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Full Length Research Paper

Environmental and genotypic effects on the growth rate of *IN VITRO* cassava plantlet (*MANIHOT ESCEULENTA*)

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Two varieties of cassava were evaluated *IN VITRO* in the screen house and culture room using five different media treatment. Each treatment was replicated 12 times and observed for 5 weeks before sub-culturing. There were significant differences in the growth rate of plantlets in different media, which suggests an interaction between treatments and environments. However, F-probability on survival shows that TMS 188/00106 was significantly different from TMS 083/00125 in the culture room than in the screen house. The study confirms that cassava tissue culture can be raised in the screen house but suggests that plantlet should be allowed to survive in the culture room before transferring to the screen house for further growth.

Key words: Explants, plantlets, sub-culturing, nodal increase.

INTRODUCTION

Cassava is a major food crop in sub-Saharan Africa and other parts of the world. Vast potential growing areas and increased demand for food and feed open challenges for increased production. A limitation, however, is the low multiplication ratio of 1:10 using traditional production methods, which results in little progress in replacing susceptible or nonadapted varieties, or in expanding cassava into new areas. Rapid multiplication techniques are known and have been the subject of training courses and extension campaigns (Otoo, 1996).

Tissue culture offers a unique opportunity to mass propagate plant materials especially, disease free plantlets. Vegetative propagation through tissue culture has played vital roles in the mass production of vegetative propagating materials (Ajithukumar and Seemi, 1998). It is faster and requires less space than that required for conventional methods of preparing cuttings.

The use of various tissue culture methods for rapid multiplication of improved cassava clones is a good option because of their very high multiplication ratio. It is however often hampered by inadequate number of trained personnel and absence or inadequacy of a unit to immediate executes the multiplication. The method is quite expensive to establish and maintain in a short time, considering the current socio-economic status accorded root vis-à-vis grain crops (Earrnet, 2003). Tissue culture also provides large quantities of disease-free seedlings for farmers. Tissue culture has made possible the mass production of disease-free and uniform plants. The techniques thus bring farmers the great benefit of high-quality planting materials of new high- value crops (Dalmacio, 1992).

Electricity is presently epileptic in Nigeria and the cost of running a power plant is very exorbitant. There is need for alternative way of raising tissue culture plantlets. Many of the rural community where the greater percentage of farmers, especially cassava growers, resides have inadequate source of electricity. This research will contribute immensely to the production of planting materials in the rural areas; it will increase food production, generate employment and alleviate poverty. Since less power is required, the available power can be freed for use in other industrial activities.

In addition, newly developed cassava varieties can easily be mass propagated and distributed to farmers in the rural areas with less transportation cost. Tissue culture can be practiced in the rural area where electricity is inadequate and this will reduce transportation cost and

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Table 1. Summary of ANOVA for the five treatments used in the *in vitro* cassava plantlet tissue culture.

Parameters	DF	Sum of square	Mean square	Variation Ratio	F-Probability
Survival	4	14.1374	3.5343	7.87	<001
Shoot	4	7.7280	1.9320	3.12	0.008
Root	4	13.349	3.337	3.12	0.160
Node	4	2.6084	0.6521	0888	0.474
Leaves	4	75502	1.8875	2.77	0.029
Height	4	8.4901	2.1225	3.56	0.008

*Significant at 1% level of probability

encourage the high yielding and adapted varieties to be planted everywhere.

This study aims at the possibility of using screen house to maintain *in vitro* cultures and rapidly propagate important vegetative crops with less contamination at a reduced cost. Nigeria has just introduced cassava into the international market and the need to rapidly produce diseases free planting materials to meet the growing local and international demands was a propelling factor for this investigation. Secondly, the space in many laboratories cannot accommodate the commercialization of major crops as demanded by big time farmers, hence the need to look beyond the laboratory environment. Furthermore, the provision of electricity in the laboratory and the cost of maintaining a generating plant for regular power supply are very high in Nigeria and other developing countries. Even if the government can afford it, the private laboratories and seed companies may not be able to break even. *In vitro* culture in the screen house will not only reduce the cost of production, it will also enhance quick acclimatization.

An effective multiplication and distribution network system has been developed in Uganda. About 80,000 hectares of land are planted to at least four improved cassava varieties throughout the country. Many national programs have identified improved cassava lines over the last 5 years. However, the system of multiplication and distribution of planting material is often inefficient. The multiplication of cassava is a slow process. Production of planting materials is indispensable in the overall structure of research for conservation of variety purity and supply of planting materials of preferred high yielding cultivars to stem multipliers and producers (Acheapong, 1982; CIAT, 2001; Henshaw, 1982).

MATERIALS AND METHODS

Two genotypes of cassava TMS 188/00106 and TMS 083/00125 were obtained from the International Institute of Tropical Agriculture (IITA) while five different medium were prepared using Murashige and Skoog (1962) with minor adjustments as follows:

Treatment 1 (T_1) - Liquid only.
Treatment 2 (T_2) - Liquid with 50% normal agar (2 g/l).
Treatment 3 (T_3) - Liquid media with filter paper embedded.
Treatment 4 (T_4) - Media with normal agar (4 g/l).
Treatment 5 (T_5) - Liquid media with filter paper projecting out.

The pH was taken and dispensing was done at the rate of 3 ml before autoclaving. The subculturing was done the following day. One hundred and twenty test tubes were used for each variety with 12 test tubes per treatment. A complete set of 60 test tubes with 5 treatments of 12 replicates was placed in the laboratory while the second set was placed in the screen house at the same day for TMS 188/00106; the same procedure was adopted the following day for TMS 083/00125. Data was recorded weekly for 5 weeks before sub culturing. The second generation was observed for only two weeks to ensure the sustainability of the observation made during the first generation. The observation on explants survival was scored on a scale of 0 – 3 as follows:

0 - Dead
1 - Alive but not growing
2 - Growing slowly
3 - Growing very well

There were six parameters recorded during the investigation. These include survival rate, shoot development, root growth, nodal increase, leave development and increases in height. Survival rate was observed for two weeks only while the other five parameters were scored continuously for the rest three weeks consecutively. Only the screen house explants were subculture after 5 weeks to ensure the sustainability of the findings. The subculture materials from the screen house explants were also placed in both screen house and culture room (laboratory). The same set of observation was carried out on the responses of the explants to the culture medium and environment as in the first generation explants.

RESULTS AND DISCUSSIONS

The summary in Table 1 indicates that out of the six parameters studied, F-probability on survival is significantly different for all the media used. Observation shows no significant difference on the five treatments for shoot, root, node, leaves and height development. Although there were some effects on the survival of the explants, the laboratory plantlets grows better in liquid and liquid with filter paper embedded media than when placed in the screen house. This might be due to high temperature recorded at the time of placement (32^{o} – 36^{o}C compared to 22 – 25^{o}C in the laboratory), which indicates an interaction between treatment and environment (Table 2).

Obviously, the laboratory supports the survival of explant in the liquid medium and liquid medium with embedded filter paper. On the other hand, survival is lowest

Table 2. Summary of environmental effect on *in vitro* cassava tissue culture.

S/N	Survival		Shoot		Root		Node		Leaves		Height	
	SH	LAB	SH	LAB	SH	LAB	SH	LAB	SH	LAB	SH	LAB
I	1.77	2.41	1.92	1.93	0.73	1.55	2.00	1.93	1.92	1.93	1.92	1.96
II	1.67	1.44	1.56	1.42	1.67	1.33	1.86	1.63	1.67	1.46	1.61	1.33
III	1.02	1.81	1.21	2.04	0.46	1.42	1.46	2.17	1.29	2.00	1.21	2.04
IV	1.73	1.27	1.83	1.00	1.00	0.75	2.04	1.29	2.00	0.96	1.79	1.00
V	1.73	1.27	1.88	1.13	1.63	1.04	2.13	1.29	1.79	1.13	1.92	1.17
CV	42%		49%		89%		48%		51%		48%	
Lsd	0.38.4		0.4470		0.2987		0.4889		0.470		0.439	
Std	0.193		0.2266		0.5892		0.2479		0.2383		0.2228	

SH = Screen House.
LAB = Culture room in the Laboratory.

Table 3. Summary of genotypic effect on the *in vitro* growth rate of cassava tissue culture.

S/N	Survival		Shoot		Root		Node		Leaves		Height	
	G_1	G_2	G_1	G_2	G_1	G_2	G_1	G_2	G_1	G_2	G_1	G_2
I	2.37	1.51	2.25	1.34	1.29	0.77	2.29	1.39	2.29	1.30	2.21	1.46
II	1.67	1.37	1.46	1.53	1.79	1.05	1.67	1.84	1.54	1.60	1.46	1.47
III	1.13	1.71	1.21	2.04	0.71	1.17	1.25	2.38	1.13	1.17	1.29	1.96
IV	1.54	1.46	1.33	1.50	1.00	0.75	1.79	1.54	1.54	1.42	1.42	1.38
V	1.54	1.46	1.58	1.42	1.00	1.67	1.63	1.79	1.42	1.5	1.54	1.54
CV	43%		50%		92%		48%		52%		51%	
Lsd	0.3898		0.4494		0.5889		0.4791		0.4684		0.4600	
Sed	0.1976		0.2278		0.2985		0.2429		0.2375		0.2332	

G_1 = Genotype 1 = TMS 188/00106.
G_2 = Genotype 2 = TMS 083/00125.

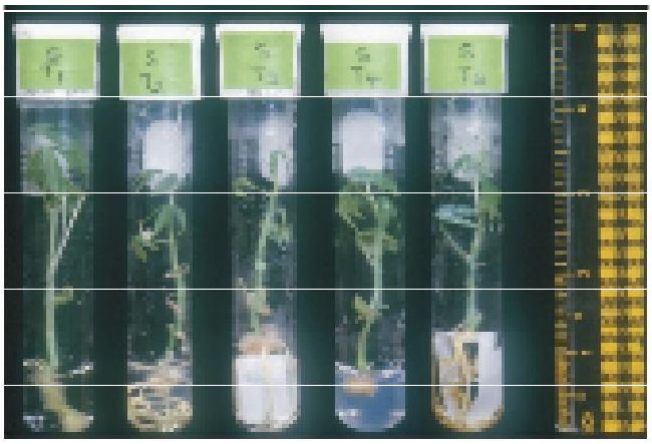

Figure 1. Screen house performance of the 5 treatments of cassava tissue culture. T_1 - Liquid only; T_2 - liquid with 50% normal agar (2 g/l); T_3 - liquid media with filter paper embedded; T_4 - media with normal agar (4 g/l); and T_5 - liquid media with filter paper projecting out.

in the screen house with liquid medium containing embedded filter paper. This suggests that before the ex-plants can be transferred to the screen house, there is need to ensure their survival in the laboratory. For T_2 (liquid with 50% normal agar), T_4 (media with normal agar, 4 g/l) and T_5 (liquid media with filter paper projecting out), the survival was significantly higher in the screen house than in the laboratory. On the other hand TMS 083/00/25 survived better in the liquid media with embedded filter than TMS 188/00106. The survival rate of TMS 188/00106 was also better in liquid medium with 50% normal agar (2 g/l), media with normal agar and liquid media with filter paper projecting out than TMS 083/00/25. This suggests that for long storage before sub culturing, laboratory may be ideal while for short time storage and immediate rapid mass propagation screen house may be adopted.

Table 3 shows that TMS 188/00106 survived better in liquid media than TMS 083/00125. No significant different between the two genotypes in most of the media except on survival in liquid media only. Figure 1 Shows that screen house plantlet grow relatively uniform for all the five treatments while Figure 2 indicates that plantlets in T1 grew faster than others in the culture room. There is equal height for plantlets in T1 in Figure 3 except that

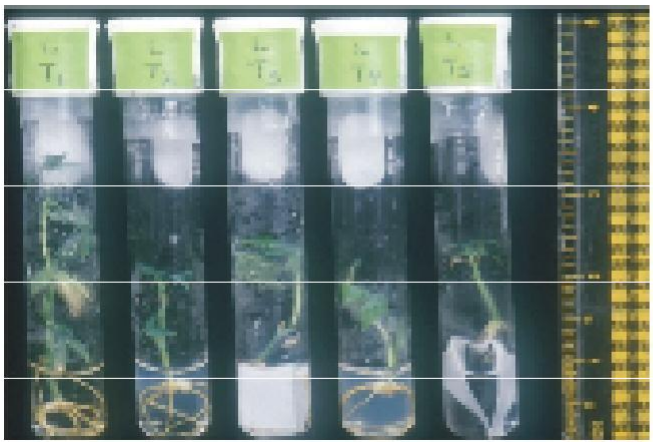

Figure 2. Laboratory performance of the 5 treatments of cassava tissue culture. T_1 - Liquid only; T_2 - liquid with 50% normal agar (2 g/l); T_3 - liquid media with filter paper embedded; T_4 - media with normal agar (4 g/l); and T_5 - liquid media with filter paper projecting out.

Figure 3. Comparism of treatment 1 (liquid only) of cassava tissue culture in two environments. S = Screen house and L = laboratory.

rooting was better in the laboratory than those placed in the screen house. Figures 4, 5 and 6 shows a faster growth rate in screen house for T5, T3 and T2 respectively than their counterparts placed in the laboratory.

It can therefore be concluded that when the need arises, in vitro plantlets of cassava can be raised adequately in the screen house and even be raised faster than the laboratory as long as the temperature does not exceed 40^{O}C (Ng, 2002).

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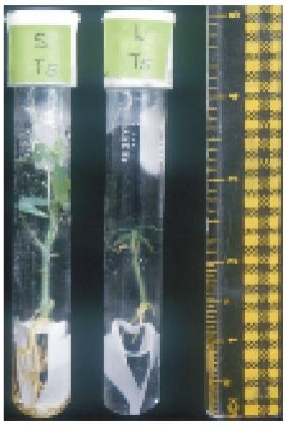

Figure 4. Comparism of treatment 2 (liquid with 50% normal agar, 2 g/l) of cassava tissue culture in two environments. S = Screen house and L = laboratory.

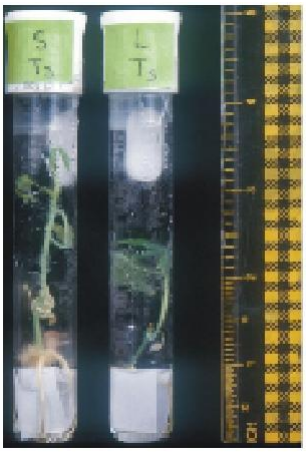

Figure 5. Comparism of treatment 3 (liquid media with filter paper embedded) of cassava tissue culture in two environments. S = Screen house and L = laboratory.

Figure 6. Comparism of treatment 5 (liquid media with filter paper projecting out) of cassava tissue culture in two environments. S = Screen house and L = laboratory.

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