observed in the study by Gantiva Ramírez et al. (2020). Under excess water conditions (150% and 200%), TCO_2 decreased compared to field capacity, despite relatively high levels of An, CO2d, and CO2n. This decline in total assimilation may be attributed to oxygen-limited root environments caused by water saturation, which can inhibit root function and reduce the efficiency of carbon metabolism (Tan et al., 2018). Interestingly, the Fv/Fm ratio, an indicator of photosystem II efficiency, remained high under both field capacity and excess water conditions compared to drought stress. Under drought conditions, the Fv/Fm ratio may decrease due to ROS-induced damage to photosystem II (PSII). Stomatal closure also reduces CO_2 flow for photosynthesis. Additionally, the accumulation of excess energy in the chloroplasts can further damage PSII (Takagi et al., 2017). As a result, the Fv/Fm ratio declines because the plants are unable to utilize light for photosynthesis efficiently. All these assimilation processes are reflected in the TA variable, which accumulates in vanilla leaves. Severe drought leads to a drastic reduction in TA compared to other treatments. These findings align with those of Gantiva Ramírez et al. (2020) who reported that water-deficient vanilla plants accumulate lower acid levels in their leaves due to inhibited photosynthesis.

The morphological characteristics and quality of vanilla beans showed clear responses to different water regimes. Under severe drought (25% water stress), plants exhibited a significant reduction in node formation (Δ Node), shorter node length, and fewer leaves (Δ Leaf). These limitations were likely due to restricted water availability, which impaired cell division and elongation. As a result, vanilla beans under drought stress had a lower fresh weight (W10F) and reduced vanillin content. The decrease in vanillin production can be attributed to the disruption of metabolic processes and biosynthetic pathways under water stress.

Vanillin biosynthesis in *Vanilla planifolia* occurs mainly through the phenylpropanoid pathway, beginning with phenylalanine, which is deaminated by phenylalanine ammonia-lyase (PAL) to form cinnamic acid. Subsequent reactions catalyzed by cinnamate 4-hydroxylase (C4H) and 4-coumaroyl-CoA ligase (4CL) generate intermediates such as p-coumaroyl-CoA that ultimately lead to vanillin formation (Dong et al., 2025). Under drought stress, activation of the phenylpropanoid pathway is a common response as plants accumulate phenolic compounds for antioxidant defense (Li et al., 2025; Peña Barrena et al., 2024). However, this metabolic shift may divert carbon and energy toward general stress-related phenolics rather than vanillin-specific precursors. Upregulation of PAL and related enzymes under drought can enhance lignin and flavonoid biosynthesis, reducing substrate availability for vanillin synthesis. Therefore, the observed decline in vanillin content may reflect a redirection of metabolic flux within the phenylpropanoid pathway, accompanied by altered expression of key regulatory genes such as *4CL*, *COMT*, and transcription factors (e.g., *MYB*, *NAC*, *WRKY*) involved in secondary metabolism (Dong et al., 2025). This study is consistent with the findings of Barreda-Castillo et al. (2023) who reported a reduction in the number of leaves in vanilla plants under drought stress. Similarly, Wang et al. (2019) noted that dragon fruit, another CAM plant, experienced inhibited stem and node growth when subjected to drought stress.

Under moderate drought (50%) and field capacity (100%), vanilla plants exhibited substantial improvements in all morphological parameters. There was a marked increase in node formation, longer node lengths, and more leaves. This led to a higher fresh bean weight (W10F) and vanillin content under field capacity, indicating that optimal hydration promotes both vegetative

growth and the production of key quality compounds. In excess water conditions (150% and 200%), vanilla plants showed similar morphological growth to those at field capacity, with no significant differences in node formation, node length, or leaf production. However, a slight decline in W10F and vanillin content was observed under these conditions. This reduction may be due to waterlogging, which can restrict root oxygen availability and negatively affect nutrient uptake and secondary metabolite synthesis, even though the plants appeared to maintain normal growth.

5. Conclusion

Varietas kacang hijau memperlihatkan pola The study demonstrated that vanilla plants exhibited reductions in photosynthetic efficiency, leaf water potential, and biomass accumulation under water stress conditions. Excess water saturation also slightly reduced these parameters. These results highlight the vulnerability of vanilla to both drought and waterlogging stresses, emphasizing the importance of developing water-tolerant varieties to ensure sustainable production. It is essential to identify and develop vanilla varieties with greater tolerance to water scarcity to maintain sustainable production in the face of climate change. This will enable farmers to better safeguard their livelihoods and ensure the long-term viability of vanilla cultivation in Indonesia.

6. Acknowledgement

The author would like to express sincere thanks to The Center for the Management of Herbal Simplicia for providing the greenhouse facilities for our research, and to the Samigaluh Village field extension officers for their valuable support.

7. Declaration of Conflicting Interests

The authors have declared no potential conflicts of interest concerning the study, authorship, and/or publication of this article.

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