

Short Communication

***In vitro* culture of *Telfairia occidentalis* under different cytokinins and auxin combination**

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***Telfairia occidentalis* is a tropical vine and has been a good source of iron rich vegetable to man. It is normally propagated through seeds but the seeds are recalcitrant in nature. The vegetative propagation of *T. occidentalis* has been difficult hence there is a need to develop an *in vitro* method. Nodal cuttings of *T. occidentalis* was cultured on Murashige and Skoog (MS; 1962) basal media supplemented with IBA, IAA, NAA, BAP, and kinetin at different concentrations. Among all the growth hormones used, IBA and BAP combination gave the best result for both rooting and shooting while BAP in combination with NAA showed the highest number of nodes. The result showed that *in vitro* growth of *T. occidentalis* is hormone specific.**

Key words: *Telfairia occidentalis*, hormones, IBA, IAA, NAA, BAP, kinetin *in vitro*.

INTRODUCTION

Telfaria Occidentalis (fluted pumpkin) is a tropical vegetable grown in West African. It is widely consumed in tropical regions (Fagbemi et al., 2005). The seed is rich in protein. The largest diversity in plant populations can currently be found in Imo state in South-Eastern Nigeria when it is commonly grown. *T. occidentalis* is a dioecious plant although occasional monoecious plants have been observed (Akoroda, 1990). The female plant produces big leaves and seed for future planting while the leaf of the male is small. However the female plant is preferred with respect to leaf and fruit production (Akoroda, 1990). The vegetable is usually propagated through seed and the seed are recalcitrant hence will fail to germinate when kept dry.

In other to solve the problem of limited seeds available to produce the many seedlings required, Esiaba (1982) developed a technique where the germinated seeds with young seedlings (approximately 7 cm long) are carefully

split into two cotyledon parts, each cotyledon with one or two plants. Therefore, from one seed up to four seedlings can this be obtained, but only about two are likely to survive, thus enabling a farmer to double his or her plant population. Akoroda (1990) also found that seeds germinate better and faster in sawdust than in soil or sand (7 days instead of 10 -14 days). Such seedlings also split more easily than those grown in the field.

Fluted pumpkin has several production problems to which biotechnology can provide solutions. The natural diversity or genetic base is not very wide and available morphotypes have not been characterized. Molecular characterization would provide solution for establishment of genetic relationship among fluted pumpkin germplasm. Molecular markers like RFLP, RAPD, AFLP and micro satellites are potent tools in this direction. Similarly, sex-linked markers would help distinguish female plants from male ones at early stage of growth. New plants can be obtained through somatic embryogenesis from pedicels; stem, leaves roots and other explants and this can solve the problem of recalcitrant seed. This technique can make possible the production of artificial seeds in *T. occidentalis*. Seeds/fruits for consumption purposes, with stronger, more vigorous shoots and which are tolerant to water stress and resistant to *T. occidentalis* mosaic virus (Opabode and Adeyooye, 2005).

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Abbreviations: NAA, Naphthalene acetic acid; BAP, benzylaminopurine; IBA, indolebutyric acid; and IAA, indole-3-acetic acid.

Table 1. Response of *T. occidentalis* on MS basal medium supplemented with cytokinins and auxins.

Explants	Medium (mg/L)	No of nodes	No of roots
Nodal cutting	BAP 0.05 + NAA 0.02	6	-
	BAP 0.05 + NAA 0.03	4	-
	BAP 0.05 + NAA 0.1	7	-
	BAP 0.05 + IBA 0.1	3	2
	BAP 0.05 + IBA 0.02	5	1
	BAP 0.05 + IAA 0.01	1	-
	BAP 0.05 + NAA 0.01	2	-
	Kinetin 0.5+ NAA 0.01	2	- (Premature senescence)
	Kinetin 0.5 alone	2	- (Premature senescence)
	MS alone	-	- (Callus)

MATERIALS AND METHODS

The experiment was carried out at the tissue culture laboratory of National Center for Genetic Resources and Biotechnology in Ibadan, Nigeria. Seeds were extracted from ripened, mature fruits of *T. occidentalis*, which were collected from National Center for Genetic Resources and Biotechnology in Ibadan, Nigeria. The extracted seeds were planted in the potted soil in the screen house. The explant (that is) nodes were cut from the seedlings four weeks after planting.

Nodal explants were obtained from 4 - week's old seedlings grown aseptically. The explants were surface-sterilized in 15% NaOCl + 2 drops of Tween 20 per 100 ml for 25 min. They were then cut with a sharp sterile knife into single node cuttings. Three or four explants from a seedling were cultured on the prepared medium to which either naphthalene acetic acid (NAA) or benzyl-aminopurine (BAP), indolebutyric acid (IBA), indole-3-acetic acid (IAA) and kinetin had been added. Different concentrations were investigated for each of the auxins and cytokinins. The basal medium used comprised of Murashige and Skoog (MS) (1962) macro and micro-elements, vitamins (Nitsch and Nitsch, 1965), 3% sucrose, 10 mg/L ascorbic acid, 0.1 g/L myo-inositol, and 0.02 g/L cysteine. Cultures were incubated in the dark at $25 \pm 2^\circ\text{C}$ for duration of six weeks for shooting and rooting induction. The number of roots and nodes were counted and recorded on the sixth week.

RESULTS AND DISCUSSION

Among all the growth hormones used, IBA (0.05 mg/L) + BAP (0.01 mg/L) combination gave the best result for both rooting and shooting while the highest number of nodes was observed in BAP (0.05 mg/L) + NAA (0.01 mg/L). The application of kinetin both in combination with NAA and alone resulted in premature senescence with lower number of nodes. This is in agreement to the findings of Balogun et al. (2002) who showed that kinetin is not a suitable hormone for regeneration of *Telfairia* especially if it will be kept *in vitro* for a long time. However BAP (0.05 mg/L) + IAA (0.01 mg/L) combination resulted in lowest number of nodes and MS alone produced callus without regenerating into a plantlet (Table 1). The result shows that *in vitro* growth of *T. occidentalis* is hormone specific.

In vitro propagation of *T. occidentalis* should be encouraged to provide pathogen-free starting material for genetic improvements and cultivar breeding. The conservation of *T. occidentalis in vitro* is a solution against genetic erosion of the crop genetic base. Biotechnologies to induce and express genetic variability like pollen and ovary culture, anther and microspore culture, meristem culture, somatic embryogenesis, somaclonal variation and embryo culture could produce cultivars with a higher proportion of female to male plants, capable of producing more seeds/fruits for consumption purposes, with stronger, more vigorous shoots and which are tolerant to water stress and resistant to *T. occidentalis* mosaic virus.

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