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Full Length Research Paper

A study on the effect of plant growth regulators (PGRs) on fluted pumpkin (*Telfairia occidentalis*)

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Root and stem explants of fluted pumpkin were cultured in medium containing different types and concentrations of plant growth regulators (PGRs). The explants were observed for callus, root and shoot formation parameters after four months. Differences among explants, plant growth regulators and their interaction were not significant for number of roots per plantlet. Callus formation was higher in stem than root explants. Stem explants formed more callus in medium containing naphthalene acetic acid than that containing indole acetic acid while the trend was opposite with root explants. Root explants did not form shoots, leaves or nodes in any of the PGR regimes, while a concentration of 1.5 mg/l benzylaminopurine (BAP) induced the highest numbers of shoots, nodes and leaves per stem explant. There was no callus, shoot, node and leaf formation by both explants when cultured in medium without PGRs while root formation was minimal.

Key words: Fluted pumpkin, *Telfairia occidentalis*, micropropagation, plant growth regulators.

INTRODUCTION

Fluted pumpkin (*Telfairia occidentalis*), commonly known as ugu in Nigeria, is a nutritious vegetable of Tropical West Africa widely cultivated by women in Eastern Nigeria for its leafy shoots and immature edible seeds (Akoroda, 1990). The seeds have lactating properties and are in high demand by nursing mothers. The leaves are rich in iron and used to cure anaemia while also being high in protein content (Okoli and Mgbeoku, 1983). The plant also contains considerable amount of antinutrients (Ajibade et al., 2006).

A major constraint to pumpkin production however, is that there are separate male and female plants (dioecy) such that the sex cannot be known until after flowering which takes about 4 months after planting (Schippers, 2000). Seed propagation has more than fifty percent chance of being the less desirable male type, whose shoots are coarser and less succulent than the female and does not set fruit (Ajibade et al., 2006). Hence, female leaves and plants are in higher demand by housewives and farmers respectively since they bring additional monetary returns. Therefore, any production research that evolves ways of early sex identification is of high priority, as this will enable farmers to adjust the sex ratio and increase productivity. Another production constraint is the *Telfairia* mosaic virus (TeMV), which is seed-borne and is therefore transmitted from generation to generation by mere planting (Anno-Nyako, 1988). The seeds are also recalcitrant and can only be kept viable and transported inside the large fruit that ranges from 20 – 50 cm in length and 10 – 20 cm in width (Balogun et al., 2002). Further, there is a serious shortage of seeds for both planting and consumption.

Improvement of *Telfairia* by conventional breeding has been difficult since offspring arise from separate plants. Hence, no two plants are genetically identical due to 100% cross pollination (Schippers, 2000). The natural diversity of *Telfairia* is small (Schippers, 2000), such that somaclonal variation will also be useful in introducing desirable changes in plants that can be incorporated into breeding programmes (Opabode and Adebooye, 2005). To overcome these constraints, there is an urgent need to develop propagation systems that are vegetative, circumventing the use of seeds as propagules and allowing the fixing of genes in hybrids.

Micropropagation, the propagation of plants *in vitro*, is an alternative means of vegetative propagation. It is used in rapid multiplication of vegetatively propagated crops, plants with long generation period or low propagation rates and to produce virus - free plants (Ng, 2000).

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Table 1. Mean square values of root and callus formation parameters of different explants of *Telfairia* cultured at different plant growth regulator (PGR) regimes.

Source of variation	Degree of freedom	Roots per Explant	Callus length (cm)	Callus breadth (cm)
Explant	1	0.00^{ns}	19.84^{***}	11.28^{***}
PGRs	10	0.01^{ns}	1.77^{***}	0.99^{***}
Explant x PGR	10	0.02^{ns}	2.25^{***}	1.19^{***}
Error	43	0.02	0.12	0.05
Total	64			

***: significant at p= 0.001; ns: not significant.

However, efforts on micropropagation of ugu have been sparse. Conservation of *Telfairia* biodiversity using plantlets *in vitro* will reduce the space required for storage, facilitate germplasm transportation due to small size of plantlets while providing a rich source of biodiversity for breeders and farmers. We propose to investigate the effects of different plant growth regulator regimes on growth of *Telfairia in vitro*, with a view to developing protocols for micro propagation of sex-identified seedlings of *Telfairia.*

MATERIALS AND METHODS

One pumpkin of *Telfairia* was purchased from the Alesinloye local market in Ibadan, Nigeria. The seeds were removed and washed in tap water. They were then disinfected in 70% methylated spirit, 35 and 17.5% commercial bleach respectively and rinsed in three changes of sterile distilled water. The embryos were excised along with some endosperm (1 cm in length) and cultured in Murashige and Skoog (1962) medium containing (per litre) 30 g sucrose, 0.1 g inositol, 30 mg kinetin, 7 g agar set at a pH of 5.7. When the embryos had germinated (three weeks after culturing), the plantlets were transferred into rooting medium, composed of the same constituents as in germination medium except that kinetin was reduced to 20 mg while 10 mg of indole acetic acid was added per litre of medium. Rooting was achieved two weeks later, the roots and the stem were then cut into portions, 1 cm in length and cultured in petri plates on eleven media which differed in type and concentration of plant growth regulators.

The media contained Murashige and Skoog, (1962) medium to which 30 g of sucrose and 0.1 g of inositol were added per litre of medium. The cytokinins used were benzylaminopurine (BAP) and kinetin (KIN) while naphthalene acetic acid (NAA) and indole acetic acid (IAA) were the auxins used. The constituents of the media are:

i) BAP1: 1.5 mg per litre of BAP.
ii) BAP1IAA1: 1.5 mg/l BAP, 1.5 mg/l IAA.
iii) BAP1IAA2: 1.5 mg/L BAP, 3.0 mg/l IAA.
iv) BAP1NAA1: 1.5 mg/l BAP, 1.5 mg/l NAA.
v) BAP1NAA2: 1.5mg/l BAP, 3.0 mg/l NAA.
vi) BAP2: 3.0 mg/l BAP.
vii) BAP2IAA1: 3.0mg/l BAP, 1.5 mg/l IAA.
viii) BAP2NAA1: 3.0 mg/l BAP, 1.5 mg/l NAA.
ix) KIN1NAA1: 1.5 mg/l KIN, 1.5 mg/l NAA.
x) KIN2: 3.0 mg/l KIN.
xi) Control: No plant growth regulator.

The experiment was a 2 (root and stem explants) x 11 (PGR regimes) factorial in completely randomized design. There were six replicates per treatment combination. Four months after culturing, data were collected on length and breadth of callus, numbers of roots, shoots, nodes and leaves per culture. Square root transformation was done for data that were counts. None of the root explants produced shoots, stem or leaves, so the effects of the PGR regimes on these three traits was based on only the stem explants. Analysis of variance was done using SAS and means were separated at p = 0.05.

RESULTS AND DISCUSSION

Differences among explants, PGRs and their interaction were not significant for number of roots per plantlet but significant for length and breadth of callus formed (Table 1). Stem explants formed significantly longer and wider calli than root explants (Table 2). Among the root explants, the longest and widest calli were formed in media containing only BAP (0.70 and 0.37 cm, respectively) or in combination with IAA (0.43 and 0.23 cm, respectively) while no callus was formed in any of the other media. Stem explants produced the largest calli in medium containing 3 mg/l of BAP and 1.5 mg/l of NAA (2.9 cm long, 2.13 cm wide), although this was not significantly different from medium containing BAP and NAA at 1.5 mg/l each. Callus formation by stem explants in media containing sole BAP or in combination with IAA was also minimal, except when the IAA concentration doubled that of BAP. Thus, stem explants responded more to NAA than IAA while the trend was opposite with root explants in terms of callus formation (Table 2). Regarding the cytokinins, both root and stem explants responded more to BAP than KIN (Table 2).

Auxins have been reported to induce callus formation in tissue culture of plants (Tisserat, 1985), while NAA and IAA promoted excessive callus formation in water melon (Compton and Gray, 1993). A high degree of callusing was reported in single node explants of fluted pumpkin cultured in medium containing both kinetin and NAA (Balogun et al., 2002). This study has shown, in addition, that for good callus formation, BAP combined with NAA is optimum for stem sections. This can be used as initiation medium for plant regeneration via somatic embryogenesis which will be useful in plant transformation.

There was neither callus formation nor root, shoot, node and leaf formation from both explants when cultured in medium without PGRs. This shows that *Telfairia* is highly responsive to plant growth regulators (Table 3 and 4).

Table 2. Mean values of root and callus formation parameters of different explants of *Telfairia* cultured at different plant growth regulator (PGR) regimes.

PGR regimes	Callus length (cm)		Callus breadth (cm)		Number of Roots	
	Root	Stem	Root	Stem	Root	Stem
BAP1	0.70a	0.47d	0.37a	0.30e	0.33	0.00
BAP1IAA1	0.43ab	0.00d	0.23ab	0.00e	0.33	0.00
BAP1IAA2	0.10b	2.27b	0.03ab	1.87ab	0.00	0.00
BAP1NAA1	0.00b	2.53ab	0.00b	1.53bc	0.00	0.00
BAP1NAA2	0.00b	0.00d	0.00b	0.00e	0.00	0.00
BAP2	0.00b	1.60c	0.00b	0.80d	0.00	0.67
BAP2IAA1	0.30ab	1.60c	0.07ab	1.43c	0.33	0.00
BAP2NAA1	0.00b	2.90a	0.00b	2.13a	0.00	0.33
KIN1NAA1	0.00b	2.23b	0.00b	1.73bc	0.00	0.00
KIN2	0.00b	0.13d	0.00b	0.10e	0.00	0.00
Control	0.00b	0.00d	0.00b	0.00e	0.00	0.33
Mean	0.14B	1.25A	0.06B	0.90A	0.09NS	0.09NS
S.E	0.04	0.13	0.02	0.10	0.04	0.04

Values in each column followed by the same letters are not significantly different at p=0.05.

Table 3. Mean square values for shoot parameters of stem explants of *Telfairia occidentalis* cultured at different plant growth regulator regimes.

Source of variation	Degree of freedom	Number of shoots/explant	Number of nodes/explant	Number of leaves/explant
PGRs	10	0.35**	0.75**	0.96**
Error	21	0.08	0.25	0.36
Total	31			

Values are square root transformations of the original values. **: significant at p= 0.01; ns: not significant.

Table 4. Mean values of shoot formation parameters of stem explants of *Telfairia occidentalis* cultured at different plant growth regulator regimes.

PGR regimes	Number of shoots/explant	Number of nodes/explant	Number of leaves/explant
BAP1	4.00a	6.67a	9.00a
BAP1IAA1	0.00b	0.00b	0.00b
BAP1IAA2	0.33b	0.33b	1.00b
BAP1NAA1	0.00b	0.00b	0.00b
BAP1NAA2	0.00b	0.00b	0.00b
BAP2	0.67b	2.67ab	2.00b
BAP2IAA1	0.00b	0.00b	0.00b
BAP2NAA1	0.00b	0.00b	0.00b
KIN1NAA1	0.00b	0.00b	0.00b
KIN2	0.33b	0.33b	0.67b
Control	0.00b	0.00b	0.00b
S.E	0.07	0.13	0.18

Means in each column followed by the same letters are not significantly different at p=0.05.

The number of shoots, nodes and leaves were highest in medium containing only 1.5 mg/l BAP with mean values of 4, 6. 7 and 9. 0 respectively; although as high as 21 shoots were recorded in some cultures (Figure 1). This was followed by medium containing double the latter concentration of BAP while the remaining 9 media did

Figure 1. Multiple shoot formation from stem explants of *Telfairia occidentalis* cultured in Murashige and Skoog medium containing 1.5mg/l of benzylaminopurine

not differ significantly. Thus, a 4 - 21 times multiplication rate can be achieved on a regular 4 month micro-propagation cycle. BAP has been reported to enhance multiple shoot formation from apical shoot bud of water melon but reduced shoot elongation (Adelberg et al., 1997). Research is on-going to perfect the protocol for rooting of shoots and transplanting to the field. The use of seedlings produced via micro propagation as propagules will also increase the quantity of seeds available for consumption.

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