(4)

NIGERIAN JOURNAL OF HORTICULTURAL SCIENCE

VOLUME 5 (2000)

Established in 1974 for the promotion of Horticultural Science in Nigeria

Research notes
Microproagation of Fluted pumkin by Enhanced Axilary shoot formation

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Abstract

In a bid to develop protocols for micropropagation of fluted pumkin *(Telfairia occidentalis)*, single node cuttings of two genotypes, *Telfairia occidentalis* white and *Telfairia occidentalis* orange (ToW-1 and ToR-1 respectively) were cultured in Murashige and Skoog medium but with four different combinations of kinetin and naphthalene acetic acid concentrations. The highest number of nodes per plantlet was obtained in the medium containing l=litre2.0mg/1 kinetin which indicates suitability for micropropagation purposes. There was however, lack of root formation. Premature senescence was also significantly predminant in ToR-1 relative to ToW1. Optimum medium for *Telfairia* micropropagation appears to be genotype dependent.

Key Words; Telfairia, micropropagation.

Introduction

The fluted pumpkin *(Telfairia occidentalis)* is a seed-propagated vegetable of Tropical West Africa cultivated for both the leafy shoots and immature edible seeds. It is most popular among the Igbos of Nigeria. It's the oily seeds are believed to have lactating properties, and are therefore in high demand by nursing mothers (Akorada, 1990). The leaves are rich in iron and used to cure anaemia.

Telfairia seeds are however recalcitrant and can only be kept viable and transported inside the large fruit. Also, the sex of the plant can not be known until after flowering and seed propagation has more than fifty percent chance of being the less desirable male type which does not set fruit. In addition, the *Telfairia* mosaic virus (TeMV), which constitutes serious constraints to its production, is seed borne (Anno-Nyako, 1988). Further, there is a serious shortage of seeds for both planting and consumption, hence the need for its vegetative propagation.

Micropropagation, the *in vitro* techniques of plant propagation, is an aternative means of asexual propagation having the most practical application out of all the other tissue culture techniques (Ng, 2000). The technique is used in rapid multiplication of vegetatively propagated crops, plants with long generation period, those with low propagation rates and in propagation of virus-free plants (Hussey, 1978; 2000). Micropropagation via enhanced axillary shoot formation (involving shoot) tip culture or single node culture) has proved to be the most generally applicable method of *in vitro* propagation, having the advantage of little callus formation and low degree of genetic abnormalities (Torres, 1989). Using this method, a 4-10 times multiplication rate can be achieved on a regular 4-8 week micropropagation cycle (Mentell *et al*, 1985).

This study was therefore conducted to develop protocols for micropropagation of *Telfairia occidentalis*. This will enhance the reservation of seeds for consumption only, allow for selection of healthy vines with desirable traits and subsequent rapid cloning for field planting. Male and female genotypes can also be selected for at planting, while available germplasm can be conserved with minimal space *in vitro* and easily transported without fear of loss of viability.

Materials and Methods

Two fruits of pumpkin, *Telfairia sp.,* were purchased from the Ojoo local market in Ibadan. The seeds of one (ToW-1) was white with dry pulp while the other (ToR-1) was orange with relatively watery pulp. The seeds were conditioned by sun-drying before planting in pots in the nursery. At the 8-10 node stage, single node cuttings were excised, surface sterillized using 70% ethanol and 25% commercial bleach for 5 and 20 minutes repectively and rinsed in three changes of sterile distilled water. Using test tubes containing 10ml of media each, 1cm long single node cuttings were then inoculated

into full strength MS media (Murashinge and Skoog, 1962) containing 30g/l sucrose, 0.1g/l myo-inostitol, 7g/l agar and four different combinations of concentrations of naphthalene acetic acid (NAA) and kinetin (kin) at a pH of 5.7. The four combinations of concentrations of NAA and kinetin which constituted the treatments were:

a: 1mg/l kinetin
b: 2mg/l kinetin
c: 2mg/l NAA + 1mg/l kinetin
d: 2mg/l NAA + 10mg./l kinetin.

Completely radomized design was used in the experiment, and the experimental treatments randomized within replicates. The cultures were then incubated at 25°C and 4000 Lux. Five weeks after culture initative, data were taken on the percentage of plantlets with roots, callus and chlorotic leaves or stem (senescenes); number of shoots and nodes per plantlet. The degree of callus formation per plantlet was also recorded on a 0-3 scale as follows:

0-no callus formed
1-25% of explant covered with callus;
3-75-100% of explant covered with callus.

Taking into consideration the fact that the distribution of the various data collected is not normal but binomial or poisson, it is more eappropriate to carry out a chi-square test rather than analysis of variance (Bailey, 1959; Wardlaw, 1985). Statistical analysis of data was therefore with the SAS computer application software which takes cognizance of the distribution of the data and is therefore more precise. Chi-square values and p-values (labelled as pr>chi) are likelihood ratio statistics for testing the significance of each effect. The poisson and binomial distributions were used in the analysis. Mean seperation was by means of orthogonal comparison between treatments whose effects were found to be significant.

Results and Discussion

The four media formulations were significantly different with respect to degree of callus formation, number of nodes per plantlet and percentage of explants with callus (Table 1). Orthogonal comparisons revealed that degree of callus formation and percentage of explants with callus were highest in the presence of both kinetin and NAA (Table 1 and 2). This is expected as auxins (NAA inclusive) have been reported to induce callus formation (Tisserat, 1985). Although the target is micropropagation, callus formation is not necessarily undesirable as root and shoot formation can be induced from callus to produce plantlets (Torres, 1989).

The highest number of nodes per plantlet was observed in medium containing 2mg/1 of kinetin alone (Table 2). As high as six nodes per plantlet was recorded in this medium in some units (Figure 1). This indicates the efficacy of the medium for micropropagation purposes, the more the number of nodes per plantlet, the higher the number of fresh plantlets that can be obtained. Earlier reports have shown that cytokinins induce shoot formation (Lydiane and John, 1996). In addition, enhanced axillary branching using stem tips and lateral buds have been reported in a number of vegetable crops includng cauliflower, cucumber, cabbage, garlic and tomato, among others (Torres, 1989).

Only the medium containing 1mg./1kinetin plus 2 mg/1 NAA had scanty root formation while in all other media, there was no root formation (Table 2). However, rooting can be achieved by testing other auxins apart form NAA at the optimum concentration of 2mg/1 kinetin (Table 2), or even after transplanting (Lydiane and John, 1996).

The two genotypes were not significantly different in all the parameters except for percentage of plantlets showing senescence (Table 1), with 21% and 57% of the ToW-1 and ToR-1 having senesced five weeks after culture initiation respectively (Table 2). This may be due to water stress resulting from osmotic imbalance, or chlorotic leaves due to inadequate nitrogen nurtrition (Lydiane and John, 1996). A high salt medium or high agar concentration may be the cause of osmotic imbalance. The threshold for tolerance to water stress and inadequate nitrogen nutrition therefore appears to differ among genotypes. However, premature senescence can be circumvented by frequent subculturing.

Developing an optimum medium for micropropagation of Telfairia occidentalis needs to be perfected, although it appears to be genotype-dependent. Work is continuing in our laboratory to achieve this goal.

Acknowledgements

This authors wish to thank the International Atomic Energy Agency and the Institute of Agricultural Research and Training, Moor Plantation, Ibadan, Nigeria for generously providing the equipments and chemical used for the project.

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Table 1: Chi-square values from likelihood ratio statistics of growth parameters of Telfairia occidentalis grown in media with different types and concentrations of growth regulators

Source	df	Degree of Callus Formation	Root Formation (%)	Number of shoots per plantle	Number of nodes per plantlet	Callus Formation (%)	Senescence (%)
Genotype	1	1.0717ns	1.4233ns	0.0954ns	0.0809ns	1.9621ns	4.2805
Media	3	14.854**	2.706ns	1.889ns	21.721***	14.157**	2.975ns
a vs b	1	0.907ns			7.109**	0.746ns	
c vs d	1	0.907ns			7.109**	0.746ns	
ab vs cd	1	13.400***			11.714***	12.198***	

***: significant at 0.5and 0.01 probability levels respectively.
a: 1mg/1 kinetin
b: 2mg/1 kinetin
c: 2mg/1NAA + 1mg/1kinetin
d: 2mg/1NAA + 10mg/1 kinetin

Table 2: Means of growth parameters of *Telfairia occidentalis* grown in media with different types and concentrations of growth regulators.

Media	Degree of Callus Formation	Root Formation (%)	Number of shoots per plantle	Number of nodes per plantlet	Callus Formation (%)	Senescence (%)
a	0.25	0.00	1.00	1.00	25.00	62.00
b	0.57	0.00	1.00	2.87	43.00	29.00
c	1.50	17.00	0.50	0.33	83.00	33.00
d	2.00	0.00	0.57	0.43	100.00	29.00
Genotype						
ToR-1	0.86	7.00	0.71	1.14	71.00	57.00
ToW-1	1.21	0.00	0.86	1.21	50.00	21.00

a: 1mg/1 kinetin
b: 2mg/1 kinetin
c: 2mg/1NAA + 1mg/1kinetin
d: 2mg/1NAA + 10mg/1 kinetin

Figure 1: A: Plantlet of Telfairia occidentalis grown in MS medium with 2mg/1 kinetin five weeks after culturing. B: Senescent plantlet of genotype Tor-1 of *Telfairia occidentalis* five weeks after culturing.

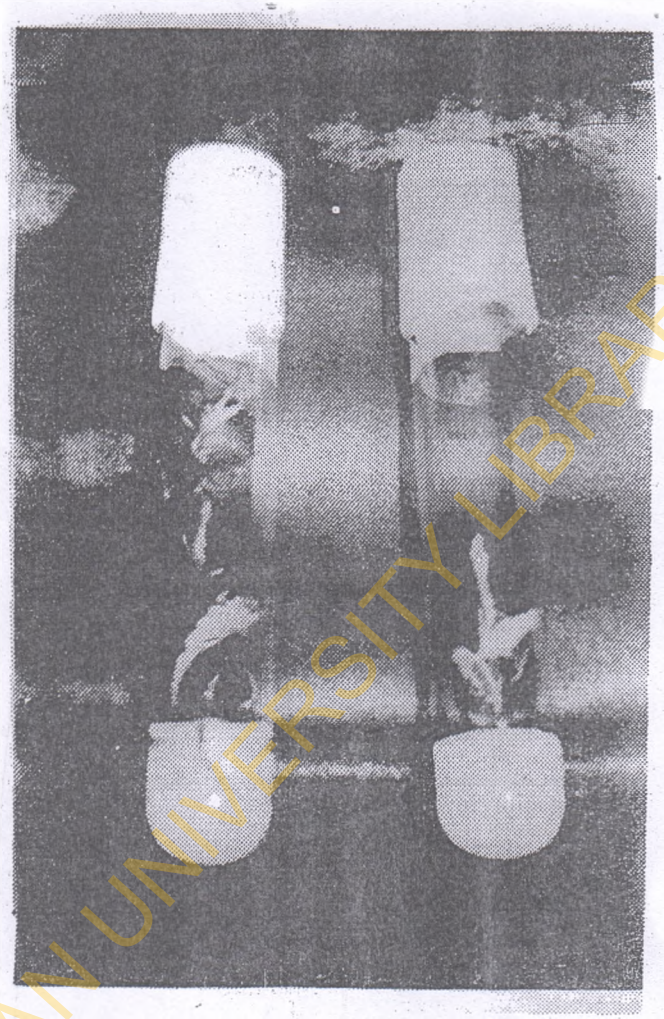

Figure 1: A Plantlet of Telfairia occidentalis grown Ms medium with 2mg/1 Kinetin five weeks after culturing
B: Senescent plantlet of genotype ToR-1 of Telfaria occidentalis five weeks after Culturing