

Cowpea and cassava leaf responses to different levels of water stress using fluorescence and reflectance spectroscopies

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ABSTRACT

Rising temperatures and increasingly recurring droughts are due to global warming. This phenomenon leads to increased water stress in crops and a drop in yield in certain cereal plants and tubers such as cowpea and cassava. Early detection of water stress is essential for the rational use of water resources and to overcome this stress. Several direct measurement techniques are used for this stress detection, such as stomatal conductance, chlorophyll content, soil moisture content, and leaf area, but their implementation is long and tedious. Fluorescence and reflectance spectroscopies could allow early water stress detection in real time. This research aims to evaluate the impact of water status in cowpea and cassava plant leaves using fluorescence and reflectance spectroscopies. Three degrees of stress of 0%, 50%, and 100% of the useful water reserve are induced on the two plants. Reflectance spectra were used to discriminate the different states of the plant. Similarly, a significant difference was observed in the variations of spectral indices such as normalized difference vegetation index, water index, and photochemical reflectance index that are more sensitive to plant water stress, especially in their growth phase. The fluorescence ratios show discrimination between the different plant water treatments. Classification using principal component analysis shows three classes corresponding to the three water treatments induced in the plants. The findings highlight a route of potential leaf-level water stress detection using noninvasive methods such as reflectance for plant health.

RESUME

L'augmentation des températures et les sécheresses de plus en plus récurrentes sont dues au réchauffement climatique. Ce phénomène entraîne un stress hydrique accru dans les cultures et une baisse de rendement chez certaines céréales et tubercules comme le niébé et le manioc. La détection précoce du stress hydrique est essentielle pour une utilisation rationnelle des ressources en eau et pour surmonter ce stress. Plusieurs techniques de mesures directes sont utilisées pour cette détection de stress telles que la conductance stomatique, la teneur en chlorophylle, la teneur en humidité du sol et la surface foliaire mais leur mise en œuvre est longue et fastidieuse. Les spectroscopies de fluorescence et de réflectance pourraient permettre une détection précoce du stress hydrique en temps réel. Cette recherche vise à évaluer l'impact de l'état hydrique dans les feuilles des plants de niébé et de manioc en utilisant des spectroscopies de fluorescence et de réflectance. Trois degrés de stress de 0%, 50% et 100% de la réserve en eau utile sont induits sur les deux plantes. Des spectres de réflectance ont été utilisés pour discriminer les différents états de la plante. De même, une différence significative a été observée dans les variations des indices spectraux tels que l'indice de végétation par différence normalisée, l'indice hydrique et l'indice de réflectance photochimique qui sont plus sensibles au stress hydrique des plantes, notamment en phase de croissance. Les ratios de fluorescence montrent une discrimination entre les différents traitements hydriques des plantes. La classification par analyse en composantes principales montre trois classes correspondant aux trois traitements hydriques induits dans les plantes. Les résultats mettent en évidence une voie de détection potentielle du stress hydrique au niveau des feuilles à l'aide de méthodes non invasives telles que la réflectance pour la santé des plantes.

I. INTRODUCTION

The demographic evolution of the world is rapid and the world population will increase from 8 to 10 billion by 2050 (United Nations, 2024). This rapid population growth is the source of several problems. Food safety and security are major challenges facing most countries worldwide (Chevallier-Le Guyader and Bock, 2020). It is up to every country to produce enough food, sustainably, to meet the needs of a growing global population. According to recent estimates, food production will double by 2050 to effectively meet needs (Van Dijk et al., 2021; Godfray et al., 2010; McKenzie and Williams, 2015). Achieving this goal is being delayed by climate change.

Indeed, climate change is causing significant environmental changes, such as droughts, the recurrence of which is accelerating the decline of forests in the West African Sahel. Drought also reduces plant cover and agricultural yields and promotes the expansion of denuded areas (Omotoso et al., 2023). The extent of climate change is also measured by a decrease and irregularity in precipitation (Kouassi et al., 2022), as well as shortening rainy seasons interspersed with more or less long dry episodes, with deficits. Over the last two decades, precipitation variability has become increasingly characterized by the frequent alternation of droughts and floods (Robinson et al., 2021; Li et al., 2023). Rainfall deficits, seasonal shifts, and extending drought duration, which are increasingly significant, affect vast territories and agricultural production basins worldwide (Wu et al., 2022). These persistent climate crises, combined with others, are the primary causes of the drop in agricultural yields and recorded food shortages (Bedair et al., 2023). Additionally, one significant impact of climate change due to rising temperatures is the alteration of water supply and demand for plant communities, which are essential for food production (Konapala et al., 2020). It has long been recognized that a lack of water availability has an adverse effect on plant growth, thereby decreasing crop productivity and yield (Jones et al., 2024; Seleiman et al., 2021). Therefore, studies focusing on early detection and warning of water stress in plants have become important (Manghwar et al., 2024; Safdar et al., 2023; Brown and Pool, 2023). The Cassava plant (*Manihot esculenta* Crantz) is one of the most cultivated root crops in the world, especially in West Africa (Mohidin et al., 2023; Adebayo, 2023). Cassava is a staple food for more than 800 million people in the tropics due to its numerous derived products (Coelho et al., 2020). Its leaves are rich in proteins. The crop offers a flexible harvest date. This allows farmers to keep their starchy roots in the soil until needed (Agbodan et al., 2020). Cassava's nature is versatile which confers its status as a crop of drought, famine, and war by the people (Immanuel et al., 2024; Burns et al., 2023;

Vernier et al., 2018). Cassava has become a major staple and a potentially significant crop (Adebayo, 2023). The solution is improving food security during climate change (Bourgoin et al., 2017; Gasparini et al., 2024). Cowpea (*Vigna unguiculata*) is an important legume, widely cultivated around the world (sub-Saharan Africa, tropical and subtropical, semi-arid and subhumid regions) (Achinewhu et al., 2003; Sebetha et al., 2015; Bassedik, 2021). Cowpea is grown for human and animal food (Djohy et al., 2023). The seeds of Cowpea contain high protein, energy, micronutrients, and macronutrients and constitute a valuable source of fiber carbohydrates, minerals, polyphenols, flavonoids, and antioxidants (Affrifah et al., 2022; Horn and Shimelis, 2020; Torres et al., 2019). Cassava and cowpea plants are of capital importance due to their numerous virtues. These plants can adapt to drought or lack of water, but still suffer from the effects (Priya et al., 2018). Water stress harms the health of these plants and agricultural yield even after re-irrigation (Ajayi et al., 2018; Bakayoko et al., 2009; Ravelombola et al., 2020). Therefore, the earlier detection of water stress that these plants undergo is crucial. Over the past decades, several direct measurement techniques such as stomatal conductance, chlorophyll content, soil moisture content, and leaf area have been used to study the water stress of plants (Nguinambaye et al., 2020; Chafika et al., 2014; Mussard et al., 2024). However, these direct methods are time-consuming and tedious to implement (Jones, 2007). Indirect and rapid optical techniques have been developed to characterize plant growth parameters (Sun et al., 2015). Leaves absorb, transmit, and reflect the incident radiation received by plants. The observed variations in spectral reflectance are primarily influenced by the optical properties of the leaf surface, its internal anatomical structure, and the concentration of key biochemical constituents such as chlorophyll and water (Peñuelas & Filella, 1998). Spectral signatures from leaf reflectance variations provide information about morphophysiological and biochemical changes in a plant, such as pigment concentrations, photosynthesis, biomass, and plant water status (Kothari et al., 2023; Li et al., 2023; Silva-Perez et al., 2018). Thus, the analysis of reflectance spectra offers an indirect means of estimating various plant physiological traits, which can be used to assess whether a plant is under stress (Katsoulas et al., 2016). Fluorescence spectra provide information on plant health. As a result, several vegetative studies, such as monitoring stress levels and the physiological state of plants, are carried out using the fluorescence technique (Lins et al., 2009; Ranulfi et al., 2016; Cui et al., 2024; Chappelle et al., 1984). The present study aims to study the potential of fluorescence and reflectance spectroscopies in characterizing the physiological and health state of cowpea and cassava plants under different levels of water stress based on in situ

measurements and spectral indices.

II. MATERIALS AND METHODS

II.1 Plant Description and Cultivation

The variety (VITOKO) of cowpea seeds was purchased from the Togolese Institute of Agronomic Research (ITRA), the most cultivated improved variety due to its high productivity and resistance to bad weather. The cuttings of Gbazékouté, one of the most cultivated varieties of Cassava in Togo, were taken from the Ecole Supérieure d'Agronomie (ESA). Pot trials were performed under a greenhouse on the ESA agronomic farm at the University of Lomé from January to March 2024. The research site is located at the geographic coordinates of 6°10'32"N and 1°12'39" E. The ambient temperature of the greenhouse varied between 27°C and 29°C while the relative humidity ranged from 70% to 85%. The cultures were done in six pots per plant. The plants were grown in 18 kg pots containing natural compost amended with sand at 10% by weight. For stress, the technique based on the useful reserve of the soil was used (Gupta and Kumar, 2023; Combres *et al.*, 1999; Doussan *et al.*, 2017). Three degrees of stress were applied, corresponding to 100%, 50%, and 0% of the useful reserve, representing plants subjected to excess irrigation (flooding), normal watering, and total cessation of watering (drought), respectively. The pots are weighed and adjusted, if possible, to maintain the stress degrees. The cultures were done on January 5, 2024, for both plants. Each pot contained one plant. The cowpea plants were cultivated under stress on January 23, 2024, two weeks after sowing the seeds. The cassava plants were stressed from February 17, 2024, and the delay is intended to allow the cassava plant cuttings to develop their first stems or leaves.

II.2 Sample collection

Samples are leaves of plants on which the measurements are carried out directly. For each type of measurement, one leaf is picked per plant and then analyzed immediately following the same protocol. Six leaves are taken from each plant, corresponding to the three stress levels. For a stress level, two leaves are picked, equivalent to two plants placed in the same state of stress. Leaves of the same shape are taken from approximately the same place for all plants.

II.3 Instrumentation

The SILVA NOVA spectrometer (StellarNet, US) is used for fluorescence and reflectance spectroscopy. The spectrometer has a spectral range of 190 nm to 1100 nm and a resolution of 1nm. Figure 1 presents the setup of reflectance measurement systems (Figure 1A), using a bifurcated optical fiber with the probe.

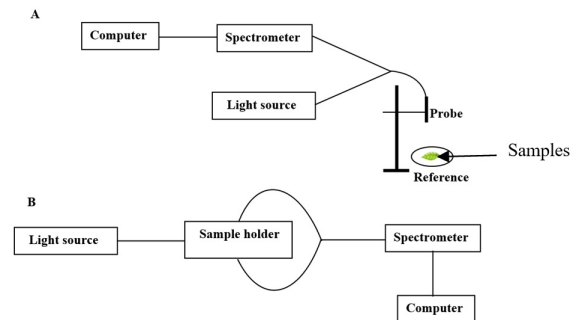


Figure 1: Measurement setups: A-Reflectance and B-Fluorescence

A white light source covering the range from 500 to 850 nm is used for illumination. Figure 1B represents the fluorescence measurement system setup, using a bifurcated F600 "Y" optical fiber connecting the spectrometer, a sample holder, and a light source. The excitation wavelength of 435 nm is used for fluorescence measurements. A quartz cuvette was used, and the leaf extract was obtained using 90° ethanol.

III. RESULTS AND DISCUSSION

III.1 Reflectance Spectra analysis

Full reflectance spectra of cassava and cowpea leaves at different dates and stress states are presented in Figure 2.

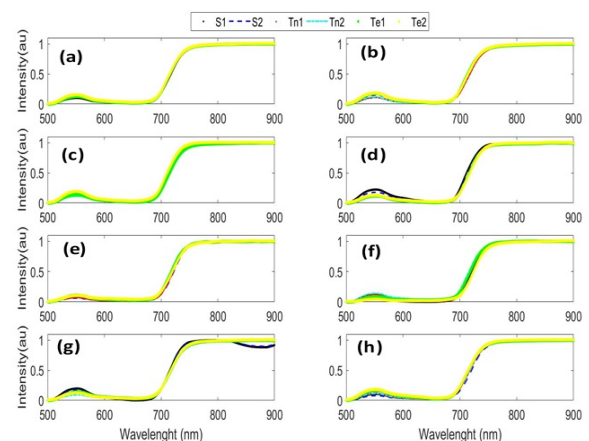


Figure 2: Full reflectance spectra of leaves at different degrees of water stress. Cassava: at (a) the onset of stress; (b) three days after stress; (c) 20 days after stress; (d) 24 days after stress. Cowpea: at (e) three days after stress (f) seven days after stress; (g) 18 days after stress; (h) 30 days after stress.

For all these spectra, the reflectance of the plant cover varied according to the water treatment regimes. The spectra (S) correspond to the plants subjected to a watering stop, the (Tn) to the control plants, and (Te) to those placed in excess watering. The reflectance spectra exhibit prominent peaks between 500 and 600 nm, a distinct trough near 675 nm corresponding to chlorophyll absorption, and a broad plateau extending from 740 to 900 nm, characteristic of near-infrared reflectance in vegetation.

Figure 3 presents the variations of the spectra between 500 and 600 nm. The reflectance spectra vary in response to water stress, with higher stress levels generally causing a decline in reflectance, attributed to reductions in chlorophyll and carotenoid concentrations as well as structural changes in leaf tissues.

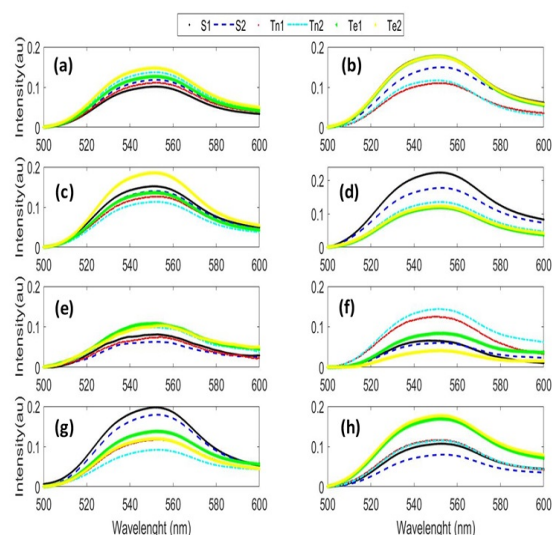


Figure 3: Reflectance spectra of leaves at different degrees of water stress. Cassava: at (a) the beginning of stress; (b) three days after stress; (c) 20 days after stress; (d) 24 days after stress. Cowpea: at (e) three days after stress (f) seven days after stress ; (g) 18 days after stress; (h) 30 days after stress

III.1.1 Cassava leaves

For cassava, two similar trends are observed from the reflectance spectra as shown in Figure 3. At the beginning of the stress (Figure 3(a)), the intensity of the first reflectance peak was practically the same for the six samples corresponding to the three degrees of stress. Three days after stress, the cultures with excess water present high reflectance values (Figure 3(b)). The same trend is observed three weeks later as shown in Figure 3(c) where high reflectance values are still noticed in plants with excess water. Figure 3(d) presents a change observed for the measurements taken in the fourth week after water

stress. The intensities of the first reflectance peaks increase as a function of the severity of the water stress at 100%, 50%, and 0%.

III.1.2 Cowpea leaves

The reflectance spectra of cowpea display several trends, especially in the spectral band from 500 to 600 nm throughout the study. For the three measurements taken in the first week, high intensities are observed in the plants (Tn) and (Te) (figures 3(e) and 3(f)). A reversal of trend occurs during the second week, with high intensities seen in the (S) plants (Figure 3(g)). At 30 days after stress, the order of increasing peak intensities is (S), (Tn), and (Te), as shown in Figure 3(h) for all measurements.

In this study, we found that reflectance peaks in cassava and cowpea leaves decreased with increasing water stress, particularly in the 500–600 nm range. This region, linked to leaf greenness and chlorophyll content, showed higher reflectance in well-watered plants, indicating better health. Our results align with recent studies showing that water stress reduces leaf pigments, lowering reflectance in the visible range (Li et al., 2024). The 500–600 nm band appears useful for early detection of water stress. Similar patterns were observed in soybean and poplar, supporting the value of reflectance as a non-destructive tool for monitoring plant health (Dai et al., 2023; Poudel et al., 2025).

III.2 Principal Component Analysis

Figure 4 shows the PCA classification for cassava and cowpea.

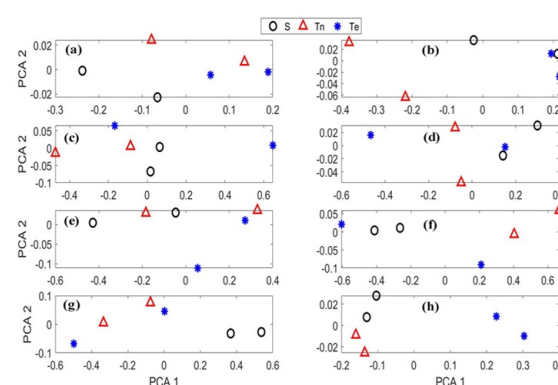


Figure 4: Classification by PCA of leaves using reflectance data at different degrees of water stress. Cassava: at (a) the onset of stress; (b) three days after stress; (c) 20 days after stress; (d) 24 days after stress. Cowpea: at (e) three days after stress (f) seven days after stress ; (g) 18 days after stress; (h) 30 days after stress

Reflectance data from cassava and cowpea leaves were subjected to Principal Component Analysis (PCA) to classify the samples according to their spectral responses under different water stress

conditions. A significant difference is noted between the two components, PC1 and PC2. PC1 enables us to identify the intrinsic relationship between the different datasets. From Figure 4(d), the data of plants (Tn) and (Te) are grouped. This shows that these cassava plants are almost in the same water state because they are watered. The same observation is made for cowpea during the measurements of the last weeks of the study (Figure 4(g) and 4(h)). The analysis separated the leaf samples based on their water stress levels, showing that using reflectance data with PCA is a useful and non-destructive way to detect plant stress early. This supports recent research where PCA was used to group plants based on how much stress they experienced. For example, Poudel et al. (2025) used PCA to tell the difference between drought-tolerant and sensitive cowpea plants. Patiluna et al. (2025) also used PCA and hyperspectral data to detect water stress in ornamental plants. In another study, Li et al. (2024) showed that reflectance data could help predict how much water is in poplar leaves. Dai et al. (2023) highlighted that combining reflectance indices with PCA improves the detection of stress in crops like soybean. These studies show that PCA is a valuable tool for monitoring plant stress and can help improve early detection in different types of crops.

III.3 Vegetative indices

Different vegetative indices were investigated using cassava leaf reflectance spectra. The Normalized Difference Vegetation Index (NDVI), Water Index (WI), and Photochemical Reflectance Index (PRI) were often used in water stress studies (Katsoulas *et al.*, 2016; Ihuoma and Madramootoo, 2017, 2019). These indices were calculated using the reflectance intensities at different wavelengths following equations 1 to 3:

$$\text{NDVI} = (\text{R800} - \text{R680}) / (\text{R800} + \text{R680}) \quad (1)$$

$$\text{PRI} = (\text{R570} - \text{R531}) / (\text{R570} + \text{R531}) \quad (2)$$

$$\text{WI} = \text{R900} / \text{R970} \quad (3)$$

The average values of these indices were obtained during measurements for four weeks, immediately after the plant was subjected to water stress.

For the Cassava plant, four weeks were divided as follows: first week: AC; second week: DE; third week: FG; fourth week: HI. For the Cowpea plant, the average values of the indices were obtained during measurements taken over five weeks, immediately after the plant was subjected to water stress. The five weeks were split as follows:

first week: A'C'; second week: D'; third week: E'; fourth week: F'G', and fifth week: H'.

III.3.1. Variation of PRI

Figure 5(I) shows how the Photochemical Reflectance Index (PRI) changed in cassava plants under three water treatments. For the plants with normal watering (Tn) and those at the end of watering (S), the PRI increased during the first two weeks, then decreased during weeks three and four, reflecting changes in water stress. In contrast, the plant with excess water (Te) showed an early increase in PRI during the first week. Overall, PRI values varied with the level of water stress. In weeks one and two, plant S had the lowest PRI, but by weeks three and four, its PRI was higher than those of Tn and Te. Figure 5(V) presents PRI trends in cowpea under the same treatments. Across all treatments, PRI decreased in the first week, rose in the second, dropped in the third, then increased again in the fourth week. In the fifth week, only plant S showed an increase in PRI. Initially, plant Te had the highest PRI, but in the last two weeks, plant S had the highest values. This shift may be due to reduced water content increasing PRI in stressed plants. Overall, PRI in cowpea followed two phases: a decrease during the onset of stress, and an increase once stress was fully established.

Under water stress, plants activate the xanthophyll cycle to dissipate excess light, increasing zeaxanthin levels and reducing reflectance at 531 nm, which lowers PRI values. This makes PRI a sensitive early indicator of drought, responding before visible stress symptoms. Studies confirm its effectiveness in detecting water stress across crops and ecosystems (Ogawa et al., 2024; Gamon et al., 1992; Suárez et al., 2008; Berni et al., 2009).

III.3.2. Variation of WI

Figure 5(II) illustrates the variation of the water index for the three degrees of stress in the cassava plant. During the first week, a decrease is observed initially, followed by an increase for all three treatments. This variation shows a decrease and an increase in the values of this index from the first week of the study for plants (S), (Tn), and (Te). On the other hand, in the second week, a decrease in WI and an increase in the third and fourth weeks are noticed. These variations are identical for the three treatments of cassava with water. The water index depends on the plant's water status. Figure 5(II) also allows us to compare the values of this index for the three water treatments of the cassava plant during the four weeks of the study. The WI values are higher in the plant (S) during the measurements carried out in the third and fourth weeks.

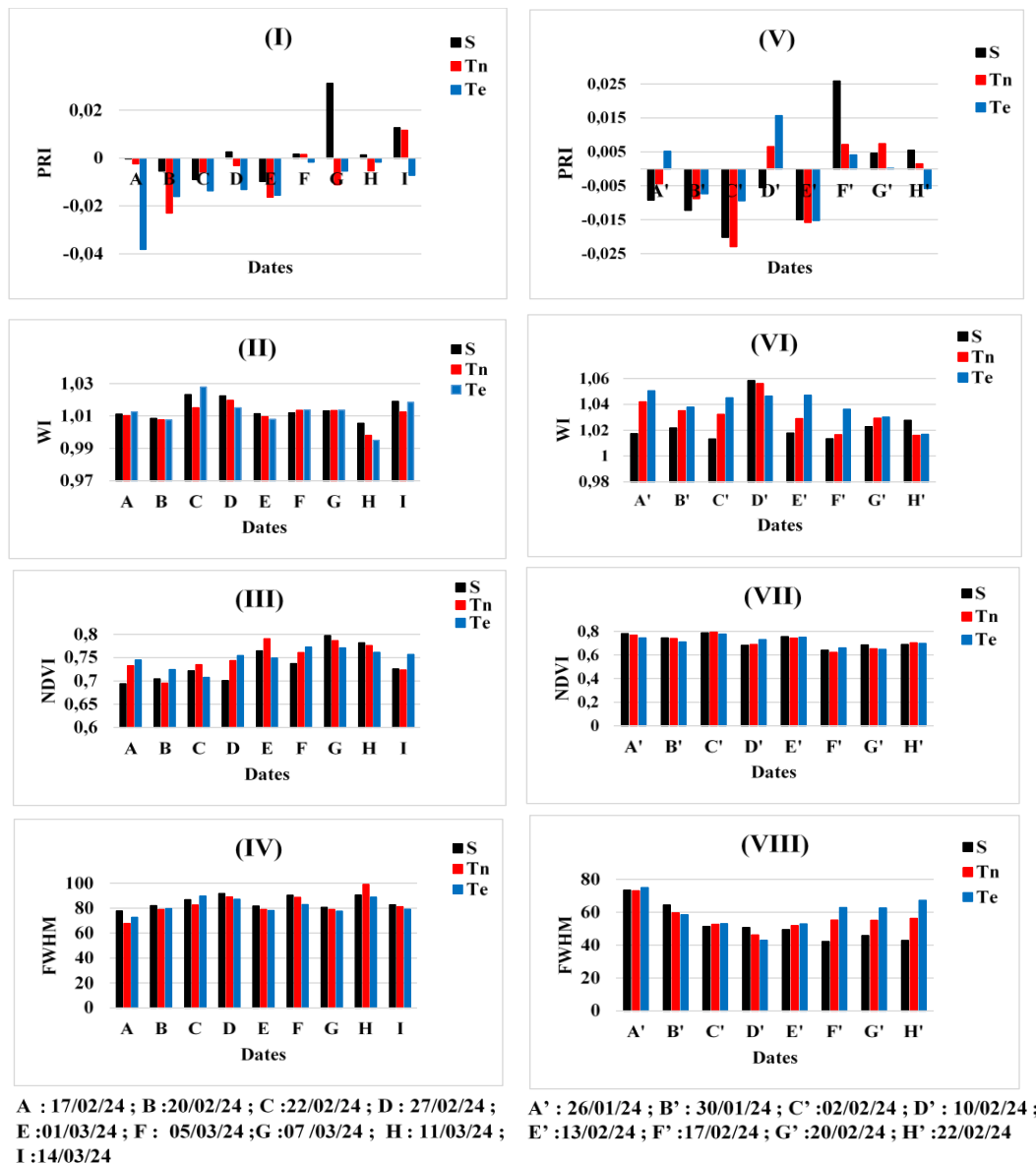


Figure 5: Effect of water stress on the values of indices: photochemical reflectance index (I, V), water index (II, VI), (III) normalized difference vegetation index (III, VII), and (IV) FWHM of the reflectance peak (IV, VIII) for cassava and cowpea, respectively. (S): water deficit; (Tn): normal watering; (Te): excess watering

These results agreed with the literature because WI increases when the water content of the plant decreases. The more the plant is in water deficit, the higher the WI values (Peñuelas and Inoue, 1999). Figure 5(VI) shows the variation of the water index for the three treatments with water on cowpea. Different patterns of WI are observed during the five weeks for these three treatments. In the first and second weeks, a discontinuity is noted across all three treatments, corresponding to either a decrease or an increase in WI. From the third week onward for the plant (Te), the WI value decreases until the fifth week. Higher WI values are observed in weeks 1, 3, and 4 when the watering degree is high. In contrast, during the second and fifth weeks of the study, WI values are higher in the plant (S). This

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leads to a shift in trend where the water index increases with the severity of water stress.

III.3.3. Variation in NDVI

For cassava, Figure 5(III) shows that the NDVI increased in the first week for the stressed plant (S), while it remained stable for the normally watered (Tn) and overwatered (Te) plants. In the second and third weeks, NDVI values increased for all three treatments, then declined in the fourth week. The values ranged from 0.64 to 0.80, indicating partial vegetation cover (Jones, 2007). Early in the experiment, NDVI was highest in Tn and Te, but by weeks three and four, it was higher in S and Te. Since the NDVI values remained close to 1, the index

appears to be less effective for detecting water stress during the early growth stage of cassava.

For cowpea, Figure 5(VII) shows that NDVI values were always above 0, confirming vegetation presence across all treatments. The NDVI followed a similar pattern for all three water treatments over the five weeks, with only minor differences. This suggests that water stress had little effect on NDVI in cowpea during the early growth phase. Therefore, NDVI may not be a reliable indicator of water stress at this stage. NDVI helps monitor how healthy crops like cassava and cowpea are by measuring how green they are from satellite images. When the plants grow well and get enough water, NDVI values go up. When they are stressed by drought or disease, NDVI goes down. Farmers and scientists use NDVI to track crop growth, detect problems early, and improve yields. This is especially useful for cassava and cowpea, which are important food crops in many tropical areas (Agarwal et al. 2025; Niyonsenga et al. 2024; Mallick et al. 2025; Ghobadi and Badehian, 2025).

III.3.4. Variation of full-width half Maximum

Figure 5 (IV) shows the variation of the full width at half maximum of the reflectance peak for the three treatments for cassava. The diagrams correspond to the average FWHM values for each type of water treatment. During the first week, the FWHM is higher in plants (S) and (Te). For the rest of the measurements taken during the last three weeks, plants (S) and (Tn) have higher FWHM values. All these results show an increase in FWHM values depending on the severity of the stress. Figure 5(VIII) presents the variation of the FWHM of the reflectance peak for the three treatments for cowpea. The diagrams correspond to the average FWHM values for each type of water treatment. Between the first week, and the third week, the FWHM is higher depending on the severity of water stress. The plants (Tn) and (Te) have higher FWHM values for the rest of the measurements taken during the last two weeks. These results show that the more the cowpea plant is in a water deficit, the lower the FWHM values.

This study shows that reflectance spectroscopy can help spot water stress early in cassava and cowpea. By measuring how plant leaves reflect light, especially around 700 nm and 531 nm, we can detect small changes caused by stress before the plants look dried or damaged. Using sensors with narrow bands makes it easier to see these changes. These hyperspectral sensors work better than normal ones and can help farmers act early to protect their crops during drought (Das Choudhury et al., 2023; Adeniyi et al., 2023; Weng et al., 2023; Ihuoma, and Madramootoo, 2019).

III. 4 Fluorescence spectroscopy analysis

Fluorescence measurements on cowpea and cassava are presented in Figure 6.

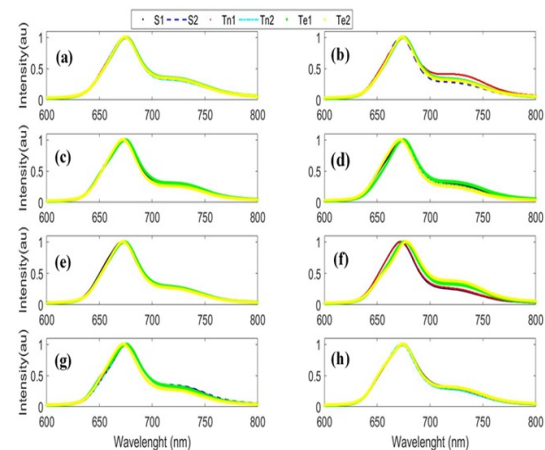


Figure 6: Fluorescence of leaves at different degrees of water stress. Cassava at (a) the onset of stress; (b) three days after stress; (c) 20 days after stress; and (d) 24 days after stress. cowpea at (e) three days after stress; (f) seven days after stress; (g) 18 days after stress and (h) 30 days after stress

The spectra (S) represent plants that had watering stopped, the (Tn) correspond to the control plants, and (Te) belong to those given excess watering. Fluorescence spectroscopy was employed to assess the physiological condition of cassava and cowpea leaves under various water treatments. The analysis focused on two key chlorophyll a fluorescence peaks: one in the red region (around 685 nm) and another in the far-red region (around 735 nm). These emission peaks offer insights into the efficiency of Photosystem II (PSII) and overall photosynthesis activity.

III.4.1. Principal Components analysis

The fluorescence data were classified using principal component analysis (PCA). Figure 7 shows the PCA classification of cassava and cowpea. The first principal component (PC1) of the fluorescence data demonstrates clear discrimination between different stress levels in both cowpea and cassava plants, indicating that fluorescence spectroscopy is sensitive to physiological changes caused by water stress. The classifications from fluorescence data during the study weeks make it possible to predict three classes corresponding to the three water treatments induced in the cowpea and cassava plants (Fu et al., 2022; Moustaka and Moustakas, 2023).

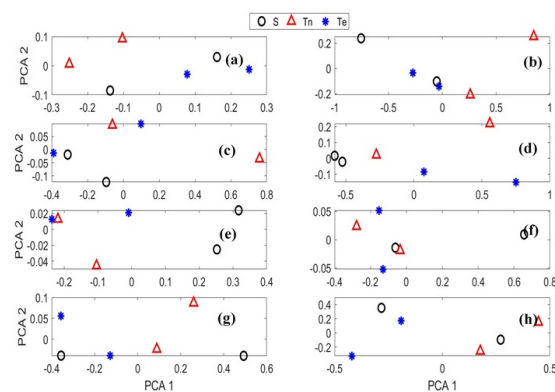


Figure 7: PCA classification from leaf fluorescence data of different degrees of water stress. Cassava at (a) the onset of stress; (b) three days after stress; (c) 20 days after stress; (d) 24 days after stress. Cowpea at (e) three days after stress ; (f) seven days after stress ; (g) 18 days after stress; (h) 30 days after stress

III.4.2. Red/far-red (RF/FR) fluorescence ratio

The intensity of two fluorescence peaks between 600 and 800nm is considered (El Fakir, 2020; Cerovic et al., 1999; Lichtenthaler and Buschmann, 1987). The first peak corresponds to red fluorescence (RF) and the second to near-infrared fluorescence (FRF). The average RF/FRF ratios for these three types of plant treatments with water were calculated. Figures 8(I) and 8(II) show the variations in RF/FRF ratios of cassava and cowpea plants, respectively. For cassava, the evolution of this ratio for all three treatments is practically the same throughout the study. Measurements at the beginning and end of the first and third weeks (AC; FG) show an increase in the RF/FRF ratio for cassava despite the degree of water treatment (Figure 8(I)). In other weeks (DE; HI), this ratio decreases despite the water level of the plant. The values of this ratio for the first and last weeks of the study for cassava show an increase for plants (S) and (Tn). A decrease is observed for the plant in overwater (Te). For cowpea, the evolution of the RF/FRF ratio is different for the three treatments. Figure 8(II) shows the same evolution for plants (S) and (Tn) during all five weeks of the study. A significant distinction is noted in the evolution of the RF/FRF ratio of the plant in excess watering (Te) from the other two plants, especially in the measurements of the fourth week (FG). The values of this ratio for the first and last measurement (A; H) for cowpea show an increase for all three plants.

The variations observed are due to the chlorophyll concentration, which differs not only from one plant to another but also from the different water treatments that the plants undergo (Munaweera et al., 2022). The RF/FRF ratio for all measurements of both plants shows higher values in cassava than in cowpea. The cassava plant has a

higher fluorescence efficiency than cowpea because the chlorophyll content is higher. The ratios of red (685 nm) to far-red (735 nm) fluorescence intensity, as the normalized difference in red and far-red fluorescence intensities, were calculated using the spectral data for both plants. These ratios were calculated based on the time of water stress for the three water treatments the plants were subjected. The results presented here for both plants are an average of the samples for each treatment type. The red to far-red fluorescence ratio (F_{685}/F_{735}) is a sensitive indicator of stress in plants, with changes in this ratio reflecting alterations in chlorophyll content and photosynthetic activity, as observed in both cowpea and cassava under water stress (Park et al., 2024; Zait et al., 2024; Legendre et al., 2021; Zhuang et al., 2020).

Cassava

Figure 9(a) presents the evolution of the fluorescence intensity ratio F_{685}/F_{735} for the cassava plant during the four weeks of water stress. Discrimination is noticed from the first week, precisely on the third day after water stress started. These variations can be caused by water stress because chlorophyll participates directly in the photosynthesis process. The normalized fluorescence difference $(F_{685}-F_{735}) / (F_{685}+F_{735})$ evolves in the same way as the fluorescence ratio for cassava. Figure 9(b) presents a similar variation where significant discrimination is noted three days after water stress (B).

Cowpea

The evolution of the F_{685}/F_{735} fluorescence ratio for the cowpea plant is shown in Figure 9(c). This ratio enables discrimination between the plant subjected to excess watering (Te) and the two others (S and Tn) for the cowpea plant. This discrimination is noticed from the seventh day (C) after the cowpea plant has been put under water stress. However, for the two plants (S and Te), good discrimination was only observed in the fifth week of the study (H). The variation in the normalized difference in fluorescence intensity is practically the same as for the fluorescence ratio observed for the cowpea plant.

Good discrimination is observed overall in the plant with excess watering of both plants (S) and (Tn) (Figure 9(d)). In certain weeks of the experiment, both the red/far-red fluorescence ratio and the normalized fluorescence difference increased in response to water stress in cowpea and cassava plants, reflecting stress-induced changes in the photosynthetic apparatus. However, in other weeks, these values decreased, indicating a non-uniform temporal response.

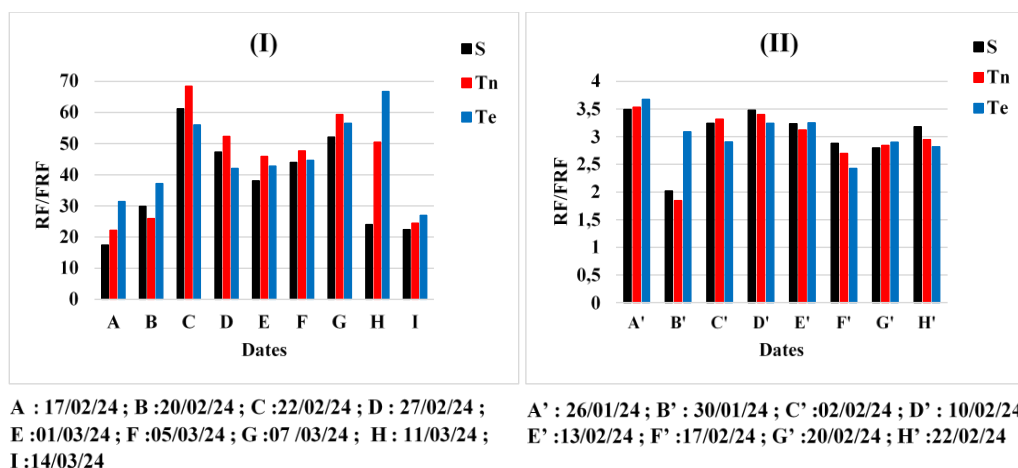


Figure 8: Evolution of the RF/FRF fluorescence ratio of (I) cassava and (II) cowpea leaves at different degrees of water stress

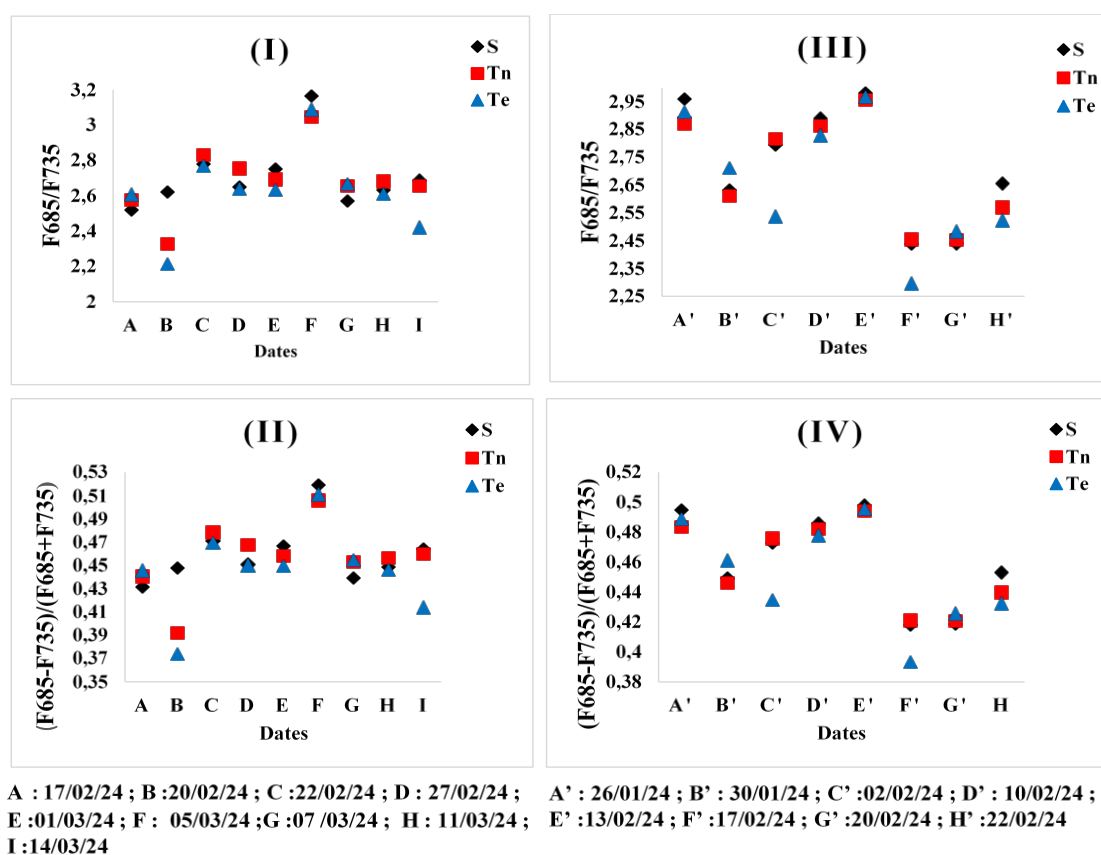


Figure 9: Evolution of the fluorescence ratios: F685/F735 and (F685-F735) / (F685-F735) of (a) and (b) of cassava; (c) and (d) of cowpea leaves, respectively at different degrees of water stress.

This variability contrasts with previous studies on maize, where these fluorescence indices exhibited a more consistent increase with stress severity, attributed to progressive chlorophyll degradation and altered energy transfer within photosystems (Park et al., 2024; Zait et al., 2024; Legendre et al., 2021). The fluctuating trends observed in cowpea and cassava may be due to species-specific physiological adaptations or differential stress tolerance mechanisms that modulate chlorophyll fluorescence responses over time (Lichtenthaler, 1988). These findings highlight the importance of considering crop-specific and temporal dynamics when interpreting fluorescence-based stress indicators (Marcassa et al., 2006).

IV. CONCLUSION

This study emphasizes the potential of combining reflectance and fluorescence techniques for early water stress detection in cowpea and cassava plants. The Photochemical Reflectance Index and the Water Index proved to be the most sensitive spectral indicators, especially during the early vegetative stage. These indices offered reliable, non-destructive assessments of plant water status under different irrigation treatments. In contrast, fluorescence ratios like F685/F735 demonstrated limited sensitivity to stress levels, probably due to effects from chlorophyll content and species-specific responses. Although this reduces their standalone diagnostic usefulness, fluorescence parameters still complement reflectance-based indices in evaluating plant physiological health. Overall, this research advances the development of a spectral model for detecting water stress. Future studies should focus on more severe and prolonged stress conditions throughout growth stages to improve the model's robustness and usability in crop monitoring and precision agriculture.

Author contribution:

Conceptualization, methodology, K.J.B.A., and M.M.D.; investigation, writing—original draft preparation, K.J.B.A., S.E.A., and K.R.B.A.; writing—review and editing, M.M.D. and M.A.M. All authors discussed the results and contributed to the final manuscript.

Conflicts of Interest:

The authors declare that they have no conflicts of interest concerning this article.

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