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Nitrogen and phosphorus as macronutrients of cocoa (*Theobroma cacao*) and their physiological functions in different planting patterns of cultivation in Central Java, Indonesia

Nitrógeno y fósforo como macronutrientes del cacao (*Theobroma cacao*) y sus funciones fisiológicas en diferentes patrones de plantación de cultivos en Java Central, Indonesia

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ABSTRACT

Keywords:
Osmolithic
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Vegetation

Plant physiological status during the growing season (specific leaf area (SLA), resorption of N and P) leads to knowing the best plant nutrition management (amount and time) based on the plating pattern. Furthermore, proline and glucose content in root tissues may provide a better technique to represent plant stress conditions. This study aimed to evaluate the SLA, the level of reabsorption of N and P from the leaf, and root proline and glucose content of cocoa plants in different seasons and planting patterns. This study was performed in the fields of Plana village, Somagede, Banyumas, 14 Central Java, Indonesia, and was conducted in December 2015 (rainy season) and October 2016 (dry season) on 7 years-old cocoa plants (*Theobroma cacao*). Three different planting patterns were observed; (1) only cocoa plants, (2) cocoa and coconut pattern, and (3) cocoa with shading trees. The results showed that different seasons and planting patterns affected each observed parameter differently. Cocoas' SLA was not significantly different in all areas for both 2015 and 2016. N resorption during the growing season did not change in 2015 and 2016 in all planting patterns, whereas P resorption had a significant change in 2016 in all planting patterns. The proline content was significantly different in June 2015, October 2015, and March 2016 in all planting patterns. The glucose content in roots showed insignificant differences in 2015 and 2016 in all planting patterns. These results also showed that SLA and glucose did respond to season and plating patterns. These parameters are suggested as poor indicators of physiological status. Furthermore, sowing cocoa plants with other types of plants can be used to help farmers and stakeholders in managing cocoa cultivation in efficient and sustainable ways.

RESUMEN

Palabras clave:
Osmolítico
Reabsorción
Área foliar específica
Theobroma cacao L.
Vegetación

El estado fisiológico de la planta durante la temporada de crecimiento (área foliar específica (SLA), reabsorción de N y P) conduce a conocer el mejor manejo de la nutrición de la planta (cantidad y tiempo) en función del patrón de siembra. Además, el contenido de prolina y glucosa en los tejidos de la raíz puede proporcionar una mejor técnica para representar las condiciones de estrés de la planta. Este estudio tuvo como objetivo evaluar el SLA, el nivel de reabsorción de N y P de la hoja y el contenido de prolina y glucosa de la raíz de las plantas de cacao en diferentes estaciones y patrones de siembra. Este estudio se realizó en los campos de la aldea de Plana, Somagede, Banyumas, 14 Central Java, Indonesia, y se realizó en diciembre de 2015 (temporada de lluvias) y octubre de 2016 (temporada seca) en plantas de cacao (*Theobroma cacao*) de 7 años. Se observaron tres patrones de plantación diferentes; (1) solo plantas de cacao, (2) patrón de cacao y coco, y (3) cacao con árboles de sombra. Los resultados mostraron que las diferentes estaciones y patrones de siembra afectaron cada parámetro observado de manera diferente. El SLA de cacao no fue significativamente diferente en todas las áreas para 2015 y 2016. La reabsorción de N durante la temporada de crecimiento no cambió en 2015 y 2016 en todos los patrones de siembra, mientras que la reabsorción de P tuvo un cambio significativo en 2016 en todos los patrones de siembra. El contenido de prolina fue significativamente diferente en junio de 2015, octubre de 2015 y marzo de 2016 en todos los patrones de siembra. El contenido de glucosa en raíces mostró diferencias no significativas en 2015 y 2016 en todos los patrones de siembra. Estos resultados también mostraron que el SLA y la glucosa respondieron a la estación y a los patrones de siembra. Estos parámetros se sugieren como malos indicadores del estado fisiológico. Además, la siembra de plantas de cacao con otros tipos de plantas se puede utilizar para ayudar a los agricultores y las partes interesadas a gestionar el cultivo de cacao de manera eficiente y sostenible.

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Cocoa (*Theobroma cacao* L.) is a species from the Theobroma genus and the Malvaceae family. This species is the most widely cultivated plant as it has high economic value (Najihah *et al*., 2018). Cocoa beans are the raw material for chocolate and cosmetics. The average cocoa production that comes from smallholder plantations from 2011 - 2018 was 649,807 t annually or around 94.32% of total national production (Ruslan and Prasetyo, 2021). In Indonesia, cocoa cultivation is conducted using agroforestry and full-sun systems (Witjaksono, 2016). Sowing protective plants to reduce direct sunlight exposure could increase cocoa production (Wartenberg *et al*., 2019). High temperatures have negative impacts on cocoa plants, such as eco-physiological stress, environmental changes, and decreased CO_2 assimilation. Shade plants play an important role to enhance cocoa plants' physiology and the quality of soil nutrients. The benefits of cacao cultivation under coconut trees are that it can increase land use efficiency, sunlight, and control Helopeltis as the main pest of cocoa (Adam *et al*., 2017). The cocoa-coconut intercropping system does not create competition for sunlight because cocoa is a plant that can grow in 40-80% shade (Latha *et al*. 2017).

Leaves are among the vital organs of a plant, which can affect plant growth and are one of the primary organs in the assimilation process that affects plant production. Furthermore, leaves are also correlated with the physiological functions of the plants, such as specific leaf area (SLA), photosynthetic capacity, nitrogen and phosphorus content, respiration rate, and leaf age (Wright *et al*., 2004). SLA is the leaf area per dry mass that is used as a parameter to assess the plant's carbon content and water status (Ali *et al*., 2017). SLA can indicate the relative growth rate of plants. Also, changes in SLA can represent the structure of leaves and their nutrient content because SLA is mostly affected by photosynthesis activity (Gong and Gao, 2019).

Macroelements, such as nitrogen, phosphorus, potassium, and calcium, play an essential role in several physiological processes (Ashraf *et al*., 2014). Resorption is a process to use nutrients efficiently by transferring nutrients from senescence leaves to young leaves (Housman *et al*., 2012). Environmental factors, such as the dry season with low water supply, affect the resorption process. The senescence leaves use 50% of their N and P components, enzymes, lecithin, and nucleic acids to be translocated to seeds, roots, young leaves, and other parts of plants via the phloem tissue (Tang *et al*., 2013;). There were indications of NPK limitation in all cropping patterns used because the NPK concentration in cocoa was lower than the ideal level without fertilization. *C. indicum* tree planting system at a distance of 8 m×16 m and *G. sepium* at a distance of 12×12 m was preferred over *C. indicum* at a distance of 8×8 m for *T. cacao*. This was because the high-density distance can lead to the depletion of organic matter. The genotype of cocoa affects the response or interaction with the cultivation environment, and will subsequently also affect the efficiency of nutrients (Rosas-Patiño *et al*., 2019).

Plants adapt to changing water content by metabolic adaptation to deal with osmotic pressure and trigger the synthesis of the osmoprotectants, which are proline, protein, and sugar. Those osmoprotectants regulate osmotic potentials and protect the cells from drought damage and regulate drought stress (Li *et al.,* 2015). Proline is the main organic osmolyte compound during abiotic stresses. Furthermore, proline plays a critical role in the osmotic regulating compound and plant structure protection. Proline accumulation is more extensive during the day than at night to protect plants from UV radiation in Barley (Fedina *et al*., 2002). The adjustment of osmotic compounds can be evaluated by the accumulation of sugars to balance water potential during drought stress in cotton (Jamal *et al*., 2015). The more plants experience water shortages, the proline level increases as a survival effort of the plant. According to Janani *et al*. (2019), cocoa clones that were subjected to drought stress and produced the highest proline content also showed high-stress tolerance, thus potentially producing cocoa.

The influence of seasons and planting patterns on cocoa growth is needed to discover the differences in physiological responses. The present study aimed to analyze differences in SLA, N, and P resorption in leaves, and proline and glucose concentration in cocoa roots in different seasons and planting patterns.

MATERIALS AND METHODS

Experimental site and design

The present study was conducted in 2015 and 2016 in Plana village, Somagede, Banyumas, Central Java, Indonesia (Type climate A based on Kuppen climate classification). Based on information from Sugito *et al*. (2019), Plana village has an altitude of 300 m, so it is classified as medium land. The sampling areas were determined by the planting patterns of the cocoa plantation (Figure 1). This land is rainfed soil and NPK fertilizer (10 kg plant^{-1}) is only applied once a year in the early rainy season from month 1 to month 2. Leaf trimming of young shoots and twigs that had many leaves according to Angela and Efendi (2015) was carried out every 2 months depending on the vegetation's density. For maintenance pruning, removed branches had a diameter of < 2.5 cm, heavy pruning was also carried out when the plant was too dense. This pruning also aimed to stimulate the growth of flowers and fruit. Insecticides were not used in this study because of the absence of insect pests on the observed cocoa plants (cocoa hybrid clone). In areas 2 and 3, the number of cocoa plants in each area was 12 trees. The age of cocoa, coconut, and other plants are 8 years, 10 years, and about 10 years, respectively. The percentage of shade plants in area 1 was 75%. Each area was about 2000 m^2 and the planting distance between cocoa plants was 3 m (Figure 1). A completely randomized design with 12 replications was used in this study.

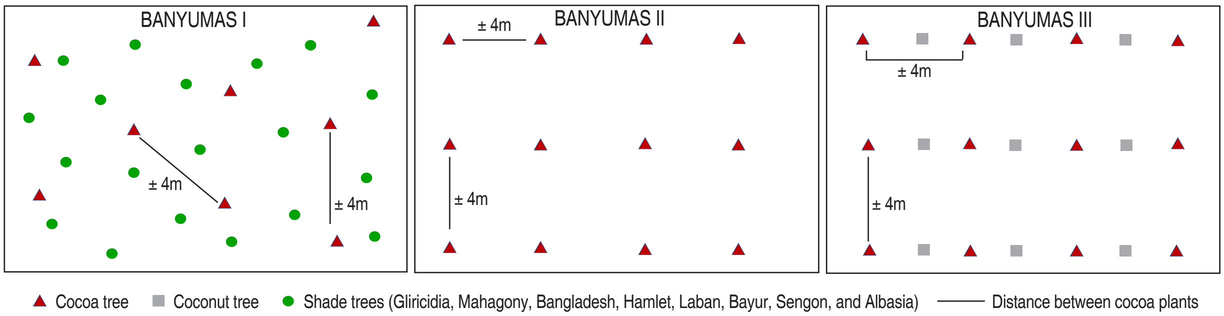

Figure 1. Three different planting patterns in sampling sites.

From 24 cocoa plants, 12 leaves per area were picked up in each area to evaluate the SLA and N and P content. The analyses on the cocoa tree roots were conducted to examine the proline and glucose content. Proline and glucose were quantified in three periods (6-7 June 2015, 31 October 2015, and 26-27 March 2016) to show a complete seasonal profile in Indonesia. According to BPS (2017), Banyumas has the highest rainfall of 113.25 mm^3 in June (rainy season) and the lowest of 0 mm^3 (dry season) in October 2015. The rainfall took place in all months observed in 2016 with 302 mm^3 of rainfall in March 2016.

Environmental and weather factors determination

The microclimate was measured from January 2015 to December 2016 every week. Air temperature, humidity, pH, temperature, and groundwater content were measured in the present study. Air temperature and humidity were measured by using a thermohygrometer. pH, temperature, and groundwater content were measured using a tensiometer from 12.00 a.m. to 02.00 p.m.

Specific leaf area (SLA) analysis

SLA is the ratio of leaf area to dry weight (cm^2 g^{-1}). SLA observations were conducted using adult cocoa leaves with a similar leaf index color according to Roderick *et al*. (1999). Leaf samples (12 leaves area^{-1}) were obtained from plant branches, wrapped in aluminum foils, and stored in a cool box to keep them fresh. The leaf area (cm^2) was measured using millimeter block paper. The leaves were dried using an oven to calculate the dry weight (cm^2 g^{-1}). SLA evaluation was conducted with three different vegetation areas in 2015 and 2016.

Analysis of nitrogen (N) and phosphorus (P)

N and P content analysis was conducted in adult and senescence leaves of cocoa plants on 26-31 January 2015 and 3-9 October 2016. The N leaf content was evaluated using the Kjeldahl method (Singh *et al.*, 2015). P content of the leaf was evaluated using the Morgan–Wolf method as a sanitizer based on Jeshni *et al.* (2017). N and P resorption measurement was conducted according to Li *et al.* (2019a) by Equation 1.

$$NR = 100\% \times \frac{(N_d - N_s)}{N_d} \qquad (1)$$

Where, NR: nitrogen resorption (%); N_d: adult leaf nitrogen (%); N_s: senescence leaf nitrogen (%)

Proline analysis

Fine root samples were obtained using a soil core at 20 cm depth in midday (12.00–14.00) on 6-7 June 2015 (early dry season), 31 October 2015 (late dry season), and 26-27 March 2016 (late rainy season). Proline accumulation was analyzed based on Bates *et al.*, (1973) method. A total of 0.5 g of dry root was homogenized with 10 mL of 3% sulfosalicylic acid for 72 h and then filtered. About 2 mL of filtrate was reacted with 2 mL of ninhydrin acid and glacial acetic acid. The samples were heated at 100 °C for 1 h. The filtrate was then placed into a glass cup with ice. A mixture of filtrate, ninhydrin, and glacial acetic acid was added to toluene and stirred for 15–20 s. The obtained red toluene with proline was measured using a spectrophotometer at 520 nm wavelength and compared to a standard proline curve to obtain the proline level of the sample.

Glucose analysis

Phenol method with glucose solution was used to determine the root's glucose content according to Chow and Landhäusser (2004). The reagents were 5% phenol solution in water, 95.5% H_2SO_4 with a density of 1.84, and a standard glucose solution. The used equipment was a spectrophotometer and a 25 °C water bath. The standard curve was made with 2 mL of a standard glucose solution with 0, 10, 20, 30, 40, and 60 µL glucose. The fine root samples were obtained similarly to proline analysis. The obtained samples were immediately heated at 60 °C for 60 s. Then, it was added with 1 mL of 5% phenol solution and mixed. After that, it was added with 5 mL of the sulfuric acid solution for 10 min, constant shaking, and placed in a water bath for 15 min. The absorbance was measured at 490 nm for hexose and 480 nm for pentose and uronic acid.

Statistical analysis

The data were analyzed using analysis of variance (ANOVA) and Duncan's Multiple Range Test (DMRT) at the significance level of 5%. The SPSS program for Windows (IBM) was used for the statistical analysis.

RESULTS AND DISCUSSION

Climatology characteristics

The climatology characteristics of the plantations' locations was shown in Table 1. The annual weekly or monthly rainfall distribution is more important than the total amount during the year because cocoa plants are more suitable in the months with not too high rainfall. Cocoa plantations generally have around 1250 to 3000-mm of rainfall every year. Based on the rainfall data of the location, the rainfall in plantations has the appropriate amount for cocoa plants' growth (Table 2). For optimal growth of cocoa plants, a maximum temperature between 30–32 °C, a minimum temperature between 18–21 °C (Sitohang and Siahaan, 2018), and relative humidity of 100% at night and between 70–80% during the day are needed conditions. Table 3 shows the organic matter, nitrogen (N), phosphate (P), and potassium (K) of the Banyumas soil (sampling site). Nitrogen, phosphorus, and potassium were 0.13-10.16%, 0.155-0.172%, and 0.03-0.1%, respectively (Table 3). Generally, the observed mineral content indicated that the soil is fertile at the sampling sites.

Table 1. Location and climatological characteristics of cocoa plantations in Plana Village, Banyumas, Central Java.

Climatological characteristics	Measurement
Altitude (masl)	105
Land slope (°)	3 – 6
Early cocoa planting	2006–2008
Air temperature (°C)	22 – 32
Relative humidity (%)	83 – 87
Groundwater content (%)	31 – 55
pH	5.8 – 6.5

Table 2. The rainfall in Plana Village, Banyumas, Central Java

Month, Year	Average rainfall (mm/month)
October – March, 2015	436.99
April – September, 2015	101.26
October – March, 2016	404.35
April – September, 2016	86.07

Source: Amini, 2019.

Table 3. Soil organic matter and soil N, P, K level.

Content	2015	2016
Organic matter (%)	2.96	2.94
N total (%)	0.15	0.15
P total (%)	0.165	0.163
K total (%)	0.065	0.048

Specific leaf area

There was no significant difference between planting patterns for specific leaf area (SLA) in January 2015. Furthermore, there was a non-significant difference in the three planting patterns in October 2016 (Table 4). At the peak of the dry season in October 2016, the SLA in plot II decreased of 8.92%, while in plots I and III increased of 24.52% and 25.76%, respectively compared to January 2015. However, the sampling was done during a relatively high rainfall period in January 2015 and a dry period in October 2016.

Table 4. Specific leaf area (SLA) of cocoa leaves.

Planting pattern	SLA (%)*	
	January 2015	October 2016
I	174.76±71.27	199.28±61.69
II	135.25±54.57	126.33±20.48
III	143.39±66.20	169.15±71.61
	***P*=0.19**	***P*=0.35**

*There is no significant difference at α=5%.
Area I: Cacao plant with several trees; Area II: Cacao without shade; Area III: Cacao plant with coconut trees.

SLA is a morphological parameter of nutrients a moisture availability that can be influenced by environmental conditions and the age of the leaf. SLA plays an important role in determining plant productivity, changes in leaf SLA indicate changes in leaf structure and nutrient content. In addition, differences in leaf SLA are influenced by soil and environmental climatic factors (Gong and Gao, 2019). Low SLA is due to the leaf's adaptation to survive, store nutrients, and avoid drought. The small size helps to regulate the temperature and water use efficiency for photosynthesis in dry and low water supply conditions (Karavin, 2013).

The increase in SLA is due to the increase in the number of cocoa leaves and may be due to a decrease in light intensity (Zang *et al.*, 2016). Low SLA values do not reflect or indicate low photosynthetic activity and vice versa. However, in a water shortage condition, the temperature around the surface of the cocoa unshaded is higher than those in the shade, which causes faster groundwater evaporation, resulting in a reduced photosynthesis rate for survival (Lahive *et al.*, 2019). According to Xu *et al.* (2008), decreasing soil moisture can reduce leaf size, and eventually affect the rate of photosynthesis. Photosynthetic rate is associated with SLA; by increasing the light capture area per mass, high SLA improves photosynthesis (Goorman *et al.*, 2011). Photosynthetic rate is associated with SLA; by increasing the light capture area per mass, a larger leaf size, and higher photosynthesis (Huang *et al.*, 2021).

Resorption of nitrogen (N) and phosphorus (P)

Table 5 shows the N and P contents of cocoa leaves. The average N content was higher in 2015 than in 2016 in areas I and II. As shown in Table 5, the N content was increased in area III during 2015 as well. The increased N content is needed by cocoa plants that grow with coconut plants. The P content was not significantly different from N content; the P content was lower in 2015 than in 2016 in three different vegetation areas. The highest P increase was found in area II at 877.78%. Moreover, the N and P content of cocoa leaves decreased in the senescence phase (Table 6).

Table 5. Nitrogen (N) and phosphorus (P) contents of cocoa leaves.

Area	January 2015	October 2016
N (%)		
I	1.21±0.20	1.12±0.24 ab
II	1.24±0.07	0.95±0.09 b
III	1.31±1.15	1.34±0.17 a
	***P*=1.43**	***P*=0.03**
P (%)		
I	0.14±0.33	0.29±0.15
II	0.17±0.32	0.16±0.03
III	0.20±0.59	0.19±0.02.
	***P*=0.13**	***P*=0.14**

Numbers in columns followed by the same letters show no difference at α=5%.
Area I: Cacao plant with several types of trees; Area II: Cacao without shade; Area III: Cacao plant with coconut trees.

Table 6. Nitrogen (N) and phosphorus (P) contents in senescence leaves of cocoa.

Area	January 2015	October 2016
N (%)		
I	0.65±0.37	0.63±0.14 b
II	0.71±0.09	0.57±0.02 b
III	0.71±0.05	0.98±0.27 a
	***P*=0.90**	***P*=0.19**
P (%)		
I	0.10±0.03	0.17±0.08
II	0.09±0.32	0.88±0.02
III	0.09±0.44	0.12±0.05.
	***P*=0.75**	***P*=0.21**

Numbers in columns followed by the same letters show no difference at α=5%.

N and P resorption of cocoa leaves was higher in 2015 than in 2016 because of the different seasons (Table 7). The rainfall in Banyumas was low (almost 0 mm^3) in October 2015 because of still early for the rainy season. The reduction in areas I–III was 3.63, 6.44, and 38.57%, respectively. Furthermore, the N resorption in area I was not significantly decreased because of the shade plant presence. In 2016, the decrease of P resorption in areas I–III was 63.43, 13.10, and 49.26%, respectively. This shows that the most significant decrease in P resorption was in area I and the lowest P resorption was in October (*P*=0.01) (Table 8).

Table 7. Resorption of nitrogen (N) and phosphorus (P) in cocoa leaves.

Area	January 2015	October 2016
N resorption (%)		
I	44.11±31.91	42.51±16.12
II	42.41±4.34	39.68±4.07
III	45.32±9.10	27.84±14.22
	***P*=0.97**	***P*=0.27**
P resorption (%)		
I	28.36±716.67	10.37±8.78 b
II	45.66±14.17	39.68±4.07 a
III	54.87±25.81	27.84±14.22 a
	***P*=0.13**	***P*=0.01**

Numbers in columns followed by the same letters show no difference at α=5%.
Area I: Cacao plant with several types of trees; Area II: Cacao without shade; Area III: Cacao plant with coconut trees.

Nitrogen plays a vital role in the growth process, leaf production, and protein content in plants. The dry season affects the absorption and transportation of N. It may reduce the transpiration and the permeability of the membrane. Besides, nutrient transfer from senescence leaves to underground organs increases nutrient use (Yang *et al*., 2016).

P absorption from the soil decreases in the dry season because of drought stress, reducing nutrient diffusion, and mass flow in grasses in the soil (Bista *et al*., 2018). The resorption ability depends on the nutrients in the soil and the environmental conditions, such as temperature and light. According to some authors, plants utilize about 5–80% N and 0–95% P through leaves (Aerts and Chapin, 2000). Furthermore, N resorption for each plant species ranges from 16 to 42%. Additionally, microclimate under the canopy increases the decomposition of N content (Sari *et al*., 2022). Area II had a good absorption of N. The root system of mature cocoa plants is stronger than the perennial or annual plant.

The most significant decrease in P resorption occurred in cocoa plants with coconut plants. Coconut trees have a root system up to 2 m wide and 0–60 cm below the soil surface making it easier to absorb nutrients than cocoa (Adam *et al*., 2017). Generally, the ability of plants to do P resorption is at 0 until 90%. It increases when the availability of P in the soil decreases (Mayor *et al*., 2014). The low rate of resorption is influenced by competition with other trees. In another study, N and P resorption were higher in plants affected by drought stress (Lobo-do-Vale *et al*., 2018). On the contrary, the P resorption tends to decrease in the present study.

Table 8. The proline content of cocoa root.

Plantibng pattern	Proline content (%)		
	June 2015	October 2015	March 2016
I	1.03±0.36 b	16.70±9.16 a	0.37±0.29 b
II	2.03±1.06 ab	10.52±5.6 7 ab	1.08±0.30 a
III	4.25±2.91 a	5.30±1.46 b	0.68±0.25 b
	***P*=0.02**	***P*=0.02**	***P*=0.03**

Numbers in columns followed by the same letters show no difference at α=5%.
Area I: Cacao plant with several types of trees; Area II: Cacao without shade; Area III: Cacao plant with coconut trees.

Proline content of cocoa root

The proline content in the cocoa plant root was increased in all planting patterns from June to October 2015 (Table 8) due to the drought. Based on the planting pattern, the proline content was significantly different (*P*=0.02) in June 2015, the difference between area I and area III was significant, 1.03% and 4.25%, respectively. In Oct 2015, the proline content in area I was significant (*P*=0.02) different and lower than area III, 16.7% and 5.30%, respectively. And in March 2016, the proline content in area II (1.08%) was significantly (*P*=0.03) higher than area I (0.37%) and Area III (0.68%).

Proline accumulation was relatively higher in cocoa plants root without shade trees compared to those with cocoa plants root in the area with coconut trees (Table 8). The increase in proline is due to drought stress during the dry season (Devaranavadagi *et al.*, 2002). Proline is a substrate for respiration, a source of nitrogen, and other metabolisms during the dry season that was reported in wheat. The accumulation of proline during drought stress does not inhibit the biochemical reactions, but it acts as an osmoprotectant in corn (Molazem *et al.*, 2010). Osmotic regulation triggers cell development and plant growth in the dry season in wheat (Keyvan, 2010). Osmotic adaptation maintains the cell's turgor to enlarge, influences plant growth and allows stomata to open to assimilating CO_2 in Festuca (Man *et al.*, 2011). The highest decrease in proline was 97.78% in area I from October 2015 to March 2016. The plants in areas with diverse vegetation have a small amount of proline because of the low transpiration process. It was because plants do not need to make osmotic adjustments like when drought stress increases proline (Robakowski *et al.*, 2020).

Glucose content of cocoa root

There were no significant differences in glucose content between planting patterns during 2015 and 2016 (Table 9). The decrease in glucose levels in area I–III was 49.81%, 32.07%, and 3.8%, respectively, showing that the highest decrease in glucose content was in area I.

Table 9. The glucose content of cocoa root.

Area	Glucose content (%)		
	January 2015	October 2016	Change (%)
I	5.28±1.21	2.65±1.15	49.81
II	5.30±1.52	3.60±1.49	32.08
III	3.42±2.07	3.29±2.34	3.80
	***P*=0.11**	***P*=0.63**	***P*=0.03**

Numbers in columns followed by the same letters show no difference at α=5%.
Area I: Cacao plant with several types of trees; Area II: Cacao without shade; Area III: Cacao plant with coconut trees.

Plants have an osmotic adjustment mechanism for leaves and root turgor for growth, water absorption, cytokinin synthesis, and photosynthesis in black willow (Carpenter *et al.*, 2008). High carbohydrate storage in leaves during the dry season can be used for the respiration process of Aleppo pine (Klein *et al.*, 2011). Besides, higher amounts of sugar during the dry season are due to the translocation of carbohydrates from senescing leaves and the increased rate of photosynthesis at the beginning of the dry season. Sugars affect the growth, development, and metabolism of leaves, shoots, roots, and other plant organs (Ciereszko, 2018). According to Tezara *et al.* (2020), each clone of cocoa has a different physiological response to drought stress. Leaf N content, chlorophyll, and photochemical activity are reduced during drought, and the metabolism is disrupted. However, certain clones managed to survive in an environment with low water availability. Research by Rosas-Patiño *et al.* (2019) revealed that liming and fertilization affect nutrient efficiency and yields, in addition, the genotype of the cocoa and climate conditions also have an effect.

CONCLUSION

In conclusion, cocoa had different responses to cocoa root proline levels, leaf glucose levels, SLA, and leaf

N and P resorption to deal with seasonal changes. Further study to correlate yields with proline or glucose concentrations is needed to determine whether sowing cocoa plants with other types of plants can be used to help farmers and stakeholders in managing cocoa cultivation to increase yields in efficient and sustainable ways.

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