

Full Length Research Paper

## Influence of spectral properties on cassava leaf development and metabolism

Ephraim Nuwamanya<sup>1,2</sup>, Patrick R. Rubaihayo<sup>2</sup>, Ssetumba Mukasa<sup>2</sup>, Samuel Kyamanywa<sup>2</sup>, Joseph Hawumba<sup>3</sup> and Yona Baguma<sup>1\*</sup>

<sup>1</sup>National Crops Resources Research Institute (NaCRRI), P. O. Box 7084, Kampala, Uganda.

<sup>2</sup>College of Agriculture and Environmental Sciences, Makerere University, Kampala, P. O. Box 7062, Kampala, Uganda.

<sup>3</sup>College of Natural and Biological Sciences, Department of Biochemistry, Makerere University, P. O. Box 7062, Kampala, Uganda.

Accepted 3 February, 2014

Cassava's importance as a food security crop in Sub Saharan Africa will be enhanced by its special traits such as tolerance to drought and high yields under hydrothermal stress. Some of the special traits which include the light reflective and absorptive properties of the leaves that depend on the surface characteristics of the leaves, are variety dependent and may influence the plants' reaction to light; hence, its photosynthetic capacity. We investigated the differences in the leaf spectral properties in different cassava varieties and related them to leaf biochemical properties using 20 cassava varieties established in a randomized complete block design in Kasese, western Uganda. Time dependent changes in leaf spectral characteristics were studied using Digimizer software and related to changes in sugar and pigment properties. Changes in the amount of reflected light were observed for the three main wavelengths used by plants (blue, green and red) with the blue being the most preferred. Total soluble free sugars exhibited a diurnal pattern from lower values (0.07 mg/g) after the dark period to higher values (0.313 mg/g) as the day progressed and was different from those of translocatable sugars such as sucrose. Chlorophyll a exhibited a curved pattern in all varieties increasing with increase in light intensity from 09:00 h (0.18 ug/g), peaking at 15:00 h (0.22 ug/g) and dropping down in concentration by 18:00 h (0.16 ug/g). Significant differences were observed in cassava varieties for the concentration of chlorophylls and carotenes. The results were obtained at a time of optimal growth conditions (four months after planting) and were used to classify these varieties into three broad groups showing that studies on spectral properties of leaves can still give a lot of insights in selection for stress tolerance under less optimal stress. The significant changes observed in the phenotype especially the foliar portion of the plant with the stay green and early recovering mechanisms of tolerance identified also tallied well with observed spectral differences. The results show that studies on plant spectral properties can be important in making inferences on the plants physiological and growth status.

**Key words:** Spectral properties, tolerance mechanisms, physiology, reflectance.

### INTRODUCTION

Cassava (*Manihot esculenta* Crantz) is the third most important source of calories in the tropics (FAO, 2010) and depicts various tolerance mechanisms to abiotic stress-

ses. For cassava, a number of studies have been carried out to understand plant response to abiotic stresses such as moisture stress (Turyagyenda et al., 2013; Alves and

\*Corresponding author. E-mail: baguma1234@yahoo.com. Tel: +256772930185.

Setter, 2004), soil salinity and related stresses (Okcgenin et al., 2010; Fregene and Setter, 2007), but still, the development of drought tolerant varieties remains a challenge. One way of solving challenges related to moisture and salinity stress susceptibility is the selection of varieties that exhibit tolerance characteristics. Most of these characteristics are phenotypic in nature (Turyagyenda et al., 2013) and are related to leaf morphology and leaf response to light (Okcgenin et al., 2010). Light mediates a number of responses including leaf growth and development which are important factors in the deployment of stress tolerance mechanisms and manifestations such as the stay green trait. Therefore, the type and amount of light used during plant growth and development determines the way plants respond to stresses like drought. In a study carried out by Samuoliene et al. (2010), it was reported that the presence of red and blue light was important in plant morphogenesis whereby absorbed light intensity influence morphogenesis through support of processes such as photosynthesis and plant development which ultimately affect total plant yield. In fact light quality, quantity and photoperiod have been found to affect whole plant growth and developmental transitions (Folta et al., 2005).

Basic plant research has shown that specific light wavelengths such as the blue light are important in plant processes such as germination and stem growth, biomass accumulation (Kim et al., 2004) and transition to flowering (Valverde et al., 2004). Red light is important in stem/shoot elongation, phytochrome responses and changes in plant anatomy (Schuerger et al., 1997). The red light also contributes to plant photosynthesis by allowing the opening of stomata at appropriate times for the plant to take up enough carbon dioxide and hence metabolic influence of the red light cannot be ignored (Okamoto et al., 1996). On the other hand, blue light is important in chlorophyll biosynthesis and chloroplast maturation, stomatal opening, enzyme synthesis and the whole process of photosynthesis (Samuoliene et al., 2010). The importance of blue light in sustainable morphological development cannot, therefore be ignored. The contributions of the two spectral wavelengths during photosynthesis have been found to be efficient in advancing the developmental characteristics associated with autotrophic growth habits. Photosynthetically, inefficient light wave lengths also impart important environmental effects to a developing plant. In particular, the green light affects plant respiration and growth via cryptochrome-dependent and cryptochrome-independent means hence affecting plant development by either slowing down or increasing the rate at which respiration and plant growth occurs (Geiger, 1994). This is through the balance between used radiation where the green light opposes the effects of blue and red wave bands (Folta and Mahrunich, 2007). Therefore, because plants have become adapted to gradually changing daily irradiance, it is advantageous to study regulation of photosynthesis

and carbon metabolism in the context of the diurnal light cycle and how this affects the way plants respond to stress in relation to its effects on photosynthesis and hence plant yield (Geiger, 1994).

Plants are exposed to the same amount of light, but the amount of reflected light intensity off the plant leaf surface may depend on a number of factors including the leaf surface properties (Barnes and Cardoso-Vilhena, 1996), its orientation and its physiological state (Blatzer and Thomas, 2005). However, it is a fact that the more the reflected intensity observed, the less efficient the plant is at conversion of received light into useful photosynthetically active radiation (PAR) that drives photosynthetic process (Geiger, 1994). The understanding of this pattern would thus be important in easing the selection process based on the use of PAR by plants. It would also allow us to understand the basis of tolerance mechanisms which are mainly based on changes in plant morphology, biomass acquisition and relatedly low productivity. Although, lot of work has been done in the area of plant spectral response (Folta and Mahrich, 2007); much has not been done for cassava and the results from such studies have not been exploited fully in this important crop.

In the present study, results from reflective spectra of the three light wavelengths of red, blue and green were studied and compared to observed biochemical and phenotypic differences in cassava varieties and the comparisons were used to select varieties showing varied responses to plant abiotic stresses.

## MATERIALS AND METHODS

### Field design and sampling plan

Twenty (20) varieties of cassava were selected based on known parameters of dry matter content, resistance to Cassava Mosaic Disease (CMD) and farmer preference. They were established in a randomized complete block design (RCBD) in Mubuku Irrigation Scheme, Kasese Western Uganda. The trial consisted of two experimental and two control blocks in plot sizes of 9 x 9 m and up to 81 plants per plot. The different cassava varieties were observed on a daily basis for two weeks at an interval of one week in between and changes in main metabolites and plant spectral response were recorded. Results were taken at 4 months after planting (MAP) representing a period of optimal growth in cassava (Alves and Setter, 2004). Each day, tagged leaf surfaces (5th leaf on individual plants) of three plants per plot were photographed at 09:00, 12:00, 15:00 and 18:00 h at a distance of 30 cm away from the leaf surface. At the same time, leaf samples were picked on the same plant at every sampling period. To maintain the metabolic state of the leaf samples, leaves were preserved in liquid nitrogen by freeze drying. The samples were used to study the relationship between metabolites, physiological state of the plant and time. The metabolites considered were sugars, chlorophylls and carotenoids within particular leaf samples.

### Cassava leaf spectral properties

Using online computer software –Digmizer (digmizer.com), leaf surface photos taken at different times were analyzed for emitted

**Table 1.** Average weather conditions at the time different photographs were taken for analysis.

Time (h)	Temperature	Humidity	Dew point	Heat index
09:00	20.53 <sup>a</sup>	78.57 <sup>a</sup>	16.65 <sup>a</sup>	21.15 <sup>a</sup>
12:00	25.08 <sup>b</sup>	65.14 <sup>a</sup>	17.92 <sup>a</sup>	25.83 <sup>b</sup>
15:00	29.5 <sup>c</sup>	44.86 <sup>b</sup>	16.0 <sup>a</sup>	29.95 <sup>c</sup>
18:00	28.26 <sup>c</sup>	53.22 <sup>ab</sup>	17.37 <sup>a</sup>	29.06 <sup>c</sup>

Values followed by the same letter in a column are not significantly different at P = 0.05.

intensity at the different time periods. The leaf surfaces which differed in area and size were harmonized by considering an approximate leaf surface of 25 cm<sup>2</sup> on the surface directly exposed to sunlight at right angles. In all cases, the leaf midrib, big leaf veins near the bottom of the leaf and near apex surfaces were avoided. The selected portion was then analyzed for specific emitted intensities for the blue, red and green light intensities using a computer. The average emitted intensity and the hue were calculated using the software. The generated data was then converted into excel sheets and the data obtained averaged for each time period for the 10 day intervals and used to make inferences on plant response to light at different times. From analysis of spectral properties, average intensities were obtained for the different reflected wavelengths of blue, red and green. These were given in unit ratios of reflected light. The data collected was subjected to analysis of variance using the Genstat Software (Genstat, discovery edition, 2011).

Mean values for the different parameters at different times recorded for the 10 day period were used to study observed variations in leaf reflectance.

#### Chlorophyll and sugar extraction

After leaf optical properties were measured, chlorophyll and sugar concentrations of the same leaves were determined. Six circular disks, each 5 mm in diameter, were punched from the leaf portion for which optical properties were measured. The disks were placed immediately into 8 ml of 100% ethanol, and pigments were allowed to extract in the dark at 30°C for 48 h. During this period, free sugars in the leaf were extracted. Pigments were determined directly from the extracted solution with the organic solvent. Reducing sugars were extracted too after the solution was exposed to light at 50°C for up to 24 h until when the solution was completely decolorized.

#### Quantification of leaf sugar and pigment contents

Leaf samples collected were analysed for their individual pigment concentration at different times of the day for the 10 day sampling periods. Pigments of interest included chlorophylls a and b (Chla and Chlb) and carotenoids. The total amount of reducing sugars included both free and bound sugars excluding high molecular weight free carbohydrate components. Quantitative measurements for chla, chlb and carotenoids were carried out using a spectrophotometer by taking absorbencies at 662, 644 and 445 nm, respectively according to Wettstein (1957). Free reducing sugars were taken to be the water extractable sugars at 30°C while bound reducing sugars were taken to be the hot ethanol extractable sugars at 70°C for 5 min. The quantities of these sugars were determined using phenol sulfuric method by Dubois et al. (1956).

#### Data analysis

For the analysis of spectral properties, average intensities were obtained for the different reflected wavelengths of blue, red and green. These were given in unit ratios of reflected light. The data collected was converted into an excel file and the resultant data obtained was subjected to analysis of variance using the Genstat Software (Genstat, discovery edition, 2011). Mean values for the different parameters at different times recorded for the 10 day period were used to study observed variations in leaf reflectance. Carbohydrate and pigment related data was obtained from the different assays for samples obtained at different days and for the different times. Mean values for each variety were recorded and relationships between different parameters were studied. Microsoft excel software was used to study the trends in the different properties of the plant studied.

## RESULTS AND DISCUSSION

### Prevailing weather conditions

Results showing the weather conditions during the experimental time are shown in Table 1. These are conditions that have direct effects on leaf properties and its ability to intercept incoming radiation and include the relative humidity, the dew point and the wind speeds. During the experimental time, the relative humidity was considerably high and varied widely during the day (80 to 88%). Day and night temperatures ranged from 19.6°C at 09:00 h to about 31.1°C at 15:00 h. This represents the normal temperature range for the growth of cassava which is between 19 to 32°C (Alves et al., 2000); although, the variations in this case had a significant effect on the thermal and solar characteristics of the cassava leaves. The curve linear trend for daily temperature determined the type of photosynthetic metabolism carried out by the cassava leaves in relation to total metabolism (Yin and Kropff, 1996). Average daily humidity decreased with the time of the day and ranged from 87% at 09:00 h and 45% later in the day at 15:00 h representing a wide range of air water saturation which affects leaf temperature, stomatal opening and carbon dioxide intake as suggested by Katahata et al. (2007). Low average humidity (39 to 48%) at 15:00 h was due to increased incoming light and low rates of air water saturation as a result of high wind speeds during the day sweeping off any existing water near plant surfaces.

**Table 2.** Variations in leaf reflectance for the different light intensities at different times of the day for the different cassava varieties. DU = digmizer units).

Intensity	09:00 (h)	12:00 (h)	15:00 (h)	%CV
Red (DU)	0.494 <sup>a</sup>	0.471 <sup>a</sup>	0.475 <sup>a</sup>	8.80
%Reflected	30.95	30.04	30.00	
Green (DU)	0.681 <sup>b</sup>	0.671 <sup>b</sup>	0.676 <sup>b</sup>	6.47
%Reflected	42.67	42.79	42.70	
Blue (DU)	0.421 <sup>c</sup>	0.426 <sup>a</sup>	0.432 <sup>c</sup>	12.54
%Reflected	26.37	27.17	27.29	
Hue	105.14±7.08	111.6±9.87	111.31±7.02	7.53

Values for DU followed by the same letter in a column are not significantly different at  $P < 0.05$ . Values for Hue are followed by the standard deviation across the row.

The dew point was high earlier (16.7%) at 09:00 h and later during the day (21.4%) at 18:00 h and reduced with increase in in-solation as the day progressed being at its lowest at 12:00 h (Table 1).

### Phenotypic basis of grouping varieties

Based on observed phenotypic characteristics such as leaf retention, biomass accumulation and general plant growth characteristics, varieties were ranked into three broad groups according to their response to moisture and heat stress. The groupings included varieties that maintained a moderately high leaf area index (LAI) during periods of high temperature and low soil moisture or stay green varieties (SGV) such as NASE 2, NASE 4, I/92/0067 and MH/96/0068. The other grouping included those that lost all their leaves immediately after onset of stress only to recover immediately with increase in relative humidity and they were named as early recovering varieties (ERV) and some varieties had both mechanisms but were not very pronounced in each case. A few varieties completely lost their leaves as stress progressed and the ones which were retained were dechlorophyllated and rendered incapable to photosynthesize. The same varieties showed little or no capacity to recover easily after stress and they were named susceptible varieties (SV).

### Variations light intensity

Since the amount of absorbed light is directly proportional to the ability of the plant to utilize photosynthetically active radiation, the less the reflected light intensity, the more photosynthetically efficient the plant or the particular plant variety. Thus, studies on the variations in the reflected light intensity were carried out to allow for selection of photosynthetically efficient and high biomass

accumulating plants which as a result would have a higher degree of tolerance to hydrothermal stress. The results for this total reflected intensity are shown in Table 2. In all cases, the percentage reflected light was high for the green wavelength (~43%) compared to the red (31%) and blue (26%) wavelengths. High reflected green wavelengths were expected due to the high chlorophyll concentration in the leaves of the cassava plant. It represented almost twice of the total reflected intensity at all times though it changed considerably with the time of the day. Daily changes in reflected light for the blue, red and green wavelengths were observed where for the green light reflected intensity was low at beginning and end of the day while it was high between 12:00 and 15:00 h. This can be attributed to the amount of light intensity received by the plant at different times of the day (Folta et al., 2005). Like the green intensity, slight increments were observed for the blue wavelength reflected intensity as the day progressed while slight reductions were observed for the red wavelength. The plants had selective usage of three different main wavelength intensities at different times of the day, although, the blue wavelength intensity was the most preferred.

The selective usage of particular wavelengths by plants has been observed among other plants (Bjorn, 2008). This is important in allowing the plants to capture and convert all energy to chemical energy hence reducing the risk of photo-oxidation (Chalker-Scott and Cahill, 2001). However, selective use of either wavelengths at any time may be due to changes in plant morphology where if plants develop thicker leaves with cuticle meant to protect the plant against irrelevant irradiation may limit the use of particular light wavelengths and in instances where plants accumulate certain pigments that are also important in protection of membranes against oxidative damage may result into increased reflected intensity corresponding to a particular colored pigment (Spalding and Folta, 2005). Such scenarios happen during stress and may lead to changes in plant leaf spectral patterns. It also shows that

**Table 3.** Variations in leaf reflectance among variety groups and total average reflectance for the different main spectral groups.

Variety group	Intensity	09:00 (h)	12:00 (h)	15:00 (h)	Average
Stay green	Red (%)	30.99 <sup>a</sup>	29.84 <sup>a</sup>	29.57 <sup>a</sup>	30.13
	Green (%)	43.89 <sup>c</sup>	42.04 <sup>b</sup>	42.83 <sup>b</sup>	42.92
	Blue (%)	25.31 <sup>b</sup>	28.12 <sup>a</sup>	27.59 <sup>a</sup>	27.01
	Average intensity	0.523	0.538	0.537	0.533
Early recovery	Red (%)	31.81 <sup>a</sup>	30.19 <sup>a</sup>	30.69 <sup>a</sup>	30.90
	Green (%)	42.81 <sup>c</sup>	44.54 <sup>c</sup>	43.05 <sup>c</sup>	43.47
	Blue (%)	25.36 <sup>b</sup>	25.27 <sup>b</sup>	26.26 <sup>b</sup>	25.63
	Average intensity	0.531	0.501	0.520	0.517
Susceptible	Red (%)	31.07 <sup>a</sup>	30.30 <sup>a</sup>	29.38 <sup>a</sup>	30.25
	Green (%)	42.43 <sup>c</sup>	42.63 <sup>c</sup>	42.73 <sup>c</sup>	42.59
	Blue (%)	26.51 <sup>b</sup>	27.08 <sup>a</sup>	27.89 <sup>a</sup>	27.16
	Average intensity	0.548	0.495	0.559	0.534

Values followed by the same letter in a column within a variety group are not significantly different at  $P = 0.05$ . Note the high green and low blue reflectance for early recovering varieties compared to the stay green varieties.

the highly absorbed blue intensity mediated most of the physiological related reactions during the day for cassava plants. Cassava leaves selectively absorb the blue intensity to mediate phytochrome dependent processes such as photo-dormancy and for chemical energy while avoiding photo-oxidation which is mainly as a result of higher light intensities such as near ultraviolet wavelengths (Chalker-Scott and Cahill, 2001).

Importantly, it was observed that diurnal changes in irradiance follow a particular pattern beginning slowly during the day and generally matching the time constants of processes related to photosynthesis later in the day (Table 3). These results into differences in the rate of photosynthesis in the time course of the day which is mediated by the ability of the plant to absorb light of a particular wave length (Weller and Kendrick, 2008). Thus, the selective use of blue intensity in cassava is also important since the blue intensity has been found to support photosynthesis and increase the total plant biomass in form of the products. In addition, the red intensity is mainly important in flowering and physiological activities related to seed formation and seed development. The combination of the two lights is important for plants to achieve specific leaf area good enough for the plants physiological activities (Yorio et al., 2001). Plants use these different intensities selectively providing for the different physiological requirements. The use of particular wavelengths allows the plant to efficiently use light balancing different processes carried out by the plant (Spalding and Folta, 2005). Thus, cassava plants at mid growth cycle tend to selectively use the blue light to increase their vegetative growth and biomass accumulation. The selective use of different intensities at different times of the day also has an impact on plant photosynthesis and morphogenesis (Chalker-

Scott, 2010). In particular, chlorophyll synthesis is mediated by light perception and it was found out that in cassava, chlorophyll concentration patterns increased with increase in the amount of absorbed photosynthetically active radiation in the blue and red range of the spectrum (Table 4). The changes in pigment concentrations result into the observed shift in biochemical and physiological alteration within the plants metabolic profiles. For example high chlorophyll concentrations result into high photosynthetic rates and hence high sugar production (Rolland et al., 2006).

The sugars and metabolites are used in biomass accumulation in the plant which directly influences the development of stress tolerance traits such as the stay green trait (Xu et al., 2000). In this respect, plants that can withstand high temperature and low soil moisture stress are those that have accumulated reserves of metabolites before stress. These metabolites (especially the free sugars) arise from photosynthesis and hence are directly proportional to the amount of active radiation used by the plant. The use of most of the photosynthetically active radiation in stay green and early recovering varieties thus makes them better suited for biomass accumulation in preparation for adverse conditions later in the growing cycle. The differences point to the fact that in selection from a number of populations, the amount and type of radiation used is important as a selection tool for stress tolerant varieties. According to Geiger (1994), the light available to leaves for photosynthesis depends both on the time course of diurnal irradiance and on factors such as leaf orientation, that affect the interception of the incident light.

The evolution of photosynthesizing organisms under cyclic diurnal irradiance has equipped plants to regulate the photosynthetic process in ways that allow carbon to

be assimilated efficiently over the wide range of diurnal cycle irradiance.

### Variations in reflected intensity in variety groups

To understand the differences between different varieties or groups of varieties, the amount of light reflected off the leaf surfaces of these varieties was determined and recorded as the reflected intensity (Table 3). Over all, the average intensity varied within the different variety groups and at different times of the day. A lot of variation ( $P < 0.05$ ) was observed for the early recovering (0.501 to 0.531) and susceptible varieties (0.495 to 0.559) compared to the stay green varieties whose average intensity ranged from (0.523 to 0.538). In both the early recovering and susceptible varieties, average reflectance was low at 12:00 h unlike the stay green varieties where average reflectance was low at 09:00 h. High average reflectance for early recovering varieties was observed at 09:00 h while for susceptible varieties it was at 15:00 h. The reflectance also varied with the time of the day for the different variety groups. At 09:00 h, the amount of reflected light was high for susceptible varieties and low for stay green varieties while at 12:00 h, total reflected light was high for stay green varieties and low for susceptible varieties. At 15:00 h, overall average intensity was high for susceptible varieties and low for early recovering varieties (Table 3). This meant that stay green cassava varieties absorbed most of the in-coming radiation early in the day while susceptible varieties absorbed most of the radiation later in the day. Early recovering varieties absorbed most of the radiation throughout the day.

Total changes in reflected light during the day were thus low for the early recovering varieties compared to both stay green and susceptible varieties implying that at optimal conditions, the early recovering varieties had high optimal absorption of photosynthetically active radiation compared to other varieties. This means that these varieties had high optimal photosynthetic rates and hence converted more of the absorbed carbon dioxide into sugars. The results thus show that the use of different wavelengths of light by different varieties may be due to differences in pigment concentration in photosystem I and photosystem II (Hogewoning et al., 2012) which results into wavelength dependent excitation of the photosynthetic apparatus hence requirement for different wavelengths at different times of the day. This timing (the use of certain PAR at particular times of the day) is important because the effectiveness of light in the regulation of plant processes varies with the phase of the diurnal cycle as determined by the plant's time-measuring mechanisms (Weller and Kendrick, 2008). This explains the differences observed in different varieties in relation to selective use of certain light intensities at different times of the day. In addition, the differences observed

may reflect the differences in the photosystem used by cassava for main photosynthetic processes where each photosystem has different components and hence differences in light energy use capabilities (Samuoliene et al., 2010). In addition, not only does this light drive photosynthesis but it also affects plant morphology, physiology, leaf display and chloroplast orientation (Spalding and Folta, 2005; Weller and Kendrick, 2008). Thus, the variation observed in different cassava varieties point to different morphological and physiological responses of cassava towards available light. The percentage reflected light also varied among variety groups but followed the same pattern with the green light wavelengths being the most reflected as expected and the blue light being the least. After 09:00 h and as the day progressed, green light was reflected highly for early recovering varieties compared to stay green varieties and susceptible varieties while the blue and red light was reflected the least in these varieties. This explains the differences in the use of light with high spectral quality among different varieties.

It also shows the adjustments carried out by the cassava plants' light capturing apparatus in order to capture light of the best spectral quality. It also highlights the ability of the light harvesting complexes in both PSI and PSII to harness light of particular wavelengths as the pigment concentration in each of these photosystems changes with time (Hogewoning et al., 2012). The pigment concentration change is accompanied by changes in the ratio of chlorophyll a and chlorophyll b; hence, a shift towards absorption of particular light wavelengths. According to Geiger (1994), spectral quality, the wavelength composition of light, is important because photons differ in their probability of being absorbed by the light harvesting complex and hence their ability to drive carbon assimilation. The various light receptors for light-mediated regulation of plant form and physiology have characteristic absorption spectra and hence photons differ in their effectiveness for eliciting these responses (Spalding and Folta, 2005). The high amount of reflected green intensity for all varieties but especially the early recovering varieties points to the ability of plants to avoid incoming green radiation which from the action spectra of most crop plants is not highly used for photosynthetic processes (Folta and Mahrunich, 2007; Nishio, 2000). There seemed to be a balance in the type and amount of PAR used in each of the varieties with the ratios of the different light intensities being the same all through. This balance is necessary since imbalance in the amount and type of light available for plant metabolism results into imbalances in plant development (Weller and Kendrick, 2008).

In relation to this, poor plant development results into poor yields and susceptibility to both abiotic and biotic stresses (Chalker-Scott, 2010). Thus, the way varieties use available irradiance or the percentage of light absorbed determines the way the plant is adapted to

**Table 4.** Diurnal changes in reducing sugar (RS) contents for the different varieties.

Variety group/RS (ug)	09:00 (h)	12:00 (h)	15:00 (h)	18:00 (h)	Daily average
Susceptible varieties	0.222 <sup>a</sup>	0.060 <sup>a</sup>	0.149 <sup>a</sup>	0.330 <sup>a</sup>	0.190
Stay green varieties	0.359 <sup>b</sup>	0.072 <sup>b</sup>	0.143 <sup>b</sup>	0.316 <sup>ab</sup>	0.223
Early recovering varieties	0.291 <sup>a</sup>	0.078 <sup>c</sup>	0.152 <sup>a</sup>	0.293 <sup>b</sup>	0.204
P -value	0.005	0.008	0.038	0.042	

Values followed by the same letter within a column are not significantly different at  $P < 0.05$ . Note that the quantified include total free sugars (monosaccharides).

different environments. From the results, the differences observed point to the fact that different cassava varieties vary genetically in their light harvesting complexes and related pigments and hence perform differently in terms of morphogenetic and photosynthetic processes. The efficiency in utilization of incoming photons results into increased output of photosynthetic products due to increased carbon dioxide fixation which in turn impacts on biomass accumulation in these plants. It was also clear that the use of different forms of light depends on the accumulation of different pigments within the light absorption apparatus of the plant at different times of the day. This also explains the time dependent differences in the way different plants respond to light. High reflected light intensities at different times of the day point to less absorbed light; hence, low rates of photosynthesis and ultimately yield (Tables 3 and 4). Properties of light that affect growth and morphology of the plant, in turn, can affect photosynthesis.

Photosynthesis generates a positive feedback system in which photo-assimilation of carbon contributes to the growth of leaves that absorb photons and assimilate carbon to contribute to further growth. The compounding aspect of the production and growth of leaves obviously is affected by plant properties such as leaf area and thickness, which are regulated by light.

#### Relationship between light intensity and production of reducing sugars

The results for changes in sugar contents with the time of the day are shown in Table 4. Reducing sugars showed a diurnal rhythm with high amounts observed during the early and late hours of the day (Table 4). By 12:00 h, the reducing sugar content was four times lower than the amount at 09:00 h or later in the day at 18:00 h. However, an increment was observed from 12:00 to 18:00 h and beyond with significant differences observed during the light and the dark period. This was the time when the amount of PAR used in form of the blue and the red intensities increased showing the importance of active PAR in reducing sugar production. Variety differences were observed at 09:00 h with the stay green varieties having higher values compared to other varieties while the susceptible varieties had lower values.

The reducing sugar values were not significantly ( $p < 0.05$ ) different at 12:00 and 15:00 h for all varieties although differences were observed at 18:00 h, the stage at which sugar accumulation was also observed. The pattern observed was so different from the pattern observed for translocatable sugars such as sucrose (Rapacz and Hura, 2002; Usuda et al., 1987). A similar pattern for sugar changes has been reported in tree species and explains the importance of sugars in the plants metabolism especially their conversion into storable carbohydrate forms (Dickson, 1987).

In this study, it was observed that export of sugars from the source leaf depends on the leaf carbohydrate metabolism at particular times of the day (Table 4). Their increase during the day shows that plants prepare for production of sucrose during the day by increasing the daily concentration of reducing sugars as photosynthesis progresses reaching the peak as the day closes. It also suggests that the process of converting manufactured glucose and fructose into sucrose and starch does not occur concurrently with simple sugar production during photosynthesis. This is the suggested model for carbohydrate metabolism in C3 to C4 plants such as cassava and other C4 plants such as maize (Usuda et al., 1987). Particularly, it is known that increments in primary products of photosynthesis result into increments in sucrose accumulation which follows a net photosynthetic rate that follows increments with irradiance as the day progresses. The sugars especially glucose 6-phosphate and fructose 6-phosphate which are monomers for sucrose change in a different pattern compared to sucrose being low at the beginning of the day only to increase as the dark hours are approached (Usuda et al., 1987). Thus, the observed increase in reducing sugars at 18:00 h led to increase sucrose accumulation. Some of the reducing sugars are changed into storable carbohydrates while others are maintained to help in the providing energy for leaf metabolism at night and during the early hours of the day (Blasing et al., 2005).

Carbon partitioning thus depends on the amount and type of organic compounds required for leaf metabolism; hence, a different pattern is observed compared to that of sucrose which is mainly destined to storage organs where it is converted into starch as was observed by Thum et al. (2004) in *Arabidopsis*. Carbon partitioning also depends on the leaf age where in highly metabolizing

**Table 5.** Daily changes in the concentrations of the different plant main pigments among the broad phenotypic classes of cassava after stress.

Variety	09:00 (h)			12:00 (h)			15:00 (h)			18:00 (h)		
	Chla	Chlb	Cart	Chla	Chlb	Cart	Chla	Chlb	Cart	Chla	Chlb	Cart
Susceptible	0.21 <sup>a</sup>	0.12 <sup>a</sup>	0.42 <sup>a</sup>	0.20 <sup>a</sup>	0.11 <sup>a</sup>	0.42 <sup>a</sup>	0.22 <sup>a</sup>	0.11 <sup>a</sup>	0.45 <sup>a</sup>	0.15 <sup>a</sup>	0.07 <sup>a</sup>	0.35 <sup>a</sup>
Stay green	0.18 <sup>b</sup>	0.10 <sup>b</sup>	0.35 <sup>b</sup>	0.19 <sup>b</sup>	0.10 <sup>b</sup>	0.39 <sup>b</sup>	0.14 <sup>b</sup>	0.07 <sup>b</sup>	0.30 <sup>b</sup>	0.18 <sup>b</sup>	0.09 <sup>b</sup>	0.40 <sup>b</sup>
Early recovering	0.17 <sup>b</sup>	0.10 <sup>b</sup>	0.34 <sup>b</sup>	0.19 <sup>b</sup>	0.09 <sup>c</sup>	0.38 <sup>b</sup>	0.26 <sup>c</sup>	0.13 <sup>c</sup>	0.55 <sup>c</sup>	0.16 <sup>a</sup>	0.08 <sup>c</sup>	0.37 <sup>c</sup>
P- value	0.0021	0.002	0.004	0.002	0.0005	0.007	0.004	0.0002	0.01	0.001	0.0003	0.002

Chla = Chlorophyll a; Chlb = chlorophyll b; Cart = carotenoids. Values followed by the same letter in a column are not significantly different at  $P < 0.05$ .

and expanding leaves, most reducing sugars (fructose and glucose) are retained overnight in the leaf to allow for leaf development in terms of night respiration, leaf construction and construction of other support organs in the plant (Givnish, 1998). If the plant does not maintain the relevant sugar reserve, the costs for net carbon gain will be high since the plant cannot form relevant support structures (Geiger and Serveittes, 1994). The reducing sugar pattern followed the *chla* pattern hence showing the importance of *chla* in cassava plant photosynthesis and production of carbohydrates (Chalker-Scott, 2010). As the *chla* content increased, reducing sugar increased in turn showing that increases in *chla* increases photosynthetic rate.

### Relationship between light intensity and pigment properties

Because of the role of pigments in leaf growth and metabolism, variations in pigment content may provide information concerning the physiological state of leaves. Chlorophyll and carotenoids concentrations tend to decline more rapidly when plants are under stress or during leaf senescence but under optimal conditions differences observed allude to plant behavior. Generally, levels of *chla* increased with time reaching the peak at 15:00 h after which they dropped significantly to twice as low the value by 18:00 h (Table 5). *Chlb* followed the same pattern although there were no significant increments between 09:00 and 12:00 h for this pigment. The patterns observed appear to be linked to increments in active irradiation as the day progresses. This is likely because it has been earlier demonstrated that increase in light intensity influences the production of the chlorophyll and carotenoids (Weller and Kendrick, 2008; Chalker-Scott and Cahill, 2001). Notable too were variety differences which were observed for the two pigments ( $P < 0.05$ ). For *chla*, values were higher for susceptible and early recovering varieties between 09:00 and 15:00 h compared to the stay green varieties. However, at 18:00 h, the value for *chla* did not show any significant differences ( $P < 0.05$ ) inbetween the susceptible and early

recovering varieties; although, the early recovering varieties seemed to maintain a higher value at this time. Notable were the high values observed for *chla* and *chlb* for early recovering varieties later in the day (Table 4).

Thus, the concentrations of different chlorophylls in the leaf varied with the time of the day but were generally at the peak by 15:00 h. They followed the same pattern with *chla* being higher in concentration compared to *chlb* in all instances. The high concentrations of chlorophylls coincided with the higher absorbed light intensities for the blue and red wavelengths. This shows the efficiency of these pigments in light capture and utilization and the role of light in morphogenesis; hence, an increase in chlorophyll concentration. Chlorophyll pigments also varied within varieties and were found to be higher for stay green and susceptible varieties during the 9 h cycle. Their concentrations also showed a varied pattern when the different varieties were compared. Changes in irradiance mediate the changes observed for pigment concentration especially chlorophylls.

It has been observed that increase in irradiance results into increase in pigment composition until the saturation point resulting into photo-inhibition (Gordillo et al., 2001). It was also observed by Weller and Kendrick (2008) that high irradiance resulted into reductions in *chla* concentrations. This explains the differences observed among different varieties of cassava in terms of pigment concentrations pointing to different saturation points for different variety groups and even individual varieties within these groups. Much as the differences observed especially for chlorophyll concentrations were not significant, the downstream effects of the differences were detectable in the reducing sugars patterns observed (Table 4).

Carter and Knapp (2001) observed differences in tree species for chlorophyll content which also resulted into differences in reflective intensity patterns in these different plants. Accordingly, the observed differences in chlorophyll concentrations serve to explain the differences observed in reflectance especially between the early recovering varieties and stay green varieties. This was also observed by Thomas and Blatzer (2005) in temperate trees grown under different intensities.



Carotenoids also showed a similar diurnal pattern as the chlorophylls. Much earlier during the day, susceptible and early recovering varieties showed higher concentrations while later in the day early recovering varieties showed higher concentrations. Changes in leaf pigment concentrations are dependent on a number of factors but mainly the physiological state of the plant (Carter and Knapp, 2001). Apart from changes in irradiance during the day as observed earlier, diurnal changes observed in different plants are also a result of changes in the photochemical efficiency of the leaves during the day depending on the amount of irradiance available. According to Farghali (1997), diurnal changes in leaf pigment content were observed in old leaves of *Senna* and coincided with increase in dry matter content during the day. This study showed that these changes are related with the accumulation of dry matter (in form of carbohydrates and carbohydrate derivatives) in plants which is a function of photosynthesis; hence, variations in the photosynthetic efficiency of different crops. According to Pollet et al. (2009), response of chlorophyll is light dependent and affects the quantum efficiency of Photo System II. The increases observed with increase in light intensity in this study allude to this. Such increments also allow increments in the concentration of sugars produced during the day (Table 4) which progresses with increase in photosynthetic efficiency.

### **Spectral property based selections for stress resilience in cassava**

A measure of successful diurnal regulation of photosynthesis is the ability of the plant to lessen the impact of environmental stresses such as high temperature stress and use what light is available efficiently. The result is a general correspondence between carbon flux through the assimilatory segment of the cycle and the course of diurnal irradiance. According to Torres and Huber (1987), the accumulation of sugars in leaves of maize showed a diurnal pattern where they accumulated progressively during the day reaching its peak by 15:00 h. In particular, this was affected by the amount of enzymes involved in sucrose metabolism. In our case, it was observed that, the amount of sugar in the leaves increased during the day as observed earlier. The high *chl a/chl b* ratios are notable of plants highly adapted to sunny conditions (Givnish, 1988). These ratios determine the amount of absorbed light and also are related to total reflectance.

Alterations in reflectance are mediated by the differences in chlorophyll content within the leaves (Carter and Knapp, 2001). Effect of stress on chlorophyll thus leads to significant changes in the reaction of the plant to the different levels of moisture and heat stress. The importance of light in plant growth and metabolism determines its role in influencing the ability of the plant to accumulate relevant biomass. Light based biomass accu-

mulation is very relevant in plant growth but importantly in plant physiological response to the environment. Thus, the differences observed in the use of light in form of PAR are important in providing a biomass based selection tool for cassava varieties that are resilient to abiotic stress but importantly hydrothermal stress.

In particular, light based production of primary metabolites such as sugars and the differences observed among varieties for production of these metabolites is important in pointing out varieties that can tolerate stress. Such varieties have a strong source and in addition deposit a lot of stored metabolites in the sink resulting into biomass accumulation and an important trait described as the stay green trait. This trait allows cassava to tolerate various environmental stresses in field conditions but importantly hydrothermal stress. For varieties that have the ability to recover after stress, the root based biomass plays a critical role in providing metabolic resources for recovery and as such, these resources are mobilized and accumulated during growth under optimal conditions. Biomass accumulation is also as a result of photomorphogenic processes that occur during cassava growth and development. These processes are driven by particular wavelengths but mainly the red and blue based wavelengths which allow development of biomass based structure for driving physiological processes and for attaining a specific leaf area index that can support physiological processes. In addition, light is key to production of relevant pigments that drive physiological processes. Thus, in this study, the importance of cassava leaves in mediating the morphological and physiological processes of the plant was elucidated. Such processes are related to the means by which cassava plants manage stress and attain various levels of tolerance to abiotic stress. The responses of leaf/leaves to available light and how it affects the physiology of the plant have also been explained in cassava.

Deductions from the results show that much as spectral characteristics of the plant are mediated by the quality of light available to the plant, there are effects of quality of light on pigment concentrations although the main effects are observed in downstream processes such as sugar metabolism and carbohydrate production by the plant which leads to growth and development. Thus, the differences in the use of available quality light have an impact on the type of response by the plant to the environment but especially under abiotic stress. In addition, leaf related traits such as chlorophyll content and average absorption of light at different times of the day if well manipulated can be important in producing stress tolerant varieties.

### **ACKNOWLEDGEMENTS**

Barbara Akoli, Sharon Acheng, Genevieve Einau and members of the Biosciences Laboratory are gratefully

acknowledged for their technical input. Cassava program staff and technicians are also greatly acknowledged. Research and preparation of the paper was supported by the BiInnovate grant.

## REFERENCES

- Alves AC, Setter TL (2004). Abscisic acid accumulation and osmotic adjustment in cassava under water deficit. *Envtal and exptal. Bot.* 51(3):259-271.
- Alves AC, Setter TL (2000). Response of cassava to water deficit, leaf area growth and abscisic acid. *Crop Sci.* 40:131-137.
- Baltzer JL, Thomas SC (2005). Leaf optical responses to light and soil nutrient levels in temperate deciduous trees. *Am. J. Bot.* 92:214-223.
- Barnes JD, Cardoso-Vilhena J (1996). Interactions between electromagnetic radiation and the plant cuticle. In: Kerstiens G, ed. *Plant cuticles: an integrated functional approach.* Oxford: BIOS Scientific Publishers. pp. 157-174.
- Bjorn LO (2008). Terrestrial day light pp 123-130 in L O Bjorn (ed) *Photobiology, the science of life and light.* 2<sup>nd</sup> Ed springer Science
- Blasing O, Gibon Y, Gunther M, Hohne M, Morcuende R, Osuna D, Thimm O, Usadel B, Scheible W, Stitt M (2005). Sugars and circadian regulation make major contributions to the global regulation of diurnal gene expression in arabidopsis. *Plant Cell.* 17:3257-3281.
- Carter GA, Knapp AL (2001). Leaf optical properties in higher plants: linking spectral characteristics to stress and chlorophyll concentration. *Am. J. Bot.* 88(4):677-684.
- Chalker-Scott L, Cahill A (2001). Plant response to natural and enhanced UV environments. pp. 231-250
- Dickson RE (1987). Diurnal changes in leaf chemical constituents and 14C partitioning in cottonwood *Tree Phy* 3:157-170.
- Dubois M, Gilles KA, Hamilton JK, Rebers PA, Smith F (1956). Colorimetric method for determination of sugars and related substances. *Anal. Chem.* 28:350-356.
- FAO (2010). FAOSTAT. (<http://faostat.fao.org/>).
- Farghali KA (1997). Diurnal variations of chlorophyll and dry matter contents of *Senna occidentalis* in response to zinc and soil moisture. *Biologia Plantarum* 40(3):419-424.
- Fregene M, Setter T (2007). Recent advances in molecular breeding of cassava for improved drought stress tolerance. In: *Advances in molecular-breeding toward drought and salt tolerant crops* (in Jenks M, Hasegawa P and Jain M, eds). Springer, Berlin, Germany. pp. 701-711.
- Folta KM, Koss LL, McMorrow R, Kim H, Kenitz JD, Wheeler R, Sager JC (2005). Design and fabrication of adjustable red-green-blue LED light arrays for plant research. *BMC Plant Biol.* pp. 5:17.
- Folta KM, Maruhnich SA (2007). Green light: a signal to slow down or stop. *J. Exp. Bot.* 58(12):3099-3111.
- Geiger DR, Servaites JC (1994). Diurnal regulation of photosynthetic carbon metabolism in C3 plants. *Annu. Rev. Plant Biol.* 45:235-256
- Geiger DR, Servaites JC (1994). Dynamics of self-regulation of photosynthetic carbon metabolism. *Plant Physiol. Biochem.* 32:173-183.
- Givnish TJ (1998). Adaptive radiation of plants on oceanic islands: classical patterns, molecular data, new insights. In Grant P, ed. *Evolution on islands,* Oxford University Press. pp. 281-304.
- Gordillo FJ, Jimenez C, Chavarria J, Niell FX (2001). Photosynthesis acclimation to photon irradiance and its relation to chlorophyll fluorescence and carbon assimilation in halo-tolerant green algae (*Dunaliella viridis*). *PhotosynRes.* 68(3):225-235.
- Hogewoning SW, Wientjes E, Douwstra P, Trouwborst G, van Leperen W, Croce R, Harbinson J (2012). Photosynthetic Quantum Yield Dynamics: From Photosystems to Leaves. *Plant Cell* 24(5):1921-1935
- Katahata SI, Naramoto M, Kakubari Y, Mukai Y (2007). Seasonal changes in photosynthesis and nitrogen allocation in leaves of different ages in evergreen under-story shrub *Daphniphyllum humile*. *Trees.* 21:619-629.
- Kim HH, Goins G, Wheeler R, Sager J (2004). Green light supplementation for enhanced lettuce growth under red and blue light-emitting diodes. *HortScience.* 39:1617-1622
- Nishio JL (2000). Why are plants green? Evolution of the higher plant photosynthetic pigment complement. *Plant Cell and Environment,* 23(6):539-548.
- Okamoto K, Yanagi T, Takita S (1996). Development of plant growth apparatus using red LED as an artificial light source. *Acta Horticulturae* 440:111-116
- Okogbenin E, Setter TL, Ferguson M, Mutegi R, Alves AC, Ceballos H, Fregene M (2010). Phenotyping cassava for adaptation to drought. In P Monneveux, J-M Ribaut, eds, *Drought Phenotyping in Crops: From Theory to Practice.* CIMMYT/Generation Challenge Programme, Mexico City. pp. 381-400
- Pollet B, Steppe K, van Labeke MC, Lemeur R (2009). Diurnal cycle of chlorophyll fluorescence in *Phalaenopsis*. *Photosynthetica,* 47(2):309-312
- Rapacz M, Hura K (2002). The pattern of changes in photosynthetic apparatus in response to cold acclimation and de-acclimation in two contrasting cultivars of oil seed rape. *Photosynthetica.* 20(1):63-69
- Samuoliene G, Brazaityte J, Urbonaviciute A, Sebaieviene G, Duchoviskis P (2010). The effect of the blue and red light component on the growth and development of frugal berries.
- Schuerger A, Brown C, Stryjewski EC (1997). Anatomical features of pepper plants (*Capsicum annum* L.) grown under red light emitting diodes supplemented by blue and far red light. *Annals Botany.* 79:273-282
- Spalding EP, Folta KM (2005). Illuminating topics in plant photobiology. *Plant cell and Environment.* 28(1):39-53
- Rolland F, Baena-Gonzalez E, Sheen J (2006). Sugar sensing and signaling in plants: conserved and novel mechanisms. *Ann. Rev. Plant Biol.* 57:675-709.
- Thum KE, Shin MJ, Palenchar PM, Kouranov A, Coruzzi GM (2004). Genome-wide investigation of light and carbon signaling interactions in Arabidopsis. *Genome Biol.* 5, R10.
- Torres W, Huber SC (1987). Diurnal Changes in Maize Leaf Photosynthesis. Leaf Elongation Rate in Relation to Carbohydrates and Activities of Sucrose Metabolizing Enzymes in Elongating Leaf Tissue. *Plant Physiol.* 83(2):189-197.
- Turyagyenda L, Kizito EB, Ferguson M, Baguma Y, Agaba M, Harvey J, Osiru D (2013). Physiological and molecular characterization of drought responses and identification of candidate tolerance genes in cassava. *AoB PLANTS: plt007* doi: 10.1093/aobpla/plt007
- Usuda H, Kalt-Torres W, Keir PS, Huber SC (1987). Diurnal changes in maize leaf photosynthesis. *Plant Physiol.* 83(2):289-293.
- Valverde F, Mouradov A, Soppe W, Ravenscroft D, Samach A, Coupland G (2004). Photoreceptor regulation of CONSTANS protein in photoperiodic flowering. *Sci.* 303:1003-1006.
- Wettstein D (1957). Formula of chlorophyll determination. *Exp. Cell Res.* 12(3):427-489.
- Weller JL, Kendrick RE (2008). Photo morphogenesis and photoperiodism in plants. 417-464, in L O Bjorn (ed), *Photobiology, the science of life and light.* 2<sup>nd</sup> Ed. Springer Science N Y [www.digimizer.com](http://www.digimizer.com) *Digimizer* Image Analysis Software
- Yin X, Kropff MJ (1996). The effect of temperature on leaf appearance in rice. *Ann. Botany.* 77:215-221
- Yorio NC, Goins GD, Kagie HR, Wheeler RM, Sager JC (2001). Improving spinach, radish, and lettuce growth under red light-emitting diodes (LEDs) with blue light supplementation. *Hort. Sci.* 36:380-383.
- Xu W, Subudhi PK, Crasta OR, Rosenow DT, Mullet JE, Nguyen HT (2000). Molecular mapping of QTLs conferring stay-green in grain sorghum (*Sorghum bicolor* L. Moench). *Genome.* 43(3):461-469.