SCREENING FOR LOW TEMPERATURE STRESS-TOLERANT CASSAVA VARIETIES USING MEMBRANE THERMOMSTABILITY

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Abstract

Assessment of tolerance to low temperature stress based upon measurements of membrane thermostability was made using young leaves of eight cassava varieties subjected to low temperatures (15/10°C) and ambient temperature (32/22°C) for 28 days (60 to 88 Day After Planting (DAP)) using growth controlled chambers. The relationship between the degree of membrane injury (%) and the different low temperature regimes at which that injury was induced was observed to be linear. The membrane injury (%) increases from a mean value of 18.6% at ambient temperature to 58.2% in temperature regime of 15/I0°C at final sampling date. Also, the data showed that membrane injury (%) increases with age. Significant differences ($P<0.05$) were observed among cassava varieties. TMS 30572 has the lowest mean value for membrane injury at the final sampling. It suggested that TMS 30572 was cold tolerant than other. Results in this study suggest that the membrane thermostability test based on electrolyte conductivity measurement is a useful screening technique for identifying low temperature stress tolerant cassava varieties.

Keyword: cassava, thermostability, temperature, membrane injury.

Introduction

Cassava (Manihot esculenta Crantz) is a common tropical root and tuber crop. It is a major staple food for about 400 million people (Nweke, 1996). It is adapted to a wide range of environments and climatic conditions, but mostly cultivated in areas with mean annual temperature above 20°C and annual rainfall above 700mm (Howeler and Cadavid, 1983; Cock, 1985). Cassava tends to perform poorly in altitudes above 1000m and mean annual temperatures below 20°C (Cock, 1985; Osiru ***et al.,*** 1995). There are several mid and high altitude agroecological zones of Africa, where the mean annual temperatures are below 20°C. Such areas are Jos and Adamawa plateaus in Nigeria, Adamawa plateau in Cameroun, central Malawi, south Burundi and eastern Madagascar among others (Carter ***et al.,*** 1992). These areas account for 14.6% of total area of cassava cultivation in Africa, and 19.9% of the total area currently under cassava cultivation (Carter ***et al.,*** 1992).

The effects of temperature on cassava are often underestimated despite reports of a

significant relationship between cassava and growing season
temperatures (Cock, 1985). Tolerance due to deviations from normal temperatures most likely involves several complex resistance and avoidance mechanisms (Sullivan, 1972). One component of temperature tolerance that has received some emphasis in recent years is cellular membrane thermostability (Martineau ***et al.,*** 1979).

The morphological, physiological and developmental changes which occur during cassava leaf growth is influenced by low temperatures. The alterations in the patterns of cell division and extension which occur during growth might be expected to produce modifications in plant form and such changes have been observed during exposure of plants to low temperature in controlled environment facilities (Krol ***et al.,*** 1984). The plasma membrane and cytoplasmic membranes of plants are composed of lipids and proteins that are often glycosylated and the composition particularly of lipid component may change in response to low temperature during growth and development of the cell (Guinn, 1971).

Recently, based upon in increase in leaf cell membrane injury due to lipid peroxidation and loss of ability to retain cell constituents. Lyons ***et al.*** (1979) and Martineau ***et al.*** (1979) devised a test of cellular membrane thermostability where electrolyte conductance following leaf treatment was used as a measure to screen crop plants for temperature / water stress tolerance. Also, Sullivan (1972) has described a technique, employing leaf discs, for evaluating thermostability. This test is based on the observation that when leaf tissue is injured by exposure to temperatures, cellular membrane permeability is increased, and electrolytes diffuse out of the cells. The amount of electrolyte conductance was used to evaluate the membrane thermostability of different cultivars. The amount of electrolyte conductance as an indicator of chilling tolerance has been applied to a wide range of crops (Martineau ***et al.*** 1979; Sullivan, 1972; Lyons ***et al.*** 1979; Bauer ***et al.*** 1990).

However, for cassava little information is available on screening for low temperature tolerance using membrane thermostability. The aim of this study was to determine if cellular membrane thermostability could be used for screening cassava tolerance to low temperature.

Materials and Methods

Plant Material: Five improved International Institute of Tropical Agriculture (IITA) cultivars: TMS 30001, TMS 30555, TMS 91934, TMS 30572 and TMS 4(2)1425; and three local cutivars: Danwaiu and Danduala adapted to midaltitude of Jos and TMEI: from the southern part of Nigeria, were used for the experiment.

Growth facilities: Cassava plants were raised in growth chambers (Model Convirons, Controlled Environments Ltd; Winning, Manitoba, Canada) at the IITA, Ibadan. In each chamber, a 12-h daylength and day/night temperature regimes of 35/25°C, 25/15°C, 15/10°C, and 10/6°C were automatically imposed. The light was supplied over each by 12 fluorescent and 16 incandescent lamps. The average photon flux density (400-700nm) was from 20 to 30 neinstein cm^{-2} s^{-1}. The relative humidity in each growth chamber were maintained between 65% and 70%. The plants were grown in plastic pots (30cm diameter, 25cm deep and containing topsoil from IITA farm site). The soil belongs to Alagba Series, Oxic Paleuestalf (Greenland, 1981).

Plant culture: Cassava stem cuttings of 0.20 m length with 10 nodes were obtained from 12 months old mother plants at the middle part of stem and were planted in the plastic pots. One stem cutting was planted per pot. Cuttings were planted inclined at 40° and watered three times a week. This experiment was a randomized complete block design with three replications. The temperature regimes (15/10°C, 20/15°C, 25/20°C day/night temperature respectively) and ambient temperature (32/22°C) formed the block and cassava cultivars were the treatments. These cuttings were raised under natural conditions for two months, until they were well established, during which the average monthly maximum temperature, minimum temperature and relative humidity ranged between 29-33°C, 19-22°C and 63-84%, respectively.

After two months after planting, they were transferred to different temperature regimes, set at 25/20°C, 20/15°C, and 15/10°C day/night temperatures respectively, while other plants remained at ambient conditions. The two most recently expanded leaves on the mainstem from each plant in both ambient and stressed plants were harvested and utilized for various estimations at 7 days intervals (60 to 88 DAP).

Electrolyte Conductance Measurement and Cellular Membrane Thermostability

Procedures used for measuring membrane thermostability (MT) at low temperatures were similar to those used by Sullivan (1972) for Sorghum with minor modifications. Leaf discs (0.5cm diameter) weighing 0.2g were washed with three portions of distilled water. With sufficient moisture being allowed to adhere to the. walls of tubes (15 x 2.5cm), the treatment tubes covered with plastic wraps were incubated at 60°C in a thermostatically controlled water bath for 20mins., and thereafter both treatment and control sets were placed at 10°C for 12 h to allow the diffusion of electrolytes into water.

After recording the initial electrolyte conductance with a conductivity meter (Model 152, Fisher Scientific, U.S.A) at 30°C, the tubes were heated at 110°C for 20 minutes, to completely kill leaf tissue cells and release all the electrolytes, and final conductance was recorded after cooling (30°C). The degree of membrane injury (%) was calculated utilising the formula of Martineau *et al.* (1979).

$$R(\%)= 1-[C,-T,/T_2)/(1-C,/C_2] \text{ x } 100$$

Where T and C refer to electrolyte conductance values for treatment and control samples, while subscripts 1 and 2 denote the initial and final electrolyte conductances respectively. R (%) refers to membrane injury which is the degree of damage as a result of low temperature injury. The ratio of initial and final conductance (i.e = T1/T2) is a relative measure of the amount of electrolyte leakage induced by the low temperature treatment and is assumed to be proportional to the amount of "injury" induced in cellular membranes.

Results

The membrane injury (%) based on electrolyte conductance due to leakage increased as the temperature decreased (Figure 1). the result indicate that in all tested varieties, the highest mean membrane injury was observed in temperature regime of 15/10°C while the lowest mean value was recorded in ambient temperature (32/22°C).

The membrane injury was fairly constant throughout the period of sampling for varieties grown under ambient temperature but those grown under different low temperature regimes, it increases as the age of the plant increases. The degree of injury occurring in cassava leaf discs tissue varied directly with the low temperature regimes inducing that membrane injury (Figure 1). Also, the data showed that membrane injury (%) increases as DAP increased (Figure 1).

Among the varieties tested at temperature regime, 15/10°C, TMS 4(2)1425 had the highest mean values of 49, 57.9, 64.9 and 68% at 67, 74, 81 and 88 days after planting respectively while TMS 30572 had the lowest mean values of 39.9, 52.6, 57.9 and 63% at 67, 74, 81 and 88 days after planting respectively (Table 1). The overall results showed' that variety TMS 30572 seem to be more resistant to cold stress than other varieties, as they recorded the lowest mean values in most of the final sampling (Table 1).

Discussion

The observed leaf membrane injury (%) increased in all varieties tested as the temperature regimes decreased. The lowest membrane injury was recorded at ambient which was fairly constant throughout the period while the highest membrane injury was observed in the most extreme temperature regime of 15/10°C. This showed that the degree of injury occurring in cassava leaf discs tissue increased at lower temperature regimes. Variation in membrane injury across the range of temperature regimes employed was linear, increasing from the ambient to lower temperatures. A similar linear response, was demonstrated in sorghum by Sullivan (1972).

The results showed a decrease in electrolyte leakage from leaf tissue cells under ambient conditions which can be related to increased membrane stability of cassava when grown under high temperatures. The greater cellular damage at lower temperature regime (15/10°C) agreed with the observations that low temperatures result in increased in permeability of cell membranes and loss of electrolyte from a tissue cause progressive deterioration of cellular membrane (Levitt, 1980; Lyons, 1973; Lyons ***et al.*** 1979). The results show that at temperature regime 15/10°C, varietal differences were significant. TMS 30572 has the lowest mean value for membrane injury at the final sampling (88 DAP). A similar cultivar differences was demonstrated in coffea arabica by Bauer ***et al.*** (1990).

It has been observed that the capacity of plants to minimize membrane damage is an important feature of tolerance to low temperature stress. The loss of ability to retain solutes is relatively small and temporary in tolerant plants while it is much greater and irreversible in sensitive plants (Couderchet and Koukkari, 1987). However, Levitt (1980) reported that membrane damage through unchecked lipid peroxidation is known to be caused primarily through loss of protection due to enzymic activities such as those of catalase and superoxide dismutase which occur during stress.

Thus, membrane thermostability is associated with tolerance to low temperature stress and the variety, TMS 30572 showing low membrane injury appeared to possess relatively tolerance at cellular level. Results in this study suggest that the membrane thermostability test based on electrolyte conductivity measurement is a useful screening technique for identifying low temperature stress tolerant cassava varieties. This identified variety can be used for further breeding programme targeted for mid-altitude and high altitude agroecological zones of Africa.

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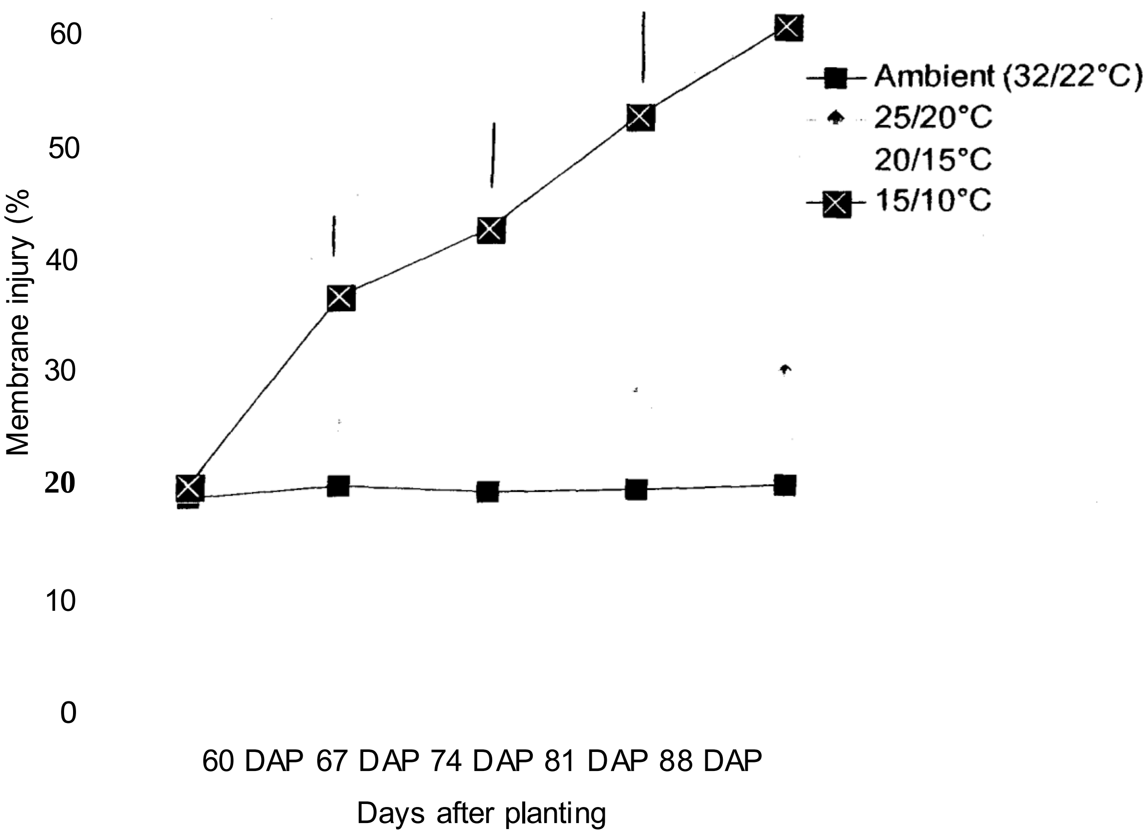

Fig. I. Effect of different temperature regimes on membrane injury of cassava varieties. Bar represents LSD at 5% level of probability

Table 1. Average Values of Membrane Injury (%) at Different Temperature Regimes

Temperature °C	Varieties	Days after planting 60	67	74	81	88
15/I0°C	TMS 30572	22.5	39.8	52.6	57.9	63
	TMS 91934	19.8	46.3	56	62.1	67.2
	TMS 4(2)1425	22.7	49	57.9	64.9	68
	TMS 30001	20.8	46.2	55	59	66.8
	TMS 30555	22.4	48	54.1	63	65
	Danduala	21.8	41.3	56	62.5	64.6
	Danwaru	22.3	48.9	56.4	63.9	65.9
	TME1	20.5	45	53.1	62.5	67.1
	Varietymean	21.8	45.56	55.14	62.4	66.2
	S.E.	0.4	1.2	0.63	0.64	0.45
	C.V.(%)	5.25	7.5	3.2	2.9	1.9
20/15°C	TMS 30572	19.2	27.8	39.9	43	46
	TMS 91934	22.4	33.1	40	45.2	48.7
	TMS 4(2)1425	18.5	30.8	39.4	45	49.1
	TMS 30001	22.6	37	40	49	50.4
	TMS 30555"	20.5	35.5	41.6	46.7	50.7
	Danduala	22.5	32.9	42	46.6	49.6
	Danwaru	20.9	33.8	40.7	47.4	50.5
	TME1	21.6	36.1	43	47	52.6
	Varietymean	21.15	33.36	40.7	46.39	50
	S.E.	0.48	1.1	0.49	0.68	0.65
	C.V.(%)	6.4	9	3.4	4.1	3.8
25/20°C	TMS 30572	19.3	23.9	25.1	24.5	26.9
	TMS 91934	21.9	22.1	27	26.2	30
	TMS 4(2)1425	19.5	21	25.9	24.5	27.2
	TMS 30001	22.57	24.9	26.2	28.8	30.6
	TMS 30555	20.8	22.4	25.5	26.9	25.4
	Danduala	22.5	25	25.9	26	29
	Danwaru	21.9	23.7	29.5	28.6	29.7
	TME1	21.6	20.4	25.7	27.3	31.9
	Varietymean	21.25	22.9	2 6.48	26.7	28.8
	S.E.	0.48	0.82	0.56	0.59	0.77
	C.V.(%)	5.9	7.5	5.9	6.1	7.5
32/22°C	TMS 30572	16.8	17.2	18.2	18.1	17.4
	TMS 91934	17.9	17.3	18.3	17.9	18.1
	TMS	17.6	18.1	18.4	18.1	18.3

	4(2)1425					
	TMS 30001	18.2	17.9	18.2	18.6	17.8
	TMS 30555	17.9	18.2	17.8	18.7	19.1
	Danduala	16.9	18.5	19.2	18.3	17.5
	Danwaru	17.3	18.1	18.4	18.4	17.9
	TME1	19.1	18.1	17.8	19.1	18.4
	Varietymean	17.3	17.9	18.1	18.3	18.1
	S.E.	0.38	0.25	0.42	0.23	0.41
	C.V.(%)	5.1	4.2	3.1	2.4	2.1