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Performance of *In Vitro* Cassava (*Manihot esculenta* Crantz) Plantlets Weaned with Locally Sourced Substrates

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Abstract— *The performance of in vitro cassava plantlets weaned on different locally sourced substrates was evaluated. Nodal cuttings were excised from healthy six weeks old OG 001cassava variety in the culture room of tissue culture laboratory of National Root Crops Research Institute, Umudike, Abia State, Nigeria. The explants were washed, sterilized and cultured in vitro. The resulting plantlets were weaned on the following substrates - top soil (TS), river sand (RS), saw dust (SD), rice hull waste (RH), 2:1 top soil plus river sand (TS +RS), 2:1 river sand plus saw dust (RS + SD), 2:1 river sand plus rice husk (RS + RH), 2:1 top soil plus saw dust (TP + SD), 2:1 top soil plus rice husk (TP + RH), 2:1 saw dust plus rice husk (SD + RH) and 2:1 peat pellet plus vermiculite (PP + VE), which served as the control. Completely randomized design was used with ten replications. Results showed that plantlets weaned on PP + VE performed better than the other treatments at the end of the weaning period with significantly ($P < 0.05$) highest survival rate (98%), plant vigour (2.6), number of leaves (5) and number of nodes (8). This was closely followed by RS with survival rate, plant vigour, number of leaves and nodes of 63%, 1.4, 1.7 and 3.5, respectively. Plantlets weaned on the other substrates performed poorly. Although plantlets weaned on peat pellet + vermiculite mixture out-performed the other substrates, river sand if properly handled could be a potential substitute for the conventional substrate in weaning cassava plantlets.*

Keywords— Cassava, In vitro, local substrates, peat pellet, vermiculite, weaning.

I. INTRODUCTION

Cassava (*Manihot esculenta* Crantz) is among major food and income security crops in sub-Saharan Africa [1]. It is a major food security crop providing about 500 calories per day for 800 million people in sub-Saharan Africa and other regions of the world [2]. Cassava is the third most important source of calories, after rice and maize for many populations in the humid tropics [3]. According to FAO [4], millions of small-scale farmers in more than 100 countries in the world now cultivate cassava with the major global production area being in Africa.

It is widely grown by farmers because of its remarkable characteristics such as reliability and cheap source of available year-round food, reasonable yields on marginal soils, tolerant to major pests, diseases and drought [5]. As a major carbohydrate crop, it is a versatile resource with potential for creating diverse products such as chips, broken dried roots meal, starch, flour and ethanol [6; 7]. The role of cassava as a traditional food for human consumption is rapidly changing to that of an efficient industrial crop in some parts of Africa. Dried cassava roots and meal are used as raw material for animal feed, while cassava starch is used for industrial purposes [8].

Cassava is traditionally propagated vegetatively using stem cuttings. High seed dormancy characteristic of cassava seeds and delayed germination limit seed propagation. Using traditional stem cutting causes loss of superior genotypes and decreases productivity as a result of low multiplication ratio (1:10) and viral, fungal and bacterial diseases [9; 1]. Yield losses of over 20% have been reported due to diseases [10; 11], hence the development and use of efficient micro-propagation techniques to produce healthy planting materials [11].

Micro-propagation techniques are used for rapid clonal multiplication of selected genotypes of diverse plant species [12]. In vitro culture has contributed significantly to crop improvement by overcoming certain limitations associated with conventional techniques [13]. Culturing of an organized tissue in the form of very small shoots or meristem has allowed the most valuable application of plant tissue culture in order to eliminate virus from infected mother plant [5]. Sesay [1] stated that until production constraints are reduced in high-yielding cassava varieties

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and cassava producers have access to disease-free planting materials the full potential of cassava will not be realized.

Tissue-cultured propagules are extremely vulnerable to environmental stress. This is due to the fact that they are produced under controlled environment, therefore the plantlets produced have small juvenile leaves with reduced photosynthetic capacity and malfunctioning stomata [14], poor vascular connection between roots and shoots and thus reduced water conduction and poorly developed cuticle or waxy layer [15]. These problems have been overcomed by weaning/ hardening of the plantlets [16].

Bonilla Morales [17] reported up to 90% loss of cassava plants from transplanting vitroplants to ex vitro environment. Nowak and Pruski ([14] reported that gradual adaptation of plants to the *ex-vitro* environment (which occurs during weaning) improved plants survival upon transfer to soil. The ultimate success of in vitro propagation lies in the successful establishment of plants in the soil [18].

However, the high cost of using peat pellet and vermiculite for weaning of plantlets has opened research on the use of alternative substrates that will be efficient and cost effective. Bonilla Morales [17] evaluated different organic substrates for acclimatization and hardening of vitroplants of cassava and reported that the substrate solid humus + husk dry rice in the ratio of 1:1 allowed the survival and adaptation of 80% of the vitroplants in comparison with solid humus + shaving (32.5%) and Bocashi (0%). Ubalua and Okoroafor [19] investigated the use of locally available substrate (river sand, saw dust and rice mill waste) as alternative to conventional substrate (jiffy peat) for hardening of sweet potato and reported 100% survival of the in vitro plants regardless of the substrate or substrate combination used. They also reported non-significant difference in plant height, number of leaves and number of nodes produced by the potato plants weaned with combination of 2:1 river sand/saw dust and jiffy peat.

Little work has been done on the use of local substrates for weaning cassava plantlets. Hence, this work was aimed at evaluating the performance of in vitro cassava plantlets weaned with locally available substrates with a view to providing alternative means of weaning cassava plantlets.

II. MATERIALS AND METHODS

2.1 Study location

This work was carried out at the Plant Tissue Culture Laboratory, Biotechnology Research and Development Center, National Root Crops Research Institute (NRCRI), Umudike, Umuahia, Abia State, Nigeria.

2.2 Plant material

Nodal cuttings measuring 1 cm long were excised from healthy six weeks old cassava variety (OG 001) in the culture room of the tissue culture laboratory of NRCRI. The explants were washed, sterilized and cultured in vitro following standard procedures in tissue culture exercise. Plantlets resulting from the culturing process were weaned using different substrates.

2.3 Preparation of substrate bags

The substrate bags were prepared using clear transparent polythene sheets of 0.28mm thickness. The sheet was cut out to a size of 20cm by 7.5 cm with a pair of scissors. Its two open horizontal ends were sealed using the impulse heat sealer. The two sealed ends were infolded and held with pins and then one of the vertical ends was sealed up using the impulse heat sealer, leaving only one vertical end open. The pins were detached and the polythene substrate bags were punctured to allow drainage of water and air passage.

2.4 Construction of humidity chamber

A clear transparent polythene sheet of width 186cm and length 125cm was cut using a scissors and rolled to a white glossy plywood board measuring 50cm in diameter. The transparent sheet was held to the edge of the board using thumb pins. The small overlapping polyethylene sheet at the base of the board was glued to the board so as to close the gaps and to avoid air passage into the humidity chamber.

2.5 Treatments and experimental design

The substrates used for the weaning process included top soil, river sand, saw dusk, rice husk, peat pellet and vermiculite. These substrates were used singly and in combination as follows: top soil (TS), river sand (RS), saw dust (SD), rice hull waste (RH), 2:1 top soil plus river sand (TS +RS), 2:1 river sand plus saw dust (RS + SD), 2:1 river sand plus rice husk (RS + RH), 2:1 top soil plus saw dust (TP + SD), 2:1 top soil plus rice husk (TP + RH), 2:1 saw dust plus rice husk (SD + RH) and 2:1 peat pellet plus vermiculite (PP + VE), which served as the control. Thus a total of 11 treatments were used. These treatments were arranged in a completely randomized design and replicated ten times.

2.6 Weaning of the cassava plantlets

The substrates were properly mixed with water and sterilized by autoclaving at 121^0C for 20 min. The *in vitro*-raised cassava plantlets were carefully removed from the culture vessel and then gently washed with distilled water to remove adhering medium on the roots. Thereafter, the cassava roots were transplanted with care onto the different sterile substrates contained in transparent polybags. One plantlet was transplanted per substrate bag. Immediately

after transplanting, the polybags containing the substrates plus cassava plants were placed in the humidity chamber and sprayed with distilled water. The humidity chamber was made airtight by closing all the open ends. The airtight clear transparent polythene humidity tent was kept under a shade to reduce water loss and irradiance during the acclimatization period.

In the morning of the fourth day, three holes about 1 cm in diameter were made on the sides of the humidity chamber to reduce the relative humidity of the chamber. In the morning of the fifth day, the relative humidity was further lowered by making an opening (window) on the lower side of the tent using a scissors. The plantlets and the humidity tent were then sprayed with water in the morning and evening of the same day. A second window was opened on the opposite side of the tent in the morning of the sixth day. A wash bottle was filled with water and six grains of NPK 20:15:15 fertilizer was added. This was sprayed on the plantlets in the morning and evening of the sixth day. On subsequent days, the plants were sprayed with water morning and evening for the period of the study.

2.7 Assessment parameters

The following parameters were determined at weekly interval for a period of six weeks: percentage survival rate, plant vigour, number of leaves and number of nodes.

A scale of 1 – 3 rating was used to determine the vigour of the plants, where:

1 represents not robust

2 represent fairly robust

3 represent robust

2.8 Statistical Analysis

Data generated were analyzed statistically using SPSS version 16.0 at 5% level of significance and significant means were partitioned using Duncan Multiple Range Test.

III. RESULTS

Plantlets survival rate was significantly ($P < 0.05$) affected by the substrates used in all the weeks sampled. Plantlets weaned with locally sourced substrates significantly had lower survival rates in relation to the conventional substrate (2:1 peat pellet plus vermiculite) with the exception of plantlets weaned on river sand only, which had comparable values with the control from weeks 1 – 4 (TABLE 1). From week 5 into the weaning period, all the plantlets had died except those weaned on peat pellet + vermiculite and river sand only. However, by the end of the weaning period, plants weaned on peat pellet + vermiculite had percentage survival rate of 98%, river sand alone had 63% while the least percentage survival of 2% was recorded in 2:1 top soil plus rice hull waste, 2:1 saw dust plus rice hull waste, top soil only and rice hull waste only.

In the case of vigour of plantlets, plantlets weaned on 2:1 peat pellet plus vermiculite consistently had significantly higher vigour in comparison with those weaned on the other substrates with the exception of plantlets weaned on river sand only at weeks 1 and 2 (TABLE 2). In the same vein, plantlets weaned on river sand alone consistently had significantly higher vigour than those on the other locally sourced substrates, which had comparable vigours.

Number of leaves produced by the cassava plantlets also differed significantly ($P < 0.05$) among the different substrates used from week 2 into the weaning period (TABLE 3). Significantly, the highest number of leaves was produced by plantlets weaned on 2:1 peat pellet plus vermiculite in all these weeks. However, plantlets weaned on river sand alone produced number of leaves that was significantly ($P < 0.05$) higher than those weaned on the other locally sourced substrates, which had statistically similar values. Aside the plantlets weaned with 2:1 peat pellet plus vermiculite, 2:1 top soil plus river sand, 2:1 river sand plus rice hull waste and river sand only, plantlets from the other substrates lost their leaves and died by week 3 into the weaning period. By week 5, the plantlets have lost their leaves and died except those weaned on 2:1 peat pellet plus vermiculite and river sand only.

Significant effect of substrates on number of nodes produced by the cassava plantlets was found in all the weeks sampled (TABLE 4). In weeks 1 and 2, plantlets weaned on peat pellet + vermiculite and river sand alone produced comparable number of nodes that was significantly higher than those on the other substrates. By week 6, plantlets weaned on 2:1 peat pellet produced significantly the highest number of nodes, followed by plantlets weaned on river sand only while the number of nodes from plantlets weaned on the other substrates did not differ significantly.

IV. DISCUSSION

The weaning of in vitro raised plants is essential for better survival and successful establishment. Direct transfer of tissue-cultured plants to sunlight (field condition) causes leaf charring and wilting of the plants [20; 16]. Weaning therefore determines the survival percentage of the plantlets in the field.

In the present study, results revealed that plantlets weaned on 2:1 peat pellet plus vermiculite showed 98% survival rate. This was significantly higher than the percentage survival recorded in the locally sourced

substrates. Following this was river sand alone, which gave survival rate of 63 % that was significantly higher than the other locally sourced substrates that recorded survival rates between 2 and 20 %. Afreen-Zobayed [21] stated that the percentage survival of in vitro plantlets during acclimatization is controlled by the nature of the supporting substrates and the intrinsic quality of the micro propagated plantlets among other factors. Studies have shown that a variety of materials including saw dust, top soil and rice mill waste are detrimental to or have no benefit for plant growth [22].

Ubalua and Okoroafor [19] reported 100% survival rate of sweet potato plantlets grown on sterile substrates (river sand, saw dust, rice mill waste, river sand/rice mill waste, river sand/saw dust, saw dust/rice mill waste and jiffy peat) and 58% survival rate on unsterilized substrates. Similarly, Bonilla Morales et al. [17] reported survival rates of 80%, 32.5% and 0% of in vitro cassava plantlets (Brazilian variety) hardened with 1:1 solid humus + husk dry rice, 1:1 solid humus + shaving 1: 1 and Bocashi and concluded that the 1:1 solid humus + husk dry rice substrate is considered suitable both nutritional level and structural component of the soil for having an adequate porosity for rooting of plant and an ideal adaptation field phase.

Plantlets on 2:1 peat pellet and vermiculite were more vigourous compared to the plantlets on the other supporting substrates where as plantlets on river sand alone had more vigour than the other locally sourced substrates. This may be attributed to the porosity of these materials. Ubalua and Nsofor [23] reported that acclimatization and growth of in vitro raised plantlets is highly correlated with substrate porosity. Savangikar [24] stated that generated plants must be vigorous and capable of being successfully transplanted in the field, and must have high field survival. It has been found that the production of high quality and vigorous plants through *in vitro* culture requires the enhancement of post-transplanting ability for water management, efficiency of photosynthesis and resistance to diseases. This is achieved by using suitable substrate for weaning process.

Overall, the plant growth parameters differed significantly ($P < 0.05$) among the various substrates used. Highest number of leaves and nodes were recorded in plantlets weaned with the conventional substrate (peat pellet/vermiculite). River sand alone weaned plantlets produced the second highest number of leaves and nodes at the end of the weaning process. Plantlets on saw dust alone lost their leaves from week 1into the weaning period. Apart from the plantlets weaned with 2:1 peat pellet plus vermiculite, 2:1 top soil plus river sand, 2:1 river sand plus rice hull waste and river sand only, plantlets on the other substrates lost their leaves and died by week 3 into the weaning period. By week 5, the plantlets have lost their leaves and died except those weaned on 2:1 peat pellet plus vermiculite and river sand alone. This is in support of the earlier report made by [22] that a variety of materials including saw dust, top soil and rice mill waste are detrimental to or have no benefit for plant growth.

Plantlets on peat pellet/vermiculite and river sand alone had competitive results on number of nodes produced at weeks 1 and 2 after which significant differences were recorded between them. Ubalua and Nsofor [23] reported enhanced rooting and growth of cassava plantlets variety TMS 98/0505 on river sand/sawdust and peat pellet/vermicultite and attributed it to the porosity of these substrates. Nodal increase of plants is associated with increases in growth. The retardation in growth as evidenced in the number of nodes recorded in the locally sourced substrates may be attributed to the low drainage property of these substrates leading to accumulation of water and consequently depletion of oxygen. The exhaustion of oxygen increases microbial activity and interferes with plant-soil-water relationship [25]. This has toxic effect on the morphological and anatomical aberration in the number of leaves. This could be the reason for the abortion of leaves, fungal attack, wilting and death of plantlets observed in the locally sourced substrates. On the contrary, peat pellet + vermiculite mixture provides good drainage and aeration to plant roots, which aided better performance of the cassava plantlets. Our findings is in agreement with the report of [19] who reported better performance of plantlets on peat pellet but disagrees with the report of [26] and [27] who reported low effectiveness of vermiculite as a substrate for acclimatization. It has been reported that good quality propagules with well developed roots and leaves are easy to acclimatize to the external environment and that any successful acclimatization protocol must ensure that the plants maintain active growth during the entire weaning period [28].

V. CONCLUSION

Survival, vigour and growth of the cassava plantlets were significantly influenced by the type/quality of the substrates used, their physical nature and possibly their nutrient composition. The leaf abortion, fungal attack, wilting and subsequent death of plantlets observed in the locally sourced substrates (excepting river sand alone) showed the inefficiency of these substrates in weaning cassava plantlets. However, plantlets grown on peat pellet + vermiculite mixture performed better than plantlets grown

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on the locally sourced substrates. Amongst the locally sourced substrates, river sand alone weaned plantlets performed better and could be a potential substitute for the conventional substrate if properly handled.

VI. TABLES

Table.1: Survival of in vitro cassava plantlets weaned on different substrates at different weeks

	Weeks						
Treatment	1	2	3	4	5	6	% Survival
PP + VE	1	1	1	1	1	1	98
TP	0.1	0	0	0	0	0	2
RS	0.8	0.8	0.6	0.6	0.5	0.5	63
SD	0.2	0.1	0	0	0	0	5
RH	0.1	0	0	0	0	0	2
TS +RS	0.6	0.4	0.1	0	0	0	18
RS + SD	0.7	0.3	0	0	0	0	16
RS + RH	0.7	0.5	0.4	0.1	0	0	28
TP + SD	0.4	0.1	0	0	0	0	8
TP + RH	0.1	0	0	0	0	0	2
SD + RH	0.1	0	0	0	0	0	2
LSD (0.05)	0.3	0.6	0.5	0.4	0.01	0.00	25

PP + PV = 2:1 peat pellet plus vermiculite; TS = top soil; RS = river sand; SD = saw dust; RH = rice hull waste; TS + RS = 2:1 top soil plus river sand; RS + SD = 2:1 river sand plus saw dust; RS + RH = 2:1 river sand plus rice husk; TP + SD = 2:1 top soil plus saw dust; TP + RH = 2:1 top soil plus rice husk; SD + RH = 2:1 saw dust plus rice husk

Table.2: Vigour of cassava plantlets weaned on different substrates at different weeks

	Weeks					
Treatment	1	2	3	4	5	6
PP + VE	2.4	2.4	2.4	2.6	2.5	2.6
TP	0.1	0	0	0	0	0
RS	2.0	1.9	1.8	1.9	1.9	1.4
SD	0.3	0.1	0	0	0	0
RH	0.1	0	0	0	0	0
TS +RS	0.8	0.3	0.1	0	0	0
RS + SD	0.7	0.1	0	0	0	0
RS + RH	1.0	0.6	0.2	0.1	0	0
TP + SD	0.5	0.1	0	0	0	0
TP + RH	0.1	0	0	0	0	0
SD + RH	0.1	0	0	0	0	0
LSD (0.05)	0.8	0.5	0.1	0.0	0.0	0.0

PP + PV = 2:1 peat pellet plus vermiculite; TS = top soil; RS = river sand; SD = saw dust; RH = rice hull waste; TS + RS = 2:1 top soil plus river sand; RS + SD = 2:1 river sand plus saw dust; RS + RH = 2:1 river sand plus rice husk; TP + SD = 2:1 top soil plus saw dust; TP + RH = 2:1 top soil plus rice husk; SD + RH = 2:1 saw dust plus rice husk

Table.3: Number of leaves produced by cassava plantlets weaned on different substrates at different weeks

	Weeks					
Treatment	1	2	3	4	5	6
PP + VE	2.7	2.7	3.5	3.6	4.7	4.6
TP	0.1	0	0	0	0	0
RS	1.7	2.0	2.3	1.9	1.7	1.7
SD	0.3	0.1	0	0	0	0
RH	0	0	0	0	0	0
TS +RS	0.8	0.4	0.1	0	0	0
RS + SD	1.0	0.3	0	0	0	0
RS + RH	0.8	0.9	0.3	0.1	0	0
TP + SD	0.4	0.1	0	0	0	0
TP + RH	0.3	0	0	0	0	0
SD + RH	0.1	0	0	0	0	0
LSD (0.05)	NS	0.5	0.1	0.1	0.1	0.0

PP + PV = 2:1 peat pellet plus vermiculite; TS = top soil; RS = river sand; SD = saw dust; RH = rice hull waste; TS + RS = 2:1 top soil plus river sand; RS + SD = 2:1 river sand plus saw dust; RS + RH = 2:1 river sand plus rice husk; TP + SD = 2:1 top soil plus saw dust; TP + RH = 2:1 top soil plus rice husk; SD + RH = 2:1 saw dust plus rice husk

Table.4: Number of nodes of cassava plantlets weaned on different substrates at different weeks

	Weeks					
Treatment	1	2	3	4	5	6
PP + VE	3.8	4.9	5.7	7.1	7.9	8.0
TP	0.5	0	0	0	0	0
RS	4.0	4.2	4.5	3.8	3.5	3.5
SD	0.6	0.6	0	0	0	0
RH	0.6	0	0	0	0	0
TS +RS	1.7	1.3	0.6	0	0	0
RS + SD	2.1	0.5	0	0	0	0
RS + RH	2.5	1.6	0.6	0.6	0	0
TP + SD	1.2	0.5	0	0	0	0
TP + RH	0.4	0	0	0	0	0
SD + RH	0.5	0	0	0	0	0
LSD (0.05)	0.5	0.8	0.2	0.1	0.0	0.0

PP + PV = 2:1 peat pellet plus vermiculite; TS = top soil; RS = river sand; SD = saw dust; RH = rice hull waste; TS + RS = 2:1 top soil plus river sand; RS + SD = 2:1 river sand plus saw dust; RS + RH = 2:1 river sand plus rice husk; TP + SD = 2:1 top soil plus saw dust; TP + RH = 2:1 top soil plus rice husk; SD + RH = 2:1 saw dust plus rice husk .

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