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Nurturing with Nature: The Efficacy of Arbuscular Mycorrhizal Fungi Microbe for Cocoa Sector Environmental Management

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Abstract

Despite the proven effectiveness of fertility-promoting microbes such as Arbuscular Mycorrhizal fungi (AMF) to minimize the adverse effects of environmental deterioration and climate change, there is limited understanding on its efficacy to improve cocoa resilience. Cocoa mortality (70%) caused by climate change induced drought within two years of transplanting to the field is a major constrain to farmers' productivity. This study assessed the effect of AMF and Potassium (K) nutrition on the biochemical characteristics of cocoa and its field survival from 2019 to 2022. A split-plot factorial design was used. The treatments were cocoa varieties (CRG8914×PA150 [V1], AMAZ15-15×EQX78 [V2], PA150×CRG 0314 [V3]), potassium (0g, 2g, 4g plant^{-1}), AMF (AMF, Non-AMF) and watering regimes (water-stressed and supplementary irrigation). Soil samples (0-20cm) from cocoa rhizosphere were examined for native AMF spores and structures using the Wet Sieving and Decantation methodology and Sucrose Centrifugation. The AMF inoculum was prepared using the trap-culture technique. Under drought conditions, V1, V2 and V3 without K had leaf chlorophyll of 19.4µmol/m^2, 20.5µmol/m^2, 19.9µmol/m^2 respectively, but with 2g KxAMF, chlorophyll increased to 21.6µmol/m^2, 26.4µmol/m^2, 26.8µmol/m^2 respectively. Phosphorus uptake and relative water content had similar pattern. *Rhizophagus irregularis* was morphologically identified as the AMF in soils under the cocoa trees. Multivariate Adaptive Regression Splines predicted leaf chlorophyll and phosphorus uptake as the key factor influencing cocoa survival and resilience. Considering the threat of drought caused by climate change on cocoa production in West Africa, the results have implications for farmers livelihood, cocoa survival and the sustainability of cocoa soils.

Keyword: climate change, water stress, fertility-promoting microbes, cocoa resilience

1.0 Introduction

Cocoa (*Theobroma cacao* L.) is a commercial cash crop cultivated in tropical regions that makes a substantial contribution to the worldwide chocolate industry and serves as a critical source of income for farmers (Adeniyi, 2019). The production of cocoa is of utmost importance to the livelihoods of farmers and the general economy of cocoa producing countries, owing to its economic significance and cultural value (Vogel et al., 2020; Hütz-Adams et al., 2016). Globally, about 4.3 million tons of cocoa is produced annually, and almost 71% of it produced mainly by smallholder farmers (Adeniyi, 2019; Carr and Lockwood, 2011). Over 70% of the world's cocoa is produced in West Africa (Côte d'Ivoire, Ghana, Nigeria, and Cameroon), with Côte d'Ivoire and Ghana considered the leading producers (van Vliet et al., 2021).

Nevertheless, there exist several impediments to cocoa production, with abiotic stressors particularly climate change induced drought, posing a considerable threat. For example, in the primary cocoa-producing region of West Africa, the yearly period of dry weather lasting from November to April leads to insufficient soil moisture. This deficiency in water negatively impacts the survival of cocoa, especially during the initial 24 months following their field planting (Anokye et al., 2021).

Climate change has worsened the negative impact of drought on cocoa seedlings survival and this is predicted to be more frequent and severe in current growing regions by 2050 (Läderach et al., 2013), thus, presenting a formidable challenge to the sustainable cultivation of cocoa worldwide. For instance, drought stress poses a significant impact on the delicate water balance in cocoa plants, resulting in unfavourable growth, development, and yield outcomes (Oyekale, 2021; Dossa and Miassi, 2023). During drought, plants are unable to keep their cells at an ideal level of hydration, which affects electron transport in the chloroplasts (Naveen et al., 2023; Sharma et al., 2023). Additionally, the lack of readily available water makes it difficult for cocoa plants to absorb crucial nutrients (van Vliet et al., 2021). It is important to highlight that, the process of nutrients uptake by plants require water, and drought stress could result in nutrient deficits in cocoa plants, further impairing their ability to grow and and produce pods (Snoeck et al., 2016; Kaba et al., 2022).

For example, flower abortion, poor pod set, and smaller-sized pods in cocoa are due to the effect of drought stress during flowering and fruit development (Carr and Lockwood, 2011). Given that drought events are occurring more frequently and intensely as a result of climate change, researching methods to decrease their detrimental effects on cocoa trees is crucial.

Among the potential remedies, the use of fertility-promoting microbes such as arbuscular mycorrhizal fungus (AMF), in combination with potassium fertilizer stand out as prospective alternatives that are worth examining.

It is important to establish that AMF colonize the roots of plants and build a network of hyphae that penetrates the soil, boosting water and nutrient uptake and enhancing the plant's resistance to various abiotic stresses (Boutasknit et al., 2020). In addition, potassium is an important macro-nutrient that is crucial for maintaining the water balance of plants and increasing their tolerance to drought stress (van Vliet et al., 2021).

Previous studies (Kwashie et al., 2023; Kaba et al., 2022; Anokye et al., 2021; Djan et al., 2018) have focused on enhancing the drought resilience of cocoa seedling using inorganic fertilizers and at the nursery stage. Per the literature review, no study has been done to assess the resilience of cocoa beyond the seedling stage. This study is novel, as it is the first time the resilience of cocoa plants has been assessed from the seedling stage to the field (up to 2 years of establishment). In addition, this study is the first to identify and explore the efficacy of native AMF in tropical soils in enhancing tree crops such as cocoa resilience to environmental stress.

The study hypothesized that the combined application of AMF and K will enhance the biochemical processes of mature cocoa trees to improve their resilience to drought stress caused by climate change.

2.0 Materials and Method

2.1 Study Location

The study was conducted at the Faculty of Renewable Natural Resource (FRNR) farm (latitude 60 43 N and longitude 10 36 W), Kwame Nkrumah University of Science and Technology (KNUST), Ghana (Figure 1). The mean annual rainfall ranges between 1,300mm and 1,600mm. The mean temperature is 26.6 ℃ and an annual humidity of 67.6% (Saah et al., 2022). The vegetation is a moist semi-deciduous forest. The soils belong to the Asuasi series, classified as forest Ochrosols. The district is bordered by the Kwabre District to the North, the Atwima Kwanwoma and Atwima Nwabiagya Districts to the West, the Ejisu-Juaben Municipal to the East, and the Bosomtwe District to the South (MOFA, 2021).

2.2 Experimental design

The research adopted a split-plot factorial (3 × 3 × 2 × 2) experimental design, in a Randomized Complete Block Design. Water-stressed conditions and under supplementary/well-watered conditions was the main plots. In addition, the sub-plots were three cocoa varieties (V1: CRG8914 × PA150, V2: AMAZ 15-15 × EQX78, and V3: PA150 × CRG 0314) and potassium application (K1: 0 g/plant, K2: 2 g/plant, and K3: 4 g/plant), and two AMF treatments (AMF and Non-AMF inoculated).

2.3 Isolation of AMF inoculum, Preparation of AMF inoculum and Inoculation of cocoa seedlings

The Wet Sieving and Decantation methodology and the Sucrose Centrifugation Technique was used after collecting soil samples at a depth of 0-20cm from the rhizosphere of five cocoa trees. The isolation process followed the recommendation of Gerdemann, (1963) and BA and Skipper, (1982).

AMF inoculum was prepared using the trap culture technique. The sampled soil (0-20 cm depth) was taken from the rhizosphere of selected cocoa trees and mixed with solarized (at an average temperature of 40℃) soil. Trap culture was done using trap crops such as maize, cowpea, and rice planted for three months after which soil samples were examined for AMF [(see Kwashie et al., 2023) for detailed procedure, the histochemical staining of the cocoa seedlings and experimental procedure).

2.4 Data collection

The growth parameters measured were stem diameter (using a vernier caliper) and height (using a meter rule). In addition, at 24 months, the number of cocoa seedlings that survived after transplanting to the field was expressed in percentage as “Field survival percentage”.

This parameter indicates the percentage of cocoa seedlings that successfully survived in the field over the course of the experiment. The leaf Relative Water Content (RWC), chlorophyll content, leaf potassium and phosphorus concentration were also measured from 6 to 24 months.

2.8.1 Measurement of Leaf Relative Water Content (RWC)

After 18 months of transplanting, the water status of the cocoa seedlings was assessed by measuring the Leaf Relative Water Content (RWC) using the Relative Turgidity Method. This measurement provides an indication of the water content in the leaves and is commonly used to assess the water status and hydration level of plants. The Leaf RWC was measured on six sampling occasions during the dry season (November-February) and also in the wet season (March and April). Samples were taken between 7 am and 10:00 am on each sampling occasion or day.

Four mature green leaves of each replicate were selected and thoroughly cleaned with cheese cloth (These leaves were usually part of the most recently hardened flush or were found below the most recent flush). Avoiding the mid-rib and major veins, 2 discs were quickly bored with a sharp 1cm inner diameter cork-borer from each selected leaf, placed in a clean plastic vial, and transferred immediately to the laboratory where the fresh weight (FW) of each set of eight (8) leaf discs from four leaves of each replicate of the cocoa was determined together using a fine balance (AL 104; Mettler Toledo, Columbus, OH, USA).

Subsequently, each set of 8 leaf discs of each selected replicate was floated on 20 ml distilled water in a 2.5 cm diameter Petri dish under diffuse light at room temperature (25 °C) for four hours after which they were removed with a clean pair of forceps, surface blotted between 2 layers of tissue paper and weighed in order to obtain their fully turgid weight (TW). The leaf discs were then dried in an oven at 60 °C for 24 hours (in order to remove only the moisture content), and cooled in a desiccator before determining their dry weight (DW).

The Leaf RWC for each set of six (6) leaf discs for each replicate was calculated by adopting the procedure recommended by Merah, (2001) and Poggi et al. (2023) as follows:

$$RWC\ (\%) = [(FW - DW)/ (TW - DW)] \times 100$$

Where:

FW: Fresh weight of the eight 1cm inner diameter leaf discs

DW: Dry weight of the eight 1cm diameter leaf discs after oven drying at 60oC for 24 hours.

TW: Turgid weight of the eight 1cm inner diameter leaf discs of each after floating in 20 ml distilled water and surface blotting for 1 min between tissue paper under a constant weight.

2.8.2 Measurement of chlorophyll content

Using CCM Plus-200 Chlorophyll Content Meter (Opti-Sciences, Inc. USA), the leaf chlorophyll content of the leaves was determined. Readings were recorded four times per replicate on each sampling day, with each reading taken between 7-8am to minimize the potential effects of diurnal variations. Fully expanded mature leaves were selected with care to ensure representative sampling. The sampling period spanned the duration of the study, and a total of six measurements were taken at regular intervals to capture changes in chlorophyll content over time.

2.8.3 Potassium (K) and phosphorus (P) concentration analysis

The plant tissues were subjected to dry ashing to destroy organic matter and dissolve the mineral elements. The procedure included weighing the sample using ceramic crucibles, ashing in a muffle furnace, and transferring the ashed samples to centrifuge tubes. Aqua Regia solution was used to rinse the crucibles and mix with the samples. The samples were centrifuged, and the supernatant was collected for further analysis.

For the determination of K and P content, flame photometry was employed using a flame photometer. The digest was diluted to an appropriate concentration for K estimation.
Standard samples, digest, and blank were measured in test tubes, and the emissions were measured in an air-acetylene flame. Calibration curves were drawn for both K and P emissions against concentration.

The calculations involved determining the percentages of K and P in the plant samples based on the measured values and the blank. The digest was diluted to a final volume of 100 ml for analysis.

2.9 Data analysis

The data were analysed using JMP statistical software (version 2020). In order to assess the impact of these treatments, the growth data were analysed using the logistic growth model. The characteristic duration was then calculated using In81/r, where r represents the growth rate of height and diameter for each treatment in the model. The logistic growth model is a widely used tool in ecological and agricultural research for modelling population growth.

It assumes that growth is initially exponential, but as the population approaches it carrying capacity, the growth rate slows down. Meyer et al. (1999) equation for the logistic growth model was used:

$$N(t) = K / (1 + e^{(-r(t-t0))})$$

Where;

N(t) represents the height of cocoa at time t; K represents the carrying capacity of the cocoa; r represents growth rate; and t0 represents the inflection point or the time at which the population growth rate starts to slow down (Figure 2). In this study, the logistic growth model was fitted to cocoa height and diameter data for each treatment, and calculated the parameters K, r, and t0 for each variety under different treatment conditions. The characteristic duration was then calculated using the formula log81/r, which represents the time it takes for the population to reach 90% of it carrying capacity. The effect of potassium fertilizer and AMF on cocoa growth was analysed by comparing the K, r, and t0 values for each treatment.

A four-way analysis of variance (ANOVA) was conducted in the statistical package R (R Core Team, 2020) for metric characteristics followed by the Fisher's test to determine which specific groups means differ at a 5% probability level.

Finally, a comprehensive analysis using Multivariate Adaptive Regression Splines (MARS) was used to determine the key factors influencing cocoa plant survival.

3.0 Result

3.1 Effect of potassium (K) fertilizer and arbuscular mycorrhiza fungi on the leaf RWC and chlorophyll content of three varieties of cocoa under water stress

Under both watering regimes, AMF inoculation combined with potassium application positively influenced the relative water content (RWC) of cocoa plants. For instance, under water stress condition, V1 exhibited a RWC of 67.7% without potassium, which increased to 82.8% with 2 g of potassium but decreased to 70.2% with 4 g of potassium. The RWC of V2 (61.1%) without potassium improved to 72.6% with 2 g of potassium and further increased to 81% with 4 g of potassium (Figure 3). The V3 had similar pattern, having an increasing trend from 64.6%, 73.9% and 83.5% as 0g K, 2g and 4g, respectively.

Under well-watered conditions, AMF x K had a decreasing effect on RWC of all the three varieties. For instance, V2 had RWC of 80.3% with 0g K but decreased to 72.2% and 75.5% with 2 g and 4g K respectively (Figure 3). However, without AMF inoculation, V1 experienced an increased in RWC from 61.5% without potassium to 81.6% with 2 g of potassium and decreased to 72.5% with 4 g of potassium. Regarding V2 without AMF inoculation, the RWC was 79.3% without potassium, decreasing to 75.3% with 2 g of potassium and remaining stable at 74.8% with 4 g of potassium.

In reference to V3 without AMF inoculation, the RWC was 81% without potassium, decreasing significantly to 75.6% with 2 g of potassium and further decreasing to 71.2% with 4 g of potassium (Figure 3).
Under water stress conditions, the combination of AMF inoculation and potassium application had a positive impact on the chlorophyll content of the cocoa varieties.

For V1, chlorophyll content increased from 19.4 μmol/m^2 without potassium to 21.6 μmol/m^2 with 2 g of potassium and further to 23.4 μmol/m^2 with 4 g of potassium (Figure 4). The V2 also recorded a higher increase from 20.5 μmol/m^2 without K to 26.4 μmol/m^2 with 2 g of potassium and 27.1 μmol/m^2 with 4 g of K (Figure 4). Similarly, V3 showed an increase from 19.9 μmol/m^2 without K to 26.8 μmol/m^2 with 2 g of K and 23 μmol/m^2 with 4 g of K when combined to AMF inoculation (Figure 4).
Contrary to the previous observation, without AMF inoculation the leaf chlorophyll content of V1 applied with 0g K increased from 16 μmol/m^2 to 19 μmol/m^2 with 2 g of K and peaked at 23.6 μmol/m^2 when K was increased to 4 g (Figure 3). Similar pattern was recorded in V2, with an increase from 22 μmol/m^2 without K to 24.2 μmol/m^2 with 2 g of K and 24.8 μmol/m^2 with 4 g of K. However, the pattern changed for V3, when K was increased from 2 g to 4g the chlorophyll content decreased from 24.7 μmol/m^2 to 18.6 μmol/m^2 (Figure 4).

Under well-watered conditions, the combined effect of AMF inoculation and potassium application also influenced the chlorophyll content of cocoa varieties, but at lower amounts than the water-stressed condition. The pattern for AMF and non-AMF was also similar. For example, without AMF V2 applied with 2g K and 4g K had chlorophyll content of 17.03 μmol/m^2 and 20.43 μmol/m^2 respectively. But compared to the same variety and K levels but with AMF the chlorophyll content increased to 27.11 μmol/m^2 and 24.74 μmol/m^2 respectively (Figure 4).

3.2 Impact of AMF inoculation on potassium (K) and Phosphorus (P) concentration of three cocoa varieties

The impact of AMF inoculation on the potassium (K) concentration of three cocoa varieties under water stress conditions was not significantly different (Table 1). However, the interaction of variety and AMF showed significant difference in the P concentration. V1 and V2 inoculated with AMF had the highest concentration of P with 0.130 mg/kg and 0.112 mg/kg respectively, but this was similar to V1 and V3 without AMF (Table 1).

Table 1 K and P concentration of cocoa varieties affected by AMF

Cocoa	**Potassium**	**Phosphorus**
Varieties	Concentration (mg/kg)	Concentration (mg/kg)
Variety 1	0.758	0.133^{b}
Variety 2	1.364	0.089^{a}
Variety 3	1.236	0.070^{a}
AMF	1.135	0.109^{a}
Non-AMF	1.037	0.098^{a}
Variety 1 × AMF	0.679	0.130^{b}
Variety 1 × Non-AMF	0.829	0.135^{b}
Variety 2 × AMF	1.406	0.112^{ab}
Variety 2 × Non-AMF	1.327	0.070^{ab}
Variety 3 × AMF	1.486	0.025^{a}
Variety 3 × Non-AMF	0.923	0.127^{b}

Means with different letters within a column are significantly different at $P<0.05$

Variety 1 = CRG8914×PA150; Variety 2= AMAZ15-15×EQX78; Variety 3= PA150×CRG 0314

3.3 Effect of K and AMF on the rate of growth and field survival of three varieties of cocoa

The growth data of cocoa (height and diameter) modelled using the logistic growth model showed that all three varieties of cocoa had different carrying capacities and growth rates for both height and diameter. The carrying capacity for height was found to be highest in V2 (746 cm) and lowest in V1 (455.78 cm) and V3 (450.36cm). A similar pattern was observed in the diameter measurement for V2 (138.67 mm) followed by V3 (129cm) and lowest in V1 (75.96 mm) (see Table 2 and Table 3).

Table 2 Summary of parameter estimate of cocoa height

Height data (cm)	**Variety**			**AMF**		**Fertilizer Application**			**Water Regime**	
Parameters	V1	V2	V3	Non-AMF	AMF	K- Level (g)			Well-watered	Water-stressed
						0	2	4		
Carrying capacity	455.78	746.00	450.36	498.21	520.03	168.19	173.50	173.83	168.64	171.81
CC StDErr	180.89	663.67	180.31	188.70	223.42	14.33	6.99	14.21	6.96	11.58
Growth rate	0.10	0.09	0.11	0.10	0.10	0.29	0.35	0.27	0.30	0.32
Inflection point	24.36	32.73	24.16	25.71	26.96	13.54	12.17	13.09	13.44	12.25

*CC StDErr is carrying capacity standard error

V 1 = CRG8914×PA150; V 2 = AMAZ15-15×EQX78 V 3= PA150×CRG 0314

Table 3 Summary of parameter estimate of cocoa stem diameter

Diameter data (mm)	*Variety*			*AMF*		*Fertilizer Application*			*Water*	
Parameters	V1	V2	V3	Non-AMF	AMF	K-Level (g)			Well-watered	Water-stressed
						0	2	4		
Carrying capacity	75.95	138.66	129.01	95.07	117.43	126.44	110.43	92.12	146.13	78.95
CC StDErr	21.69	88.16	89.01	32.30	52.41	90.42	52.13	30.57	77.31	22.55
Growth rate	0.098	0.088	0.084	0.092	0.088	0.084	0.087	0.098	0.088	0.093
Inflection point	22.07	31.72	32.03	26.07	29.59	32.42	28.45	24.41	32.35	23.61

*CC StDErr is carrying capacity standard error

V 1 = CRG8914×PA150; V 2 = AMAZ15-15×EQX78; V 3= PA150×CRG 0314

The characteristic duration, which is the time taken to reach 90% of the carrying capacity, was also found to be different for all three varieties, with V2 showing the longest characteristic duration for height (47 months) but not for diameter (49 months), V1 had 42 months and 45 months for height and diameter respectfully. V3 obtained a characteristic duration of 41 months for height and 51 months for diameter, being the longest (Table 2 and Table 3).

On the cocoa varieties survival within two years on the field, a comprehensive analysis using Multivariate Adaptive Regression Splines (MARS) revealed key factors influencing cocoa plant survival.

The model found that one knot provided the best fit in each explanatory factor (Figure 5a and 5b). For example, as chlorophyll exceeds 22.7 μmol/m^2, there was a marginal increase in cocoa plants survival.

For P, there was a similar increase (positive slope) for survival as it exceeds the threshold value of 0.102 mg g-1. For interaction plots, as both RWC and Chlorophyll decreased, cocoa plant survival increased as similarly observed in K and Chlorophyll interaction (Figure 5a and 5b).

4.0 Discussion

The cocoa varieties showed significant variation in their growth characteristics, even under similar treatments. Among the varieties, V2 had the highest carrying capacity and growth rate, indicating enhanced coping mechanisms and adaptations under drought stress. V2 trait of higher tolerance to drought stress could be due to its genetic make-up since all the varieties received similar treatments. Eliane et al. (2019) and Lahive et al. (2021) both assessed the effect of water stress in different varieties of cocoa, and observed differences among varieties, even though they used different varieties and treatments from those used in this study.

On the contrary, previous study by Kaba et al. (2022) revealed that V1 had the best mechanism for recovering from drought and subsequent survival at the seedling stage. Such discrepancies can be attributed to the variations in experimental approach, since they assessed the varieties' ability to recover after drought stress whilst this study assessed the varieties' ability to withstand drought stress. In addition, Kaba and co assessed V1 at seedlings (6 months old) and exposed the seedlings to two weeks drought stress but this study had a longer period of drought (4 months) and the cocoa trees were pre-fruiting (over 2 years) on the field.

4.1 Interactive effect of potassium application and AMF inoculation on relative water content (RWC) and chlorophyll content of cocoa varieties

The findings of this study revealed a significant interaction between potassium fertilizer application, arbuscular mycorrhizal fungi (AMF) inoculation, and cocoa varieties under different watering regimes.

In this study, the application of AMF, in combination with potassium, led to a notable increase in the relative water content (RWC) of all cocoa varieties compared to their solo applications (Figure 3). This indicates that the synergistic effect of potassium and AMF inoculation positively influences the water status of the cocoa plants under water stress conditions and suggests the potential of this combination in promoting drought tolerance in cocoa varieties. AMF's beneficial impact on plant water relations can be attributed to its ability to enhance nutrient uptake, particularly phosphorus (as evidence in Table 1), which plays a vital role in maintaining plant water balance (Porras-Soriano et al., 2009), and improving tolerance to environmental stresses, including water stress (Bahadur et al., 2019). For instance, Mathur et al. (2019) found that relative water content (RWC) of leaf was 93% in AMF inoculated wheat (*Triticum aestivum*) but declined to 27% in control (drought-stressed plants).

Potassium is also recognized as a crucial nutrient, influencing plant water relations and drought tolerance (Wang et al., 2013). The application of only potassium still had a positive impact on RWC, albeit to a lesser extent compared to cases where AMF inoculation was present (Figure 3). The positive effects of potassium and AMF on plant water relations and drought tolerance have been reported in previous studies as well. For instance, Waraich et al. (2011) demonstrated that potassium application improved water-use efficiency and reduced drought-induced damage in maize plants. Similarly, Begum et al. (2023) demonstrated that the identified Arbuscular mycorrhizal fungus (*Rhizophagus irregularis*) in this study alleviates drought stress in soybean by overexpressing the GmSPL9d gene, promoting photosynthetic apparatus and regulating the antioxidant system.

More interestingly is the fact that the findings reveal compelling insights into how AMF and K interact to modulate chlorophyll production (Figure 4), potentially impacting the photosynthetic efficiency and overall performance of the cocoa plants. For instance, under water stress conditions, a consistent and positive influence of the combined treatment of AMF inoculation and K application on the chlorophyll content of all three cocoa varieties was observed. For example, in the AMF inoculated plants, Leaf chlorophyll content increased with increasing levels of potassium fertilizer application.

If you take V1, it exhibited a notable increase in chlorophyll content from 19.4 μmol/m^2 without potassium to 21.6 μmol/m^2 with 2 g of potassium and further to 23.4 μmol/m^2 with 4 g of potassium fertilizer. V2 and V3 displayed similar trends, demonstrating the effectiveness of the combined treatments in enhancing chlorophyll production in cocoa leaves under water stress.

The AMF-mediated increase in chlorophyll content with varying potassium levels underscores the multifaceted ways in which AMF enhance nutrient utilization efficiency, contributing to improved plant performance under diverse conditions (Jajoo & Mathur, 2021). It is important to highlight that, previous studies (Rizwan et al., 2019; Khan, et al., 2019; Seleiman et al., 2020) have pointed out the important role chlorophyll plays in enhancing plants resilience to environmental stress, including drought.

However, contrary to the findings of Fotovat et al. (2007) and Chieb and Gachomo, (2023) which mentioned that photosynthetic pigment, chlorophyll is reduced under drought, this study recorded a similar chlorophyll content in both drought and watered condition (Figure 4). It is envisaged that the difference could be due to the combined effect of AMF and K, potentially increasing the levels of total chlorophyll and chlorophyll-binding proteins. For instance, the synergy of AMF and K could have prevented the accumulation of different intracellular reactive oxygen species (ROS) that are toxic to the plant organelles, thus, leading to a drastic imbalance in cellular functions such as carbohydrate degradation, chlorophyll damage (Chieb and Gachomo, 2023). The above negative effect has been reported in a number of species without AMF and K combined treatments, including sorghum (*Sorghum bicolor*), wheat (*Triticum aestivum*), Kentucky bluegrass (*Poa pratensis*), and creeping bentgrass (Wahid et al., 2007; Djanaguiraman et al., 2014).

It is noteworthy that under well-watered conditions, the influence of AMF inoculation and potassium application on chlorophyll content persisted, albeit with some variations. This suggests that the combination of these treatments can benefit cocoa plants not only in water-stressed conditions but also in well-watered environments, potentially contributing to improved overall plant health and productivity. This observation, is different from previous studies by Pons and Müller, (2022) and Sendek et al. (2019) which suggested that under good environmental condition, AMF seems to have an antagonistic or neutral effect on plants growth.

4.2 Impact of treatments on potassium and phosphorus uptake and cocoa survival

The results did not reveal significant disparities in potassium concentration across the diverse treatments. It is important to note that not every nutrient or mineral is equally promoted by the presence of AMF (Liu et al., 2019; Zhang et al., 2019). For instance, Xiao and Chen (2022) indicated that AMF increased shoot biomass, as well as N, P, Cu, and Zn uptake at every growth stage, whereas no significant difference was recorded for shoot K, Ca, and Mg uptakes. These findings highlight the selective and context-dependent nature of AMF-mediated nutrient interactions, illustrating that the effects on nutrient uptake can vary widely based on the specific nutrient and the stage of plant growth.

To emphasize the importance of AMF in phosphorus (P) nutrition, Smith et al. (2011) argued that the AM pathway plays a major role in P uptake, regardless of the extent to which an AMF plant benefits in terms of increased growth or P uptake.

The heightened phosphorus concentrations in the cocoa varieties inoculated with AMF at the different K levels (Table 1) suggest a potential positive correlation between AMF presence and enhanced phosphorus uptake. In support of these observations, Qi et al. (2022) and Venice et al. (2020) reported that AMF can form a network with plant roots to increase inorganic phosphorus acquisition by producing organic acids and phosphatases. They attributed such effect to the fact that AMF possess various key genes involved in the phosphate response signal transduction pathway.

This result (Table 1) reaffirms the findings of Smith et al. (2011) that P is critical for plant growth and makes up about 0.2% of dry weight. In addition, the result highlights the influence of cocoa genetic diversity on AMF's impact on phosphorus uptake. Varied responses across the cocoa varieties, such as those seen in V1, V2, and V3 underscore the intricate nature of plant-AMF interactions.

Another significant finding in this study was the inverse relationship between potassium rate and phosphorus concentration. Higher potassium rates were associated with lower P concentrations. This trend suggests a potential competition between potassium and phosphorus uptake pathways, where increased potassium availability might inhibit phosphorus uptake. This observation aligns with established principles outlined by Marschner (1995), who explored how interactions between different nutrients can lead to competition for uptake sites and transporters in plant roots, resulting in antagonistic interactions.

On cocoa field survival, the model determined that a single knot in each explanatory factor yielded the optimal fit.

For instance, when chlorophyll levels exceeded 22.7 μmol/m^2, cocoa plant survival demonstrated a marginal increase. A similar positive slope in survival was observed for P when it surpassed the threshold of 0.102 mg/kg (Figure 5a and 5b). Even though this is the first time such a study has been done on tree crops under field condition for a period of two years, the results resonate with previous findings (Chieb & Gachomo, 2023; Liu et al., 2015; Tariq et al., 2019; Jin et al., 2006) on the important role of phosphorus and chlorophyll in enhancing plants growth and survival under abiotic stressed conditions such as drought.

Conclusion

The sensitivity of cocoa to drought stress makes it imperative to find innovative approaches to enhance its survival under field condition. Therefore, this study established the relationships between native AMF colonization in cocoa rhizosphere and potassium rates on cocoa growth and drought resilience two years after transplanting to the field.

The findings established that the cocoa varieties vary in their growth characteristics, even under similar treatments. Among the varieties, V2 had the highest carrying capacity and growth rate, indicating enhanced coping mechanisms and adaptations under drought stress.

In addition, the findings revealed compelling insights into how AMF and K interact to modulate chlorophyll production, where, under water stress conditions, a consistent and positive influence of the combined treatment of AMF inoculation and K application on the chlorophyll content of all three cocoa varieties was observed. The AMF-mediated increase in chlorophyll content with varying potassium levels underscores the multifaceted ways in which AMF enhance nutrient utilization efficiency, contributing to improved plant performance under diverse conditions. In addition, the result highlights the influence of cocoa genetic diversity on AMF's impact on phosphorus uptake. Varied responses across cocoa varieties, such as those seen in V1, V2, and V3 underscore the intricate nature of plant-AMF interactions.

The study established that phosphorus uptake and chlorophyll content as influenced by the combined application of AMF and K were the most important factors determining the varieties' ability to survive drought under field condition.

Among the varieties, V2 had the highest carrying capacity and growth rate, phosphorus uptake and chlorophyll content, whilst the most economic K rate was 2g plant^{-1}. This reflected in the treatment "AMF x 2g K x V2" having the highest survival rate (92%) after two years.

Finally, another significant finding in the study was the inverse relationship observed between potassium rate and phosphorus uptake by the cocoa varieties. Higher potassium rates were associated with lower P uptake. This intensifies the speculation of a potential competition between potassium and phosphorus uptake pathways.

Theory and calculation

It is important to establish that most terrestrial plants, including cocoa, have a mutualistic symbiotic relationship with AMF (Xu et al., 2020; Li et al., 2023). They colonize the roots of the plant and build a network of hyphae that penetrates the soil, boosting nutrient intake and enhancing the plant's resistance to various abiotic stresses, such as drought (Boutasknit et al., 2020). AMF is currently considered an important biofertilizer and bio- stimulant in the agriculture sector; with the potential to contribute to the nutrition of its associated plants whilst enhancing their adaptation to abiotic stresses. For example, AMF symbiosis can improve nutrient uptake, root hydraulic conductivity, and water absorption of plants (Boutasknit et al., 2020; Mohammadi et al., 2022). Recent meta-analysis by Kaba et al. (2021) outlined the critical role of AMF in the cocoa sector as an alternative approach to enhancing cocoa trees survival due to environmental stresses caused by climate change.

In addition, potassium is an important macro-nutrient that is crucial for maintaining the water balance of plants and increasing their tolerance to drought stress (van Vliet et al., 2021). For instance, potassium improves water uptake and water conservation through the increase in cell membrane stability, root growth, leaf area, total dry mass and regulation of stomatal functions for plants living under drought conditions (Anokye et al., 2021). Biochemically, potassium helps in activating nitrate reductase; an enzyme critical for the production of protein in plants, regulating photosynthesis, water use efficiency, nitrogen uptake and protein building (Djan et al., 2018).

The findings in this study have implication for farmers choice of cocoa varieties, K nutrient application rate when AMF is used as a bio-stimulant under drought conditions and the UN SDG 13 (climate action) in the cocoa sector. More importantly, there is currently a high demand for cocoa beans and this is expected to continue in the future, evidence in the highest cocoa prices recorded in 2024. For instance, 1.8 million metric tons of cocoa would need to be produced to achieve a 3% increase in cocoa demand by 2025.

This implies that the current production of cocoa would need to increase by about 50% to meet the anticipated demand (Kaba et al., 2021). The practical implication of the findings in this study is the high cocoa yield on farmers field even in the wake of threads from climate change, and this translate to high income for farmers and the general improvement in the livelihood and living condition of the over 12 million people from cocoa producing countries in West Africa who depend on the cocoa sector.

Statements and Declarations

Competing Interests: The authors declare that they have no conflict of interest

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