



Field studies on chlorophyll *a* fluorescence for low temperature tolerance testing of cassava (*Manihot esculenta* Crantz)

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Abstract

Fifteen cassava (*Manihot esculenta* Crantz) genotypes were grown at two field sites of Nigeria from 1994 to 1996 (Ibadan: 28±5°C, altitude 210 masl and Jos: 18±5°C, altitude of 1260 masl) to evaluate the use of chlorophyll fluorescence in screening for cold tolerance in cassava. At 12 months after planting, the total dry tuberous root weight produced at Ibadan was significantly ($P<0.05$) greater than at the Jos Plateau location. Genotypic differences were observed among the genotypes both across locations and within locations for dry tuberous root weight. Genotypes TMS 91934, TMS 30572, TME1 and Danwaru performed better than the other genotypes in Jos. In Jos, variable fluorescence (Fv), maximal fluorescence (Fm) and chlorophyll fluorescence ratio (Fv/Fm) of cassava leaves were significantly ($P<0.05$) reduced when compared to cassava leaves grown in Ibadan. The predictive capability of fluorescence parameters was assessed by comparison with a yield-based cold susceptibility index (CSI). There was a significant correlation between Fv/Fm and CSI ($r = 0.64$, $n=15$). Genotypic differences were observed both across locations and within locations for chlorophyll fluorescence parameters. The cold tolerant genotypes TME1, TMS 30572 and Danwaru had higher Fv/Fm ratios under low temperatures whereas cold sensitive genotypes, such as Isunikankiyan and TMS 4(2) 1425 had lower Fv/Fm ratios. This work suggests that chlorophyll fluorescence might be used as a screening test for chilling tolerance in cassava.

Key words: Cassava, chlorophyll fluorescence, cold tolerance, screening, temperature.

Introduction

Cassava is a staple food for over 800 million people around the world^{1,2}. Cassava is a chilling intolerant plant^{3,4}. Exposing chilling intolerant plants to low temperature results in the inhibition of cellular activities⁵. The regulation of photosynthesis may be a necessary adaptation for enabling stress resistant plants to avoid photo-damage during exposure to low temperatures⁶. It is well known that various partial processes of photosynthesis are affected differently by changes in leaf or chloroplast temperature⁷. Chlorophyll *a* fluorescence provides an indicator of the primary photochemistry of photosynthesis⁶. It has been shown that photoinhibition occurs during low temperature stress⁸. Fluorescence may also provide information on the carbon reduction cycle. Via consumption ATP and NADPH, carbon metabolism affects the proton gradient and the Redox State of the primary electron acceptor of PSII, and thereby the fluorescence yields⁹. In *in vivo* chlorophyll *a* fluorescence may be used as a direct indicator of photosynthetic activity. Recent improvements in measurement techniques have made the fluorescence method an important tool in stress physiology and environmental research¹⁰. In durum wheat (*Triticum durum* Desf.) chlorophyll fluorescence has been used as a tool for testing drought tolerance¹¹. These authors reported an association between the drought susceptibility of different genotypes and the decrease of chlorophyll fluorescence ratio (Fv/Fm). During chilling injury, inhibition first develops on the water splitting side of PSII¹², which results in a decrease in chlorophyll fluorescence. This quenching of chlorophyll

fluorescence in chilling injured leaves has been demonstrated¹³. Plant chilling tolerance at 0°C appeared to be negatively correlated with both the loss of PSII activity in chloroplasts^{12,13} and the loss of induced chlorophyll fluorescence in intact leaf tissue¹³. Chlorophyll fluorescence as an indicator of chilling tolerance has been applied to a wide range of crops including cereals like rice¹⁵, legumes and tropical fruit species such as mango (*Mangifera indica*) and guava (*Psidium guajava*)¹⁴. However, for cassava little information is available on screening for low temperature tolerance using chlorophyll fluorescence. The aim of this study was therefore to determine if chlorophyll fluorescence could be used for screening tolerance of this crop to low temperature.

Materials and Methods

These experiments were conducted at a location in the Jos Plateau (mid-altitude) and at Ibadan (lowland-savanna zone). Two experiments were conducted during the 1994/95 and 1995/96 crop seasons at the International Institute of Tropical Agriculture (IITA), Ibadan and at the National Root Crop Research Institute field station (Vom and Heipang) in the Jos Plateau. In the 1994/1995 crop season, cassava stem cuttings were planted on 5 May 1994 and 13 May 1994 at Ibadan and Jos Plateau respectively while in 1995/1996 crop season, cassava stem cuttings were planted on 10 May 1995 and 20 May 1995 respectively, at Ibadan and Jos Plateau. These two locations represent contrasting agroecological zones (Table 1).

Table 1. Weather record of Ibadan and Jos Plateau, Nigeria. Data are means of 1994 to 1996.

Parameter	Ibadan (Non-stressed)	Jos Plateau (Cold-stressed)	
		Vom	Heipang
Longitude	2° 34' E	9° E	8° 9' E
Latitude	4° 46' N	9° 55' N	9° 38' N
Altitude, masl	210	1280	1290
Relative humidity, annual mean, %	65-90	55-85	60-85
Rainfall, annual total, mm	1545	1099	1153
Temperature, annual mean °C	28±6	18±5	18±6

The soil at Ibadan is classified as Oxic Paleustalf, Alagba soil series¹⁶ while in Jos Plateau the soil belongs to ferruginous tropical soils¹⁷.

Experimental design: Fifteen cassava genotypes were compared. They comprised eight improved IITA genotypes: TMS 4488, TMS 30337, TMS 30001, TMS 91934, TMS 4(2) 1425, TMS 30572, TMS 50395, TMS 30555; four landraces (local genotypes) commonly grown in south western Nigeria: TME1, TME11, Isunikankiyan and Oko-Iyawo; and three landraces (local genotypes) adapted to mid-altitudes: Danduala, Danrahoss and Danwaru. Stem cuttings of 0.20 m length were obtained from 12 month old mother plants at the middle part of the stem and were immersed in 0.05% of the fungicide benlate (a.i. = methyl 1-(butyl carbatomyl)-2-benzimidazole carbamate) solution. The experiments were set up in each location in randomized blocks with three replications. Each plot had six rows, 10 m long. Spacing was 1 m between rows and 0.8 m within a row. Each plot contained 72 plants. The experiments were kept free of weeds by regular hand weeding.

Data collection and analyses: Harvesting was done at 12 months after planting. Twenty-four plants were carefully removed from the center row in each plot. One border plant was left on either side of the harvested plants. The fresh weight of the tubers was noted and the samples were oven-dried for 48 hours at 80 °C prior for dry weight determination. The Statistical Analytical System¹⁸ PROC GLM program was used for data analyses. For both field seasons and at both sites combined analysis of variance was used to evaluate significant differences in dry tuberous root yield and other parameters. Fluorescence statistics were analyzed for combined locations in the second growing season. Mean comparisons were done as necessary. Correlation coefficients between pairs of data for parameters of interest were also compared.

Fluorescence measurements: Plant fluorescence measurements taken at maturity (12 MAP) are reported here. The measurement of fluorescence characteristics was carried out on two young fully expanded intact, attached leaves per genotype with a Plant Efficiency Analyzer (PEA MK2, Hansatech Instruments Ltd., Kings Lynn, England) in the morning (between 09.00 and 10.00 h). A plastic grid with hole 33 mm in diameter was clipped on the leaf. It was left for 30 min, in order for dark acclimation of that part of leaf. The measurement probe was later fitted into the hole and the readings taken.

In fluorescence measurement, the Plant Efficiency Analyzer

displays a maximal fluorescence signal (Fm) upon excitation of the chloroplasts by an array of high intensity light emitting diodes and a low level signal (Fo) giving a difference (Fv) known as the variable component of fluorescence. The chlorophyll fluorescence ratio (Fv/Fm) has been shown to be proportional to the quantum yield of photochemistry¹⁹.

Cold susceptibility index (CSI) of genotypes: The cold susceptibility index (CSI) of the 15 genotypes was calculated by analogy with the drought susceptibility index²⁰ as $CSI = (1 - Y_s / Y_{ns}) / D$

where: D = cold intensity = $1 - Y_s / \bar{Y}_{ns}$; Y_s = tuberous root yield in stressed environments; Y_{ns} = tuberous root yield in non-stressed environments; Y_s = mean tuberous root yield in stressed environments; Y_{ns} = mean tuberous root yield in non-stressed environments. Data from cassava tuberous root yield at Jos Plateau were mainly limited by low temperature; hence they were classified as low temperature stressed. The favorable environments at IITA, Ibadan were classified as non-stressed.

Results and Discussion

Significant differences ($P < 0.05$) were observed between the two locations in dry tuberous root weight (DTUB) at 12 months after planting (Table 2). Ibadan had the highest overall values for DTUB while the lowest were recorded in Jos Plateau (Table 2). Genotypic differences were observed both across locations and within locations in cassava tuberous root yield (Tables 2 and 3). TMS 30572, TME1 and TMS 91934 had better dry tuberous root weight (DTUB) than other genotypes across locations. In Ibadan, TMS 30572, TMS 50395 and TMS 30555 had the highest values for DTUB whereas on the Jos Plateau TME1, TMS 30572 and TMS 91934 and Danwaru performed better than the other genotypes. This result indicates that there is some cassava genotypes with better adaptation to mid-altitudes. This finding is consistent with earlier findings on cassava genotypes^{2,3}.

On the Jos Plateau, variable fluorescence (Fv), maximal fluorescence (Fm) and chlorophyll fluorescence ratio (Fv/Fm) of cassava leaves were significantly ($P < 0.05$) lower than those grown at Ibadan (Table 3). This implies that the lower temperature induced photoinhibition and reduced the quantum yield of photosynthesis. However there were no significant differences ($P < 0.05$) in the time at which maximal fluorescence occurred (Tm) or in the dark fluorescence (Fo) between the two locations. The lack of variation in Fo between locations, indicates that the energy distribution within the light harvesting complex and PSII chloroplast is fully intact and the light

Table 2. Tuberous root yield (Mg ha⁻¹), percentage yield reduction and cold susceptibility index (CSI) of 15 cassava genotypes in two cropping seasons, 1994 to 1996.

Genotypes	Total dry tuberous root yield (Mg ha ⁻¹)		Yield reduction (%)	Cold susceptibility index (CSI)
	Ibadan (Non-stressed)	Jos plateau (Cold-stressed)		
TMS 4(2)1425 (A)	9.2	1.1	11.9	1.29
TMS 30555 (A)	7.4	1.5	20.2	1.16
TMS 30337 (A)	5.2	1.2	23	1.13
TMS 91934 (A)	9.3	2.6	27.9	1.06
TMS 30001 (A)	7.1	2.0	28.2	1.06
TMS 50395 (A)	8.9	1.2	13.5	1.01
TMS 4488 (A)	6.2	1.9	30.0	1.01
TMS 30572 (A)	10.4	3.7	35.6	0.95
Mean (Improved genotypes)	7.9	1.9	76	1.08
Danduala (B)	2.2	1.0	45.5	0.80
Danrahoss (B)	1.8	1.0	55	0.65
Danwaru (B)	1.9	2.3	121.1	0.31
Mean (Landraces from Jos Plateau)	1.9	1.1	26	0.59
Isunikankiyan (C)	3.3	0.4	12.1	1.29
Oko-Iyawo (C)	7.4	2.2	29.7	1.03
TME11 (C)	5.9	2.8	47.5	0.77
TME1 (C)	7.1	5.1	71.8	0.42
Mean (Selected landraces from lowland)	5.9	2.6	56	0.88
Overall Mean	6.22	0.73	-	-
S.E. (15 D.F.)	2.00	0.31	-	-
C.V. (%)	45	60	-	-

A = Improved genotypes bred at IITA Landraces, B = Landraces from Jos Plateau, and C = Improved landraces from South western Nigeria selected at IITA.

absorption capacity of all chlorophyll including the reaction centers, remain constant during low temperature stress as applied here¹¹. Similar results have been observed for durum wheat cultivars (*Triticum durum*) under water stress¹¹. The technique of measuring plant chilling tolerance from the rate of decrease of chlorophyll fluorescence ratio (Fv/Fm) in chilled leaves should have applications in both physiological studies and in breeding program for the selection of chilling-tolerant plants^{12,13,15}.

Genotypic differences were observed in both locations in Fo, Fm, Fv and Fv/Fm in the second year (Table 3). TMS 4488, TME1, TMS 91934, TMS 30572 and Danwaru were the genotypes with higher photosynthetic fluorescence values than the other genotypes. In the high temperature agroecological zone of Ibadan, TMS 50395, TMS 4488, TMS 30555, TME1 and TMS 30572 had higher Fo, Fm, Fv and Fv/Fm respectively (Table 3). At the low temperature of the Jos Plateau, Danwaru, TME1, TMS 91934 and TMS 30572 had the highest values for Fo, Fm and Fv (Table 3). Also, genotypic differences were observed for Fv/Fm at Jos Plateau, TMS 91934, TMS 30572, TME1 and Danwaru had higher Fv/Fm under low temperature when compared to other genotypes tested (Table 3).

The differences in fluorescence attributes (especially in Fv/Fm) among the genotypes suggest that this technique may be a suitable test for low temperature tolerance in cassava. Thus, the ranking for low temperature tolerance would be based on Fv/Fm recorded after cold stress in cassava genotypes. Similar ranking for low temperature tolerance, using Fv/Fm values has been reported with rice genotypes¹⁵.

The genotypes with the lowest CSI values were TMS 30572, TME1, TME11, Danduala, Danrahoss and Danwaru while the most susceptible ones were TMS 4(2) 1425, TMS 30555 and Isunikankiyan (Table 2). In order to assess the predictive value for cold tolerance of the fluorescence parameters examined, we correlated Fv/Fm measure with the CSI of the genotypes. A significant correlation ($r = 0.65$, $n = 15$) between Fv/Fm and CSI was found.

In conclusion, in the high temperature environment of Ibadan, the dry tuberous root yield was greater than in the low temperature environment of the Jos Plateau. Low temperature induced photoinhibition and a concurrent reduction in leaf photosynthesis. Genotypic differences were observed for Fv/Fm and Fv/Fm was positively correlated with the CSI of the genotypes, evaluated on the basis of tuberous root yield. Thus, chlorophyll fluorescence could be a useful tool in screening for cold tolerance in cassava. Further studies are planned to select appropriate growth stage at which fluorescence parameters are the most sensitive to low temperature. This would help in identifying cold tolerant and high photosynthetic genotypes at an earlier stage than at harvest at 12 months after planting.

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Table 3. Mean values of initial fluorescence (Fo), variable fluorescence (Fv), maximal fluorescence (Fm), and chlorophyll fluorescence (Fv/Fm) at Ibadan and Jos Plateau.

Clones	Fo	Fm	Fv	Fv/Fm
<i>Ibadan (Non-stressed)</i>				
TMS 50395 (A)	519	2598	2251	0.84
TMS 30555 (A)	474	2688	2260	0.82
TMS 4488 (A)	403	2729	2460	0.82
TMS 30572 (A)	446	2609	2160	0.82
TMS 30001 (A)	474	2528	1999	0.81
TMS 4(2)1425 (A)	686	2362	1347	0.80
TMS 30337 (A)	566	2577	2011	0.78
TMS 91934 (A)	440	2533	2010	0.78
Mean	502	2578	2063	0.71
Danduala (B)	449	2511	2054	0.82
Danwaru (B)	437	2161	2121	0.82
Danrahoss (B)	474	2520	2104	0.81
Mean	453	2397	2093	0.82
Isunikankiyan (C)	415	2727	2089	0.83
Oko-Iyawo (C)	447	2660	2096	0.82
TME1 (C)	449	2644	2060	0.82
TME11 (C)	448	2433	1921	0.81
Mean	439	2616	2042	0.82
Overall Mean Ibadan	484	2546	2049	0.82
S.E. (15 D.F.)	83	195	240	0.02
C.V. (%)	18	8	11	3
<i>Jos plateau (Cold-stressed)</i>				
TMS 30001 (A)	578	2183	1605	0.73
TMS 30555 (A)	568	2114	1599	0.73
TMS 50395 (A)	291	2064	1467	0.72
TMS 30572 (A)	616	2121	1505	0.70
TMS 4(2)1425 (A)	636	2075	1439	0.69
TMS 4488 (A)	647	2145	1498	0.69
TMS 91934 (A)	624	1649	908	0.59
TMS 30337 (A)	635	1954	1227	0.55
Mean	574	2038	1406	0.68
Danwaru (B)	662	1919	1482	0.77
Danrahoss (B)	567	2032	1580	0.73
Danduala (B)	565	2256	1474	0.65
Mean	598	2069	1512	0.72
Isunikankiyan (C)	492	1956	1686	0.78
Oko-Iyawo (C)	599	2214	1803	0.76
TME1 (C)	506	2110	1604	0.76
TME11 (C)	609	2415	1806	0.74
Mean	552	2174	1725	0.76
Overall Mean (Jos Plateau)	564	2037	1473	0.70
S.E. (15 D.F.)	118	251	299	0.07
C.V. (%)	20	12	18	8

A = Improved genotypes bred at IITA Landraces, B = Landraces from Jos Plateau, and C = Improved landraces from South western Nigeria selected at IITA.

constructive comments on the manuscript.

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