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Plant water relations and photosynthetic properties of polyploid cassava grown in the Nigerian savanna

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Abstract. Cassava (*Manihot esculenta* Crantz) genotypes of varying ploidy levels (diploid, triploid and tetraploid) were investigated for their plant water relations and photosynthesis to better understand the modalities of selecting for adapted and high yielding genotypes. Data from trials conducted in the savanna agroecological zones of Nigeria were used for this purpose. Anatomy and stomatal morphology showed significant genotypic and ploidy differences. Water use efficiency, stomatal resistance and carbon exchange rates elucidated significant ploidy differences as well as genotypic differences in physiological behavior. Stomatal parameters gave an array of sensitivity and resistance to water deficit stress suggesting desirable selection indices. This paper presents satisfactory and contradictory factors associated with the use of photosynthesis and water relations in an attempt to select for improved productivity in an intensive agroecological zone-based breeding program for cassava.

Introduction

Cassava (*Manihot esculenta* Crantz) is a staple food crop in the tropical Africa. Commonly cultivated by small-scale farmers as a famine-evasive subsistence crop. The potential market for value added cassava remains largely untapped. Although post harvest factors are often cited as bottlenecks for improving value-adding process in many parts of sub-Saharan Africa low yield potential remains a serious constraint. Commonly grown cassava cultivars are diploids. Data shows low root yields (8 - 14 t ha^{-1}) in farmers' fields with local varieties on marginal soils in South America (El-Sharkawy, 1993) and an overall average yield of 11.9 t ha^{-1} of storage roots has been reported in sub-Saharan Africa (Nweke *et al.*, 2002). One of the primary objectives of the cassava breeding program at the International Institute of Tropical Agriculture (IITA) has been to improve root yields beyond the current yields on farmers' fields through germplasm improvement and enhancement. Genetic improvement thrust on cassava at IITA, specifically aims at increasing and sustaining cassava production and utilization in sub-Saharan Africa (SSA) through the development, evaluation, and promotion of improved and adapted germplasm for the lowland and mid-altitude agroecological zones. This research is done in collaboration with the National Agricultural Research and Extension systems (NARES) of SSA through informal and formal networking (IITA, 2002).

Through inter-specific crosses of cultivated cassava varieties with their related wild species, as well as by spontaneous polyplodization, polyploid cassava (tetraploids and triploids) clones were isolated at IITA (Hahn *et al.*, 1990). These polyploids were reported as superior to improved diploids with regard to plant vigor and yield potential in preliminary field testing (Hahn *et al.*, 1992; Nassar, 1992). The superiority of polyploid cassava, especially triploids, in terms of yields offers an opportunity to breed new high yielding cassava varieties (Hahn *et al.*, 1990). However, understanding the physiological basis for such superior performance could assist cassava breeders to precisely target adaptational genes since selection by combining desirable physiological characteristics could increase the efficiency of a cassava breeding program (Ekanayake *et al.,* 2000). An area of major focus in searching for new sources of yield increase has been through enhancing the photosynthetic efficiency of crop plants (Hesketh *et al.*, 1982). Therefore, basic understanding of photosynthesis of polyploids could increase our understanding of how they achieve productivity gains. Thus the objectives of this study were to evaluate polyploid cassava for their gas exchange characteristics and water relation parameters of physiological significance to improve our understanding of yield performance of polyploids.

Materials and Methods

Field experiment. The experimental sites and trials conducted are given in Table 1. Sites included IITA-Ibadan research farm in derived savanna zone (lat. 7°30'N, long. 3°54'E, altitude 150 mass, mean annual temperature

Table 1: Genotypes used and experimental details.

Experimental Type and Season	Agro-ecological zone	Location	Diploids	Triploids	Tetraploids	Total
Field trials						
1991/92 season	Derived savanna	IITA-Ibadan	TMS 91934, TMS 4(2) 1425	18	7	27
1991/92 season	Southern Guinea savanna	Mokwa	TMS 30572, TMS 4(2) 1425, TMS 63397	7	18	30
1991/92 season	Northern Guinea savanna	Zaria/Samaru	TMS 30572, TMS 4(2) 1425, TMS 63397	7	9	18
PYT (1994/95 season)	Derived savanna	IITA-Ibadan	4 diploid checks			97
1994/96 season &1995/96 season	Derived savanna	IITA-Ibadan	TMS 30572, Pubscent variant of TMS 4(2) 1425)	TMS 89/00003-10, TMS 89/00003-1	TMS 87/00018-42, TMS 89/00003-4	6
Container experiments 1994/95 season	Derived savanna	IITA-Ibadan	TMS 30572, Pubscent variant of TMS 4(2) 1425)	TMS 89/00003-10, TMS 87/00018-42,	TMS 89/00003-1 TMS 89/00003-4	6
1995/96 season	Derived savanna	IITA-Ibadan	TMS 30572, TMS 4(2) 1425)	TMS 89/00003-1, TMS 84/00316,	TMS 89/00003-10 TMS 81/01623	6
Lab experiments	Derived savanna	IITA-Ibadan	TMS 30572, TMS 4(2) 1425), TMS 91934	2	2	7

PYT – Preliminary yield trial.

28°C, average annual rainfall of 1252 mm, and Ferric luvisol soils); Mokwa in southern Guinea savanna (lat. 9°18'N, long. 5°04'E, altitude 210 mass, mean annual temperature 30°C, average annual rainfall of 1232 mm), and Samaru/Zaria (lat. 11°110'N, long. 7°35'E, altitude 686 mass, average annual rainfall of 1140 mm, and Ferric luvisol soils) in northern Guinea savanna zone. The experiments were laid out in RCB design with three/four replications. Stem cuttings were planted inclined and 1 m apart on ridges that were spaced at 1m between-rows. Cuttings that did not sprout were replaced 2 weeks after planting with extra plants that were planted at the same time in adjacent rows. All plots were kept weed-free and sprayed with herbicides when necessary.

Container experiments. In container experiments, stem cuttings were planted inclined in black polyethylene bag/containers filled with 100 kgs of sieved soil (Oxic paleustalf), spaced at 1m X 1m and laid out in completely randomized design with four replications. The plastic containers were kept in an open field throughout the growth period (6 months after planting) and regularly watered every two days except when it rained.

Photosynthetic and water relation measurements. Gas exchange characteristics of single attached leaves were made between 3 and 6 months for plants in both container and field experiments. The central lobe of established young fully expanded leaves; 4th - 7th from the top of the branch canopy were enclosed in a 1-L ventilated acrylic plastic photosynthetic chamber of a LI-COR Model 6200 portable photosynthetic system (Li-cor, Lincoln, NE, USA). Measurements were taken during midday between 1100 and 1500h when solar irradiation exceeded 1500 µmol m^{-2} S^{-1} (El-Sharkawy and Cock, 1990) Five measurements were made with a total of 5 leaves per clone from each plot. Immediately following gas exchange measurements, the enclosed leaf was excised and leaf area determined with Li-cor LI-3100 leaf area meter (LI-COR Ltd., Lincoln, NE, USA) then oven dried to a constant weight for specific leaf weight determination. Porometry was used on same plants as where photosynthesis was measured using Li-cor model 1600 according to protocol described earlier (Ekanayake *et al.*, 1996).

Data analyses. The data were analyzed by analysis of variance (ANOVA) was carried out for each variable and means were separated by least significant difference (LSD) at 0.05 significant level (SAS Institute, 1996). Regression of CER and storage root yield curves were constructed by ploidy level and evaluated for significance with student t-test.

Results and Discussion

Leaf photosynthetic characteristics. Leaf gas exchange rates in container-grown (Table 2) and field-grown plants (Table 3) from the derived savanna zone revealed that triploids had higher carbon exchange rates (CER) than tetraploid cassava clones. CER of triploids in general though were higher than that of the average for diploids did not significantly differ from one of the best adapted and high yielding diploid, TMS 30572 in its optimum environment. Although CER in triploids were higher than the means of the two diploids tested in containers and rainfed field experiments, the CE rates were in the range reported for diploids in other studies (El-Sharkawy *et al.* 1990). In addition, the observed CE rates under sub-optimal growing conditions were lower than its maximal net photosynthetic rates (40 - 50 µmol CO_2 m^{-2} s^{-1}) for cassava grown in near-optimum conditions with a saturating solar radiation greater than 1800 µmol m^{-2} S^{-1} in photosynthetic active radiation range. In the field, CER values about 27 - 36% lower than those recorded in the container experiment were partly because of stressful conditions (limiting water supply) that prevailed during some of the measurement period. There were great variations in CER among clones of different ploidy levels (Table 3) planted in a preliminary yield trial (PYT, 1994/1995 season, Table 1) in the derived savanna zone. The CER values ranged from 5 - 15 µmol m^{-2} s^{-1} for most diploids, 21 - 30 µmol m^{-2} s^{-1} for triploids, and 16 - 20 μmol^{-2} ^{-1}s for tetraploids. It was noteworthy that about 13.5% of the diploids tested were in the range of higher CER (21 - 30 µmol m^{-2} s^{-1}) suggesting that it is possible to improve on CER at this higher range by selecting suitable polyploids. More importantly this analysis suggested that not all polyploids were superior to diploids while the potential for use of triploids based on CER is interesting. It was apparent that there were some desirable genes in diploid cassava, which breeders could continue to extract to step-up productivity. Triploids had the highest fresh storage root yields (Table 4).

Positive correlations were observed between CER and fresh storage root yield (R = 0.787; n = 95) across all genotypes of different ploidy levels established in the PYT

Table 2: Comparative leaf photosynthetic gas exchange data and water use efficiency values for cassava genotypes of various ploidy levels when grown in containers (1994/95 and 1995/96 seasons).

Genotype §	Carbon exchange rate (CER)(mmol CO_2 $^{m-2}s^{-1}$)	Stomatal conductance (g)(mol $H_20m^{-2}s^{-1)}$)	Water use efficiency (WUE) (mmol $C0_2$ $mol^{-1-}H_20$)
1994/95 season			
Diploids			
TMS 30572	27.93	2.29	12.21
TMS 4(2)1425P	19.30	1.76	10.99
Triploids			
TMS 89/00003-1	27.75	2.30	12.03
TMS 89/00003-10	27.30	2.21	12.35
Tetraploids			
TMS 87/00018-42	22.68	2.25	10.08
TMS 89/00003-4	20.05	2.29	8.88
Clone mean	24.17	2.18	11.09
LSD (0.05)	4.36	N S	3.32
1995/96 season			
Diploids			
TMS 30572	28.64	3.08	10.38
TMS 4(2)1425P	22.20	1.83	12.10
Triploids			
TMS 89/00003-1	31.15	7.56	6.79
TMS 89/00003-10	30.00	17.50	2.75
Tetraploids			
TMS 84/00316	17.77	2.34	8.22
TMS 81/01623	20.63	5.13	6.53
Clone mean	25.10	6.20	7.80
LSD (0.05)	3.40	10.1	7.05

§ Diploids are currently commonly grown cultivars across West African countries.

trial (1994/1995 season data) of the breeding program. Linear regression equation for storage root yield (t ha-1; Y) was Y = 6.7336 + 1.2443X, where X was CER ($\mu mol^{-2}s^{-1}$). These results suggest that high yield cassava could be selected in early stages of the conventional breeding cycle through screening for high CER. This observation is in agreement with

Table 3: Comparative leaf photosynthetic gas exchange data for cassava genotypes of various ploidy levels when grown in the field (1994/95 season).

Genotype §	Carbon exchange rate (CER)(mmol CO_2 $^{m-2}s^{-1}$)	Stomatal conductance (g)(mol $H_20m^{-2}s^{-1)}$)	Water use efficiency (WUE) (mmol $C0_2$ mol^{-1} H_20)
Diploids			
TMS 30572	17.77	0.79	22.49
TMS 4(2)1425P	13.13	0.86	15.27
Triploids			
TMS 89/00003-1	16.94	0.73	23.21
TMS 89/00003-10	18.03	0.69	26.13
Tetraploids			
TMS 87/00018-42	15.39	0.69	22.30
TMS 89/00003-4	14.48	0.74	19.57
Genotype mean	15.96	0.75	21.50
LSD (0.05)	3.66	NS	2.32

§ Diploids are currently commonly grown cultivars across West African countries.

Table 4: Average fresh storage root yield (t ha^{-1}) of field-grown cassava genotypes (1994/95 season).

Genotype §	Storage root no $plant^{-1}$	Storage root yield (t ha^{-1})
Diploids		
TMS 30572	6.9	30.3
TMS 4(2)1425P	8.5	29.0
Triploids		
TMS 89/00003-10	7.6	35.4
TMS 89/00003-1	6.7	32.7
Tetraploids		
TMS 87/00018-42	6.9	22.9
TMS S89/00003-4	4.3	24.1
Genotype mean	6.82	29.07
LSD (0.05)	1.9	8.2

§ Diploids are currently commonly grown cultivars across West African countries.

reported positive correlations of photosynthesis and root yield in cassava diploids only (El Sharkawy *et al.*, 1990) and suggested that high yielding cassava could be selected through photosynthesis measurements if light interception is not limiting. The potential to use C-cycle enzymes and biotechnological tools to step-up the photosynthetic productivity in cassava using triploids is an area that is under-exploited.

Leaf morphology and anatomy. Anatomy and morphology of stomata based on specific leaf weight (SLW), leaf palisade thickness, and stomatal density on the adaxial and abaxial surfaces of leaves showed significant ploidy and genotypic differences (Table 5). The SLW of tetraploids was 26% larger than the diploids while triploids had intermediate SLA values. Concurrently tetraploids also had thicker leaf palisade layers than the diploids and triploids tested. Stomatal density was highest in the diploids as compared with the higher ploidy levels. Data clearly indicated intermediate leaf morpho-anatomy of the triploid cassava that also contributed to its superior water relation ability as presented in the next section, although significant regression relationships could not be constructed.

Leaf water relations. There were significant seasonal differences in WUE of container-grown plants irrespective of the ploidy level or genotype (Table 2). Significant genotypic differences were observed across all ploidy levels and among various triploids and tetraploids in the field (Table 3) as well as in container-grown plants (Table 3) for leaf water use efficiency trait. In the derived savanna site triploids were 30.7% better than diploids and 17.8% better than tetraploids for WUE. Nevertheless within ploidy groups there were individuals better in WUE than triploids. Leaf stomatal conductances across ploidy groups however were not significantly different (Table 2 and 3) except in one instance (Table 2). Leaf diffusive resistance and transpiration rates were notably higher irrespective of the

Table 5: Leaf morphological and anatomical traits of cassava genotypes of various ploidy levels.

Genotype	Specific leaf weight (mg cm^{-2})	Leaf palisade thickness (µm)	Stomatal density (no mm^{-2}) §	
			Adaxial leaf surface	Abaxial leaf surface
Diploids				
TMS 30572	5.33	66.00	61.0	615.0
TMS 4(2)1425P	4.33	55.89	61.0	586.0
Triploids				
TMS 89/00003-1	5.10	67.60	40.0	551.0
TMS 89/00003-10	5.48	66.70	39.0	542.0
Tetraploids				
TMS 84/00316	6.08	69.50	37.0	345.0
TMS 81/01623	6.15	79.80	33.6	393.0
Clone mean	5.40	67.58	45.30	505.2
LSD (0.05)	0.61	-	10.6	104.0

§ Measured on adaxial surface of leaves at the veins.

Table 6: Leaf water relation traits (mean ±s.e.) of cassava genotypes of various ploidy levels using porometry as affected by agroecological zone.

No. of genotypes	Ploidy level	Diffusive resistance(s cm^{-1})	Transpiration ($\mu g\ cm^{-2}\ s^{-1}$)	Leaf Temperature (^0C)
IITA-Ibadan (Derived savanna)				
2	2x	2.14 ± 0.11	7.26 ± 0.01	33.14 ± 0.33
18	3x	3.90 ± 0.90	5.27 ± 0.96	33.06 ± 0.22
7	4x	3.61 ± 0.86	4.94 ± 1.38	33.06 ± 0.17
Mean		3.21	5.82	33.13
Mokwa (Southern Guinea savanna)				
3	2x	7.38 ± 0.25	7.61 ± 0.32	-
7	3x	8.82± 0.34	7.11 ± 0.13	-
18	4x	8.16± 0.56	7.07 ± 0.21	-
Mean		8.12	7.26	-

Denotes data not available; measured at midday.

ploidy level in the southern Guinea savanna site as compared to the derived savanna site (Table 6). Similarly triploids had higher resistances than diploids or tetraploids suggesting their comparatively better adaptation to the Guinea savanna. At both locations transpiration rates were high indicating non-water stress conditions at the time of measurement. According to these experimental data, higher ploidy levels (especially tetraploids) did not necessarily indicate better water relation and adaptation characteristics to drought-prone savanna agroecologies. WUE of triploids were also better than diploids as reported in the same ecology in previous reports (Ekanayake *et al.*, 1996; Ekanayake and Githunguri, 2000).

Conclusions

In conclusion, inter-specific hybridization and spontaneous polyplodization have resulted in the production of polyploids with favorable physiological traits (photosynthesis and water use). These promise higher yield levels than the potential productivity of present generation of diploids cultivated across SSA.

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