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Chlorophyll Fluorescence Analysis for Assessing Water Deficit and Arbuscular Mycorrhizal Fungi (AMF) Inoculation in Cassava (*Manihot esculenta* Crantz)

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Abstract: Arbucsular Mycorrhizal Fungi (AMF) are implicated as phyto-stimulators of various physiological processes of its symbiotic plants. The influences of AM fungi and water deficit on quantum yield of photochemistry (Fv/Fm) of photo-system II of cassava leaves were therefore investigated. Field experiment was conducted at Ajibode village while the semi-controlled experiment was conducted at the International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria. One improved cassava clone (TMS 4(2) 1425) and a landrace (TME1) were investigated. The soil is Oxic Paleustalf in both fields and controlled experiments. The controlled experiments were arranged factorially in a complete randomized design, while the field experiment was a randomized complete block design. The plants were adequately watered at alternate days. After two months, one half of the plants in each treatment were subjected to water stress. All treatments were replicated three times. Results obtained from chlorophyll fluorescence probe indicated that inoculated AM fungi enhanced photochemical efficiency of light reactions of the photosystem II (PS II) in intact cassava leaf tissues both under irrigation and water stress conditions. Water deficit levels based on more negative leaf Ψ had adverse effects on chlorophyll fluorescence of both genotypes. However, the adverse effect of water stress was significantly reduced by AM inoculation. Fv/Fm values could be a potentially useful criterion in selecting for drought tolerance and AM fungi efficiency of cassava.

Key word: AM fungi · cassava *(Manihot esculenta* crantz) · chlorophyll fluorescence · chlorophyll pigments · photochemical efficiency · quantum yield · sterilized soil

INTRODUCTION

Cassava is a tropical crop and has become one of the dominant starchy staples in sub-humid and humid lowlands. It is mainly cultivated on small farms in a variety of infertile soils and in environments subjected to varying periods of drought stress [1]. Cassava is commonly known to possess a good capacity to withstand drought stress [2, 3] irrespective of the cultivar used. Despite this ability, water stress still reduces its net biomass production [4] greatly below its maximum yield potential. Inoculation of cassava with Arbuscular Mycorrhiza (AM) fungi has also been shown by various studies to enhance its yield in soils low in available nutrients [5-8]. However, the influence of AM precisely on cassava photosynthetic mechanisms as it relates to growth and yielding ability has not been fully investigated for those cultivars recently selected by breeders and are targeted for sub-Saharan Africa's cassava growers. Physiological processes of cassava are noted as sensitive to moderate to severe drought stress [9-11] reported a decrease in cassava chlorophyll production during water stress condition. A decrease in chlorophyll synthesis will definitely have detrimental effect on the quantum yield of PSII of cassava and consequently affects its yield performance because quantum yield of PSII in plants can be directly related to their stress physiology. The relative quantum yield of PSII can change with abiotic factors such as water deficit, light, temperature, etc. and therefore quantifying quantum yield of PSII can provide important information about plant-environment relationship.

Leaf chlorophyll fluorescence probe is a powerful and sensitive intrinsic measurement of the photosynthetic process [12, 13] that can be used to detect the influence

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of various environmental stress factors. It is used to determine how light use efficiency for photosynthesis occurs at the cellular level. The intensity of fluorescence is directly related to the concentration of excited chlorophyll molecules, which suggests that a change in the fluorescence yield be related to a change in the efficiency of photosynthesis, therefore, providing a measure of leaf photosynthetic ability of plants. The onset of stress injury is always accompanied by a decrease in chlorophyll fluorescence. According to Sthapit [14], the ratio of variable fluorescence (Fv) to maximal fluorescence (Fm) is termed as photochemical efficiency of PSII (Fv/Fm), is directly related to its quantum efficiency and is therefore used as a good diagnostic probe for measuring cold stress. A healthy leaf generally gives a Fv/Fm value of about 0.80.

Although cassava is reputed for its drought tolerance, limited information is available on its mechanisms of drought tolerance associated with photosynthetic light use and in particular for the cassava germplasm presently advocated for culture in the food-starved sub-Saharan African regions [2, 15-18]. The objective of this study was hence to determine the influence of AM fungi inoculation and water-deficit stress on the photochemistry of cassava influencing its growth and yielding potential. The ultimate objective of this and associated studies is to provide information for the cassava breeding programs where photosynthetic ability and tolerance to water deficit are part of the potential selection tools to improve the crop for greater productivity.

MATERIAL AND METHODS

The experiments were carried out at the International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria located in the transition zone (derived savanna) between tropical rain forest and humid savanna with a mean annual rainfall of between 1200-1500mm, which is bimodal in nature. The dry period starts in November and may last till mid-April. Total class A pan evaporation is 1550-1600mm. The mean annual air temperature ranges from 20-30°C (min) to 24-34°C (max). The relative humidity is usually high. Mean monthly relative humidity at lowest was 61% (February) and above 83% in August.

Semi-controlled experiment: Semi-controlled experiments were conducted at IITA, using large plastic containers. The topsoil (Oxic Paleustalf) was obtained from the IITA Farm. It was thereafter steam sterilized in a gas-propelled furnace in order to produce sterilized soil for the container experiments. The sterile soil was then filled into large polyethylene bags (standard garbage). Each container received 50kg of the sterilized soil. The containers were laid out factorially in a complete randomized design with three replications. The containers were spaced 1m by 1m. Two cultivars tested included TMS 4(2) 1425 (improved cultivar) and TME1 (a local landrace). They were planted separately in each container.

The experiment was sub-divided into three levels for the mycorrhizal treatment. One level was inoculated with *Glomus mosseae* (Nicholson and Gerdeman) Gerderman and Trappe, the second set was inoculated with *G. clarum* (Nicholson and Schenck). The third served as the control (no mycorrhizal inoculation). The inoculated containers received 20g of crude inoculum (which consisted of soil, root fragments of the host plant used to trap the mycorrhizal, spores and hyphae) each. Inoculation was done by placing the crude inoculum directly under the stakes in the polyethylene containers. All plants were watered every 48 hours until two MAP. Each treatment was later sub-divided into two sets at 2 months after planting (MAP). One set received adequate water supply (watered every alternate day to field capacity) while the other set was subjected to complete water stress for 2 months. Water stressed containers were covered with transparent polyethylene sheets at the advent of rainfall to prevent rain water from entering the containers.

Field experiment: The field experiment which was arranged in a randomized complete block design was conducted at Ajibode. The moulds were spaced 1m x 1m. One cassava stake was planted in each mould. The stakes were either inoculated with *G. moseae* or not, though, the indigenous mycorrhizae were not destroyed. The experiment received adequate watering for the first three months. Watering was withheld after three months in water stressed plots for another three months. The stress was terminated in the field experiment after 3 months of continuous water stress and the plants were afterwards watered adequately, particularly from rainfalls till harvest at 12 MAP.

Chlorophyll fluorescence and leaf water potential measurements: Chlorophyll fluorescence measurement started at 1 Month after Planting (MAP) and continued until the sixth month in controlled experiment. Measurements were carried out every week. Three

leaves were selected from each plant for measurements. Chlorophyll fluorescence was also replicated three times thereby giving nine leaves per treatments. This was measured with a Portable-photosynthetic Efficiency Analyzer (PEA) model (Hansatech Inc. Co., UK). [17]. The leaves were pre-adapted to dark period for one hour by fixing special tags on each leaf blade before measurements were taken. Young matured leaves (5th position from the top) were tagged in each plant to be measured prior to the commencement of data collection. After one hour of dark adaptation, the sensor cup was fitted on the leaf for measurement. Values of Fo and Fm were recorded. Values of Fv were estimated as the difference between Fm and Fo. Measurements were taken twice in a day, both in the morning (10 am) and in the afternoon (3 pm). Early morning and mid afternoon leaf Ψ were measured in representative leaves (three leaves per plant) with a pressure chamber apparatus (Soil Moisture Instruments Co. Santa Barbara, CA., USA)

Growth and biomass analyses: Destructive samplings were carried out at 3, 6 and 9 MAP to determine the biomass production. The plants were separated into fibrous roots, tubers, rootstocks, stems, petioles and leaves at harvests. Their fresh weights were taken using a Metler balance. Total leaf area was estimated at each harvest with a leaf area meter (Model 3000, Licor Inc. USA). Plants heights were measured with a meter rule. The samples were dried to constant weights in an oven at 70°C for 72 h.

Estimation of AM colonization rates: Roots samples were collected at each harvest from three replicates for each treatment for determination of AM root colonization rates. These roots were stored separately in different McCartney bottles filled with 50% ethanol. The samples were later washed in running water and cleared in 10% KOH. They were again rinsed in three changes of water. The clean samples were further bleached in H_2O_2 (3ml of 20% NH_4OH and 30 ml of 3% H_2O_2) for 30 min. under room temperature. The samples were later acidified in 1% HCl for 3im. After this, the HCl was poured off and the roots stained in trypan blue solution prepared by mixing 500ml of glycerol, 450ml of water, 50ml of HCl and 0.05g of trypan blue. The samples were then left overnight after which the stain was poured off. The samples were later stored in glycerol for further investigations. Percentage root colonization was estimated using grid-line intercept method of Giovanetti and Mosse [19].

Statistical analysis: Data were either analyzed as a factorial CRD or split plot RCBD. Analysis of variances (ANOVA) was carried out and means were computed and compared using the t-test [20].

RESULTS

Root colonization and plant biomass production: The root colonization of the inoculated plants was significantly higher (at P = 0.001) than the non-inoculated plants at 3 and 6 months after planting (Table 6 and 7). Colonization of the AMF inoculated plants ranged above 80% irrespective of the AMF species, cultivars or water regime. However, there were low levels of colonization (18.3 to 32%) in the non-inoculated cassava plants.

The dry tuber weight of the mycorrhizal inoculated cassava was significantly higher than the non-inoculated ones in both the field and semi-controlled environments (Table 6 and 7). The cassava biomass production was significantly reduced by water stress. The number of leaves and leaf area production were greatly reduced by water stress (Table 6 and 7). Exception was found in TMS 4 (2)1425 under semi-controlled environment where the non-inoculated plants produced higher leaf area than the AMF inoculated in both well and water stress treatments.

Diurnal water stress effects on leaf water potential: In the landrace genotype, variations between treatments for leaf Ψ were minimal in both early morning and mid afternoon in water stress condition. However, the

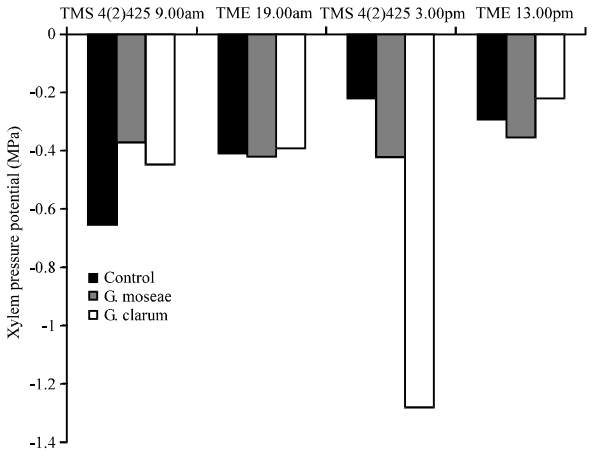

Fig. 1: The effect of mycorrhizal inoculation on xylem pressure potential of two cassava cultivars under water stress condition at 3 MAP

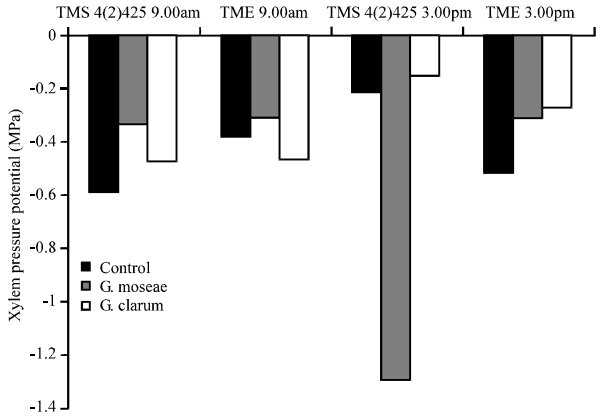

Fig. 2: The effect of mycorrhizal inoculation on xylem pressure potential of two cassava cultivars under irrigation at 3 MAP

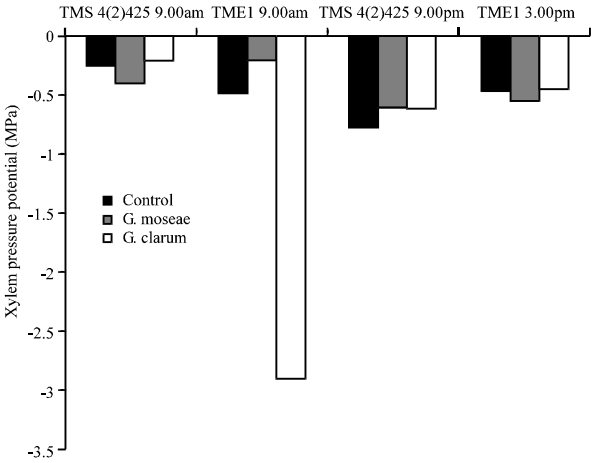

Fig. 3: The effect of mycorrhizal inoculation on xylem pressure potential of two cassava cultivars under water stress condition at 6 MAP

variations between treatments for leaf Ψ were large in the improved cultivar, both in the morning and afternoon under water stress condition at early growth stage (Fig. 1). In the morning in improved cv. the leaf Ψ of non-inoculated was lower than the inoculated counterparts, but in the afternoon the inoculated counterparts maintained lower leaf Ψ than the non-inoculated (Fig. 1). This implies that the improved cultivar was sensitive to biotic and water tresses. This trend of result was also observed in the irrigated cassava (Fig. 2). However, in the afternoon under well watered condition, *G. mosseae* inoculated improved cv. had the least leaf Ψ while those of *G. clarum* inoculated and non-inoculated were similar. The non-inoculated leaf Ψ was lower than

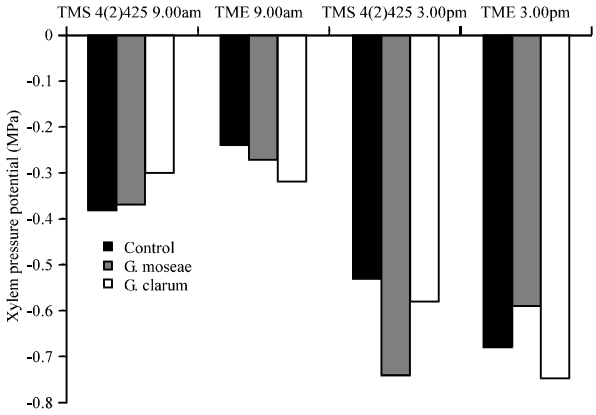

Fig. 4: The effect of mycorrhizal inoculation on xylem pressure potential of two cassava cultivars under irrigation at 6 MAP.

those of the inoculated cassava in the land race under irrigation. All these adjustment are to maximize water use efficiency of the genotypes (Fig. 2).

There were minimal variations observed in the leaf Ψ of the genotypes that were previously water stressed at the sixth months after planting (Fig. 3 and 4). *G. clarum* inoculated cassava exhibited the lowest leaf Ψ in the morning. In the continuously irrigated cassava (Fig. 4), the leaf Ψ were similar in all the treatments, though there was a genotypic variation. Non-inoculated, improved cv. maintained the lowest leaf Ψ in the morning, a contrary situation was recorded for the landrace where inoculated had the least. However, the inoculated improved cv. had the least Ψ in the afternoon (Fig. 4).

Effects of AM inoculation and water regimes on cassava photochemistry: Table 1 shows the maximum quantum yield of PSII photochemistry (Fv/Fm ratio) of matured cassava leaves at six (during stress period) and 12 (post stress period) months after planting (MAP) under the field condition. The Fv/Fm was adversely affected by water stress at the end of sixth months. The Fv/Fm values from the well-watered cassava plants did not present significant differences with water stressed counterparts at 12 MAP (after the stress was removed). The effect of the stress was alleviated after the removal of the stress.

Table 2 depicts the influence ofwater stress and AMF inoculation on photochemical photosynthetic efficiency (Fv/Fm) of matured cassava leaves at one MAP as recorded at two-time period during the day (at 10am and 3pm). It was observed that *G. clarum* inoculated TMS 4(2) 1425 had the lowest quantum yield at noon. There

Table 1: The influence of water stress and irrigation (control) on leaf photochemical efficiency (Fv/Fm) of TMS 4(2) 1425 (Improved cultivar) and TME1 (Landrace) at 6 and 12 months after planting under field conditions

Treatments (cultivar and watering regime)	TMS 4(2) 1425 (Improved cultivar)		TME1 (Landrace)	
	6 MAP (during water stress)	12 MAP (post water stress period)	6 MAP (during water stress)	12 MAP (post water stress period)
Irrigation (control)	0.75b	0.73a	0.72b	0.70a
Water stressed	0.71a	0.71a	0.66a	0.69a

Values followed by the same letter in the same column are not significantly different at P<0.05

Table 2: Effects of water stress and AMF inoculations on photochemical efficiency (Fv/Fm) of TMS 4(2) 1425 (improved) and TME1 landrace cultivars) at mid-morning (10 am) and afternoon (3 pm) at 1 MAP

Treatment (AM) Application	10 am		3 pm	
	TMS 4(2) 1425 (Improved cultivar)	TME1 (Landrace)	TMS 4(2) 1425 (Improved cultivar)	TME1 (Landrace)
+ *G. clarum*	0.73a	0.76a	0.79b	0.76a
+ *G. mosseae*	0.77b	0.80b	0.75a	0.79a
Control (no AM fungi)	0.77b	0.73a	0.75a	0.78a
ANOVA Mycorrhiza	*	*	ns	ns

Values followed by the same letter in the same column are not significantly different at P < 0.05. ns = Not significant at P< 0.05; * = Significant at P<0.05

Table 3: Effects of water stress and AMF inoculations on photochemical efficiency (Fv/Fm) of TMS 4(2) 1425 (improved) and TME1 (landrace) cultivars at mid-morning (10am) and afternoon (3pm) at 3 MAP. Water stress period was one month at this growth stage

Treatment	Water Stressed		Well Watered	
	TMS 4(2) 1425 (Improved cultivar)	TME1 (Landrace)	TMS 4(2) 1425 (Improved cultivar)	TME1 (Landrace)
10.00am				
+*G. clarum*	0.79b	0.81b	0.80b	0.82b
+*G. mosseae*	0.80b	0.81b	0.80b	0.81ab
Control (no AM fungi)	0.70a	0.69a	0.76a	0.80a
Treatment effects				
Wr	**	***	**	**
M	***	***	***	*
Wrxm	***	***	***	**
3.00 PM				
+ *G. clarum*	0.78ab	0.80a	0.80b	0.81a
+ *G. mosseae*	0.79b	0.80a	0.80b	0.81a
Control (no AM fungi)	0.75a	0.78a	0.77a	0.80a
Wr	*	*	**	**
M	**	**	**	ns
Wrxm	**	*	**	ns

Values followed by the same letter In the same column under each hour are not significantly different at P < 0.05. ns = Not significant at P<0.05; * = Significant at P<0.05; ** = Significant at P< 0.01; *** = significant at P<0.001

was however no significant difference between the Fv/Fm values of *G. mosseae* inoculated and non-inoculated plants. The highest value was recorded in *G. mosseae* inoculated plants. The situation with regard to the ratio was different in the afternoon from that of the morning; with *G. clarum* inoculated TMS 4(2) 1425 having the highest value while the values were similar in non-inoculated and *G. mosseae* treated TMS 4(2)1425. The values in the three treatments were also similar in TME1 plants in the afternoon. In the mid morning, *G. clarum* inoculated and non-inoculated TME 1 had similar values, while the *G. mosseae* treated TME 1 had the highest Fv/Fm value.

Table 3 shows the values of Fv/Fm in TMS 4(2) 1425 and TME 1 leaves when inoculated with AM fungi under water stressed and well watered conditions at 3 months

Table 4: Effects of water stress and AMF inoculations on photochemical efficiency (Fv/Fm) of TMS 4(2) 1425 (improved) and TME1 landrace cultivars) at mid-morning (10 am) and afternoon (3 pm) at 4 MAP. Water stress period was two months at this growth stage

	Water stressed		Well watered	
Treatment	TMS 4(2) 1425 (Improved cultivar)	TME1 (Landrace)	TMS 4(2) 1425 (Improved cultivar)	TME1 (Landrace)
10.00 am				
+ *G. clarum*	0.79b	0.81b	0.80b	0.83b
+ *G. mosseae*	0.80b	0.81b	0.80b	0.82b
Control (no AM fungi)	0.70a	0.70a	0.75a	0.80a
Treatment Effects				
Wr	**	***	**	**
M	***	***	***	*
Wrxm	**	*	*	*
3.00 PM				
+ *G. clarum*	0.78b	0.80b	0.80b	0.81a
+ *G. mosseae*	0.79b	0.80b	0.81b	0.81a
Control (no AM fungi)	0.74a	0.77a	0.76a	0.80a
Wr	**	**	**	**
M	***	***	***	ns
Wrxm	**	***	**	**

Values followed by the same letter in the same column under each time are not significantly different at $P < 0.05$. ns = Not significant at $P < 0.05$; * = Significant at $P<0.05$; ** = Significant at $P< 0.01$; *** = significant at $P<0.001$

Table 5: Effects of water stress and AMF inoculations on photochemical efficiency (Fv/Fm) of TMS 4(2) 1425 (improved) and TME1 landrace cultivars) at mid-morning (10am) and afternoon (3pm) at 6 MAP. At this stage the stressed was already terminated and the plants exposed to two months of post stress recovery

	Previously water stressed		Well watered	
Treatment	TMS 4(2) 1425 (Improved cultivar)	TME1 (Landrace)	TMS 4(2) 1425 (Improved cultivar)	TME1 (Landrace)
10.00 am				
+ *G. clarum*	0.62a	0.80b	0.79b	0.80b
+ *G. mosseae*	0.70b	0.73a	0.76a	0.76a
Control (no AM fungi)	0.72b	0.72a	0.75a	0.74a
3.00 pm				
+ *G. clarum*	0.56b	0.81c	0.76a	0.78a
+ *G. mosseae*	0.58ab	0.69a	0.82b	0.79a
Control (no AM fungi)	0.61a	0.76b	0.77a	0.78a

Values followed by the same letter in the same column under each time are not significantly different at $P<0.05$

after planting. The Fv/Fm values recorded under water stressed condition were lower than those under well watered. This was more apparent in non-inoculated plants particularly in the morning. The inoculated TMS 4(2) 1425 and TME1 exhibited the lowest Fv/Fm values in the morning in both water-stressed and well-watered conditions. In the afternoon, it was only in the TMS4 (2) 1425 that significant differences occurred in Fv/Fm values obtained for the three treatments. The AMF inoculated TMS 4 (2) 1425 had higher values than the un-inoculated counterpart both in the water stressed and well watered conditions. The values in all treatments were similar in TME1 in the afternoon under both water regimes. This indicates differences in their genotypic response to adjust to varying light intensities and temperatures. The Fv/Fm values were higher in TME1 than TMS 4(2) 1425.

The effects of AM fungi and drought on the photochemical photosynthetic efficiency (Fv/Fm) of TMS 4(2) 1425 and TME1 leaves at four MAP (i.e. 2 months of water stress) are shown in Table 4. In the morning, the Fv/Fm values of both *G. clarum* and *G. mosseae* inoculated plants were similar, though higher than those of non-inoculated plants under both well watered and water stressed conditions. Similar results were obtained in the afternoon, except that the values were higher in the afternoon.

Table 6: Root colonization and biomass production of TME 1 and TMS 4(2)1425 in response to watering regimes as affected by *G. mosseae* inoculation at 12 MAP under field condition

Watering regime (Wr)	Mycorrhizal inoculation (M)	Root colonization (%)a	No of leaf/plt	Dry tuber wt kg/plt	Dry shoot wt kg/plt)	No fibrous root	Leaf area (m2/plt)
TMS 4(2)1425							
Irrigated	with	93.7a	925.3a	1.7a	1.03a	27.0b	3.8a
	without	72.2c	762.8b	1.3b	0.7b	31.7b	3.2b
Stressed	with	82.9b	146.4c	0.96c	0.63b	25.1b	0.8c
	without	65.7b	149.6c	0.6d	0.55b	54.05a	1.04c
ANOVA							
Wr		**	**	***	***	ns	**
M		***	ns	*	*	ns	***
Interaction							
Wr*M		**	ns	*	ns	ns	ns
TME1							
Irrigated	with	90.2a	836.7a	1.6a	0.7b	39.0b	4.08a
	without	53.8b	185.0b	0.94b	0.98a	77.5a	2.6b
Stressed	with	82.9a	140.5c	0.97b	0.73ab	14.5c	0.64c
	without	36.9c	146.5c	0.45c	0.52c	37.5b	0.95c
ANOVA							
Wr		*	*	*	*	ns	**
M		**	*	*	ns	ns	*
Interaction							
Wr*M		*	ns	ns	ns	ns	ns

The mean in each column under each cultivar with the same letter are not significantly different at P = 0.05

[A] = Retransformed mean data. ns = Not significant at P< 0.05; * = Significant at P<0.05; ** = Significant at P< 0.01; *** = Significant at P<0.001

Table 7: Root colonization and biomass production of two cassava cultivars in response to mycorrhizal inoculation and watering regimes at 6 MAP in sterilized soil

Watering regime (Wr)	Mycorrhizal inoculation (M)	Root colonization (%)a	Dry shoot wt g/plt)	Fibrous root wt g/plt	Dry tuber wt g/plt)	Root/ shoot ratio	Leaf area (m2/plt)
TMS 4(2)1425							
Irrigated	*G. mosseae*	94 .3a	92.1a	357.9a	330.0a	3.3a	0.69c
	G. clarus	98.5a	89.2a	245.7b	221.8b	4.5a	0.85ab
	without	39.2b	63.3b	194.5c	170.9b	4.9a	1.12a
Stressed	*G. mosseae*	97.5a	86.3a	39.6d	172.2b	4.2a	0.62b
	G. clarus	98.7a	66.7b	34.2d	40.4c	5.6a	0.45c
	without	10.1c	70.0b	18.1d	55.7c	4.9a	1.2a
ANOVA							
Wr		ns	*	***	***	ns	ns
M		***	*	**	*	ns	*
Interaction							
Wr*M		ns	ns	***	***	ns	ns
TME1							
Irrigated	*G. mosseae*	98.3a	86.9ab	460.1a	445.9a	5.8a	1.06a
	G. clarus	98.1a	87.8a	343.7b	396.0a	3.8a	0.86b
	without	51.9b	64.7c	229.6c	218.7b	4.5a	0.42c
Stressed	*G. mosseae*	96.0a	78.3b	38.1d	77.5d	4.5a	0.43c
	G. clarus	97.2a	92.8a	37.0d	47.6d	4.6a	0.39c
	without	25.8c	48.3d	15.6d	135.9d	4.3a	0.31c
ANOVA							
Wr		*	**	***	***	ns	***
M		***	***	***	***	ns	***
Interaction							
Wr*M		*	**	***	**	ns	**

The mean in each column under each cultivar with the same letter are not significantly different at P = 0.05

[A] = Retransformed mean data. ns = Not significant at P< 0.05; * = Significant at P<0.05; ** = Significant at P< 0.01; *** = Significant at P<0.001

The quantum yield of photochemistry of PSII of the cassava leaves at 6 months after planting (two months after the stress was removed) is depicted in Table 5. The values were similar in *G. mosseae* inoculated and non-inoculated TMS 4(2) 1425 that were water stressed (morning readings). However, that of *G. clarum* inoculated plants had significantly lower values. Contrary to this, the values obtained for *G. clarum* inoculated TME1 was significantly higher than those of *G. mosseae* inoculated and un-inoculated, which values were similar. *G. clarum* treated plants also exhibited higher Fv/Fm values under irrigation in both cultivars, while the values of those under the other two treatments were similar. However, in the afternoon, *G. clarum* inoculated TMS 4(2) 1425 exhibited the lowest Fv/Fm values under both watering regimes, while the values of the other treatments were statistically ($P<0.05$) similar. TME1 inoculated with *G. clarum* had the highest value followed by un-inoculated, while *G. mosseae* inoculated had the lowest value under water stress. Nevertheless, the values were similar in all the treatments in TME 1, under irrigation in the afternoon. ANOVA revealed that effect of combination of AMF and water regime was significant on the photochemistry of the two cassava cultivars.

DISCUSSION

The data indicated that the two AMF species used colonized the roots of the two cultivars effectively without any genotypic preference. AMF inoculations enhanced the tuber yields of TME 1 under water stressed condition at three months after planting. This corroborates the finding of Howeler *et al*. [21], Fagbola *et al*. [22] and Oyetunji *et al.* [23]. However, the vegetative growths of these cassava cultivars were not enhanced by the two AMF species used under water deficit, but their effects were felt on the cassava vegetative growth when they were adequately irrigated. Nevertheless, the leaf areas of the cassavas were greatly enhanced by the inoculation of the AMF. The *G. clarum* species was able to improve cassava tuber yield (in both cultivars) more than *G. mosseae* species. This suggests a genotypic difference between the two AMF. *Glomus mosseae* appeared to support vegetative growth than *G. clarum*. It is known that different species of AM fungi differ in the type of benefits they confer on growth and development of plants [21, 17].

The effect of water stress on the cassava productivity was significant. This findings supported that of Howeler *et al.* [21] and Oyetunji *et al*. [23]. Vegetative growth and tuber yield of the two cultivars were reduced by water deficit, supporting the findings of Osiru *et al*. [24]. The percentage reduction ranged from 91 to 732. The effect of water deficit on the shoot dry weight was minimal compared with its effect on tuber and fibrous root weights. This corroborates the earlier findings of El-Sharkawy *et al*. [25] and Osiru *et al*. [24]. This suggests that cassava used larger proportions of it's assimilates for maintenance rather than storage during water deficit period.

Water stress was found to reduce the quantum yield of PSII photochemistry as reflected in the Fv/Fm ratios obtained in both field and semi-controlled trials. This finding corroborates that of Ekanayake *et al*. [11] when reporting effects of drought on cassava chlorophyll productions. This suggests that electron transport from PSII to PSI in cassava was adversely affected by water deficit. This has been established for other plant species that the amount of chlorophyll fluorescence indicates thyllakoid membrane integrity and the relative efficiency of electron transport from PSII to PSI [26-28]. The quantum yield of PSII photochemistry of cassava plants was consistently higher in the well water regime.

Photochemical quantum yield was enhanced by AM association, though this was not apparent at the early stage of cassava growth (i.e. one month after planting). As the cassava plants grew older their PSII efficiency were enhanced by the inoculated AM fungi up to sixth months of age according to this study. The effects of the AM fungi were more pronounced under water stress condition. The implication is that AM fungi can alleviate the adverse effect of water stress on quantum yield of PSII photochemistry in cassava. This indicates that AM inoculation can enhance drought tolerance in cassava by ameliorating to some degree the injury done to the photosystems reaction centers. A clonal difference was also observed between the cassava genotypes tested. The improved cultivar responded more to mycorrhizal inoculation than the landrace at early growth stage. However, the latter consistently had higher photochemical quantum yield than that of the improved cultivar. This was the exact findings of Ekanayake *et al*. [11] on chlorophyll synthesis in cassava.

The efficiency of PSII was observed to be always higher in the mid-afternoon than during the mid-morning. This could not be due to the quality of the available photons. The reason for this observation could be that the internal leaf temperature has an influence on the photochemistry efficiency of cassava because the effect of photon flux density that can account for these

differentials had been removed. The only condition left that can cause this variation between morning and afternoon in Fv/Fm values was the internal leaf temperature. This was in line with the findings of Schreiber and Armond [27].

It was not clearly established whether one strain of the AMF used was better than the other in terms of enhancement of cassava quantum yield. However, *G. mosseae* appeared to enhance PSII efficiency under water stressed condition than *G. clarum*. It was evident from this study that AMF conferred stress tolerance (particularly water deficit and light) on these cassava cultivars. AMF inoculation was advantageous to cassava particularly during water stress [21]. The adverse effect of water deficit on photochemistry of cassava was ameliorated by AMF association. This was reflected in the biomass production of these cassava cultivars. However, the improved cultivars were more responsive than the landrace in terms of tuber productivity. *G. clarum* appeared to be more efficient in enhancement of cassava tuber yields.

CONCLUSIONS

It could be concluded from this study that there were enhancements of quantum yield of PSII of cassava by *G. clarum* and *G. mosseae* inoculations. It was established from this study that one of the mechanisms employed by AM association in bringing about increase in photosynthetic rate during water stress is alleviating the quantum yield of PSII photochemistry, which consequently leads to quantum yield of non-cyclic electrons of photosystems [29, 30]. There may be a potential to increase the quantum yield of PSII of cassava through inoculation with these effective *Glomus spp*. It was also deduced that these AM fungi alleviated the adverse effect of water deficit on cassava photosynthesis. All these might be due to improved P nutrition or gene mediated, which requires further study.

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REFERENCES

1. El-Sharkawy, M.A., 1992. Drought tolerance. Cassava for Africa, Asia and Latin America. Bioscience, 43: 441-451.
2. El-Sharkawy, M.A. and J.H. Cock, 1987. Response of cassava for water stress. Plant and Soil, 100: 345-360.
4. Connor, D.J., J.H. Cock and G.E. Parra, 1981. The response of cassava to water shortage. 1. Growth and yield. Field Crop Res., 4: 181-200.
5. Howeler, R.H., 1996. Mineral nutrition of cassava. In Mineral nutrient disorder of root crops in the Pacific. Craswell, E.T., C.J. Asher and J.N. O'Sullivan (Eds.). Proceedings of workshop, Nuku'alofa, Kingdom of Tonga, 17-20 April 1995. ACIAR Proceedings, 65: 110-116.
6. Osonubi, O., O.N. Bakare and K. Mulongoy, 1992. Interactions between drought stress and vesicular-arbuscular mycorrhiza on the growth of Faidherbia albida (Syn. Acacia albida). Biol. Fertl. Soils, 14: 159-165.
7. Osonubi, O., I.E. Okon, O. Fagbola and I.J. Ekanayake, 1998. Mycorrhizal inoculation and mulching applications for continuous cassava production in alley cropping systems. In: Roots crops for poverty alleviation. Akoroda, M.O., I.J. Ekanayake (Eds.). ISTRC-AB, Nigeria, pp: 190-194.
8. Oyetunji, O.J., O. Osonubi and I.J. Ekanayake, 2003. Contribution of an alley cropping system and arbuscular mycorrhizal fungi to maize productivity under cassava intercrop in the derived savannah zone. J. Agric. Sci., 140: 1-6.
11. Ekanayake, I.J., O.J. Oyetunji, O. Osonubi and O. Lyasse, 2004. The effects of arbuscular mycorrhizal fungi and water stress on leaf chlorophyll production of cassava (Manihot esculenta Crantz). J. Food, Agric. Environ., 2: 190-196.
12. Oquist, G. and R. Wass, 1988. A portable microprocessor operated instrument for measuring chlorophyll fluorescence kinetics in stress physiology. Physiolgia Plantarum, 73: 211-217.
13. Govindjee, 1995. Sixty years since Kaustky: Chlorophyll fluorescence. Australian J. Plant Physiol., 22: 1-10.
14. Sthapit, B.R., 1993. Chlorophyll fluorescence analysis (CFA) for assessing cold tolerance at anthesis in Nepalese indigenous rice genotypes. IRRN 18: 1.
15. Connor, D.J. and J. Palta, 1981. Response of cassava to water shortage. III. Stomatal control of plant water status. Field Crops Res., 4: 297-311.

16. Baker, G.R., S. Fukai and G.L. Wilson, 1989. The response of cassava to water deficit at various stages of growth in the subtropics. Aust. J. Agric. Res., 40: 517-528.
17. Ekanayake, I.J., M.C.M. Porto and A.G.O. Dixon, 1994. Response of cassava to dry weather: Potential and genetic variability: In African Crop Science Conference. Adipala, E., M.A. Bekunda, J.S. Tenywa, M. Ogenga-Latigo, W.W. Mugah, (Eds.). African Crop Science Society, Kampala, Uganda, 1: 115-119.
18. Ekanayake, I.J., M.C.M. Porto and A.G.O. Dixon, 1996. Performance of various cassava clones in the dry savanna region of Nigeria. In Tuber Crops: Problem, Prospects and Future strategies. Kurup, G.T., P. Palaniswamy, G. Padmaja, S. Kabeerathama, S. Pillai and V. Mohan (Eds.). Primlani for Oxford and IBH Publishing Co., Pvt Ltd., 66 Janpath, New Delhi, 110001, India, pp: 207-215.
20. SAS Institute, 1996. Statistical analysis system for windows. Release 6.10. SAS Institute, Cary, North Carolina, USA.
21. Howeler, R.H., E. Sieverding and S. Saif, 1987. Practical aspects of mycorrhizal technology in some tropical crops and pastures. Plant and Soil, 100: 249-283.
26. Krause, G.H. and E. Wei's, 1984. Chlorophyll fluorescence as a tool in plant physiology. 11. Interaction of fluorescence signals. Photosynthetic Research, 5: 139-157.
27. Schreiber, U. and P.A. Armond, 1979. Heat induced changes of chlorophyll fluorescence in isolated chloroplasts and related damage at the pigment level. Biochin. Biophs. Acta, 502: 138-151.
28. Johnson, J.D., R. Tognetti and P. Paris, 2002. Water relations and gas exchange in poplar and willow under water stress and elevated atmospheric CO_2. Physiologia Plantarum, 115: 93-100.
29. Genty, B., J.M. Briatntais and N.R. Baker, 1989. The relationship between the quantum yield of photosynthesis electron transport and quenching of chlorophyll fluorescence. Biochimica et Biophysica Acta, 990: 87-92.
30. Flagella, Z., D. Pastore, R.G. Campanile and N. Di Fonzo, 1995. The quantum yield of photosynthetic electron transport evaluated by chlorophyll fluorescence as an indicator of drought tolerance in drum wheat. J. Agric. Sci., Cambridge, 125: 325-329.