Journal of Applied Biosciences 49: 3415– 3424

ISSN 1997–5902

Effect of phytohormones and genotype on meristem and shoot tip culture of *Telfairia occidentalis* Hook F.

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Originally Submitted on 19th November 2011. Published online at www.m.elewa.org on January 29, 2012.

ABSTRACT

Objectives: The aim of this work was to investigate the effect of plant growth regulators (PGR), genotype and explant type on *in vitro* shoot induction, elongation, and multiplication in *Telfairia occidentalis*.

Methodology and Results: In this study attempts were made to induce shoots from meristem and shoot tip culture of *Telfairia occidentalis* Hook F. Shoot apical meristems with 1-2 leaf primordia and shoot tip explants were aseptically isolated and cultured on ½ N6 medium supplemented with different combinations of 2mg/l BAP, IAA and kinetin. Explants were derived from seedlings of two *T. occidentalis* genotypes grown in sterilized soils inside vials in the laboratory. Regeneration response were examined based on five parameters – callus formation, shoot length, number of shoots per explant, number of leaves per explant and number of nodes. Shoot induction, elongation and multiplication were most effectively promoted by the medium supplemented with 2.0 mg/lBAP + 2.0 mg/l IAA for shoot tip culture, while 2.0 mg/l BAP was most effective for shoot regeneration from meristem culture. Genotype I was significantly higher than Genotype II in shoot bud regeneration response ($p<0.05$). Culture of explants from shoot tip led to better shoot regeneration in comparison to explants from meristem. Callus formation/induction was also influenced by explants and media interaction.

Conclusion and Application of Findings: In vitro shoot induction from meristem and shoot tip culture of T. *occidentalis* has been demonstrated. These *in vitro* culture procedures would be useful for developing uniform clones or micropropagation and could also form the basis for *in vitro* storage of explants and subsequent regeneration of plantlets after long term conservation in this species. The recalcitrant nature its seeds makes alternative means of genetic resources conservation very necessary. More importantly, meristem culture technique is useful for developing virus-free clones and avoids the limitations imposed by conventional mode of planting. Calli produced could also be excellent targets for genetic transformation and improvement of this species.

Key words: *Telfairia occidentalis*, shoot tip, Meristem culture, in vitro, multiple shoot, Virus elimination, Indigenous leafy vegetable.

INTRODUCTION

Telfairia occidentalis Hook F., a tropical plant, belonging to the family Cucurbitaceae, is an important leaf and seed vegetable and a local medicinal plant. It is naturally found in the humid

part of West African countries like Nigeria, Cameroon and the Republic of Benin. It is well known for its high nutritional, medicinal and economic potentials and widely cultivated in the Eastern part of, Nigeria. The plant is dioecious, however, monoecious ones have also been observed. The female plant is preferred by farmers to the male plant because it produces seeds for later planting (Akoroda, 1990).

Telfairia occidentalis has many economic values. The leaves and stems are succulent and tasty. This makes it the most popular vegetable to millions of people, ranking as one of the three most widely eaten vegetable at homes and in restaurants across Nigeria. The seeds are nutritious, widely consumed in Nigeria and are processed into seasonings, high-protein cake, marmalade, infant weaning foods, flour bread supplement and different local fermented foods. They are also a good source of edible oil. However, immature seeds are preferred to mature ones when eaten cooked or roasted because anti-nutrient characteristics increase with maturity (Abiose, 1999).

Moreover this species has been reported to have several medicinal and health benefits. In rats fed on *Telfairia occidentalis*-supplemented diet, a significant increase in weight and reduced oxidative brain damage was reported (Iweala and Obidoa, 2009; Kayode *et al,* 2010). It has been established that the plant is useful in the treatment or management of anaemia (Ajayi *et al,* 2000) and diabetes (Eseyin *et al,* 2007). The seeds, believed to have lactation-promoting properties are in high demand by nursing mothers (Schippers, 2000). The roots have high alkaloid content and their extracts are therefore used for controlling pest and rodents (Akubue *et al,* 1980; Ajibesin *et al,* 2002). The ability of the plant to combat certain diseases may be due to its antioxidant and antimicrobial properties and its minerals (especially iron), vitamins (especially vitamins A and C) and high protein contents (Kayode *et al,* 2009; Kayode and Kayode, 2010). Leaves of *T. occidentalis* can be beneficially used in heart diseases, hypertension, hypoglycemia, diabetes and even in fatal cases of meningitis. They have been effectively used in lowering blood cholesterol, increasing heamoglobin and preventing blood clothing (Iweala and Obidoa, 2009).

One major constraint to the production of this species is that it can only be propagated by seeds. Unfortunately these seeds are recalcitrant, that is, they cannot be stored for long period because they are sensitive to both desiccation and chilling. Another constraint is that it is beset with bacterial, fungal and several viral diseases. The plant is often infected by *Telfairia* Mosaic Virus (TeMV), and to a lesser extent, the Pepper Veinal Mottle Potyvirus (PVMV) and the T-strain of Cucumber Mosaic Virus (CMV) which are seed borne and are therefore transmitted from generation to generation by mere planting. (Anno-Nyako, 1988). Another constraint is that there are separate male and female plants, such that the sex cannot be known until after flowering (Schippers, 2000). Although these constraints had been noted long ago, they have persisted because little attention had been paid to propagation and conservation problems, compared to the utilization of fluted pumpkin. This is largely due to lack of expertise and information on handling the equivocally recalcitrant seeds of fluted pumpkin (Odiaka and Schippers, 2004).

Micropropagation through meristem culture has proved to be the most generally applicable method of *in vitro* propagation, having the advantage of developing virus-free clonal stocks. It is believed that diseases are transferred through vascular bundles and since meristems lack vascular bundles, they are expected to be free of disease when cultured. Shoot tip has the advantage of being able to regenerate plantlets faster than meristem because it is larger in size. However, it is not able to produce plantlets that are free from viral attack. This research is aimed at investigating the effect of plant growth regulators (PGR) and the response of two genotypes of *Telfairia occidentalis* on *in vitro* shoot induction, elongation, and multiplication using meristem and shoot tip explants of *Telfairia occidentalis*.

MATERIALS AND METHODS

Plant Materials: This research was carried out in the Tissue Culture Laboratory of the Institute of Agricultural Research and Training, Nigeria between February and July, 2010. Two mature fruits of *Telfairia occidentalis* of different genotypes were purchased from the market at Ojoo in Ibadan and brought to the Laboratory. They were maintained under standard laboratory conditions.

Explants preparation and surface sterilization: Each pod was cut open and washed in water to remove the protective cover, the pulp. The seeds were placed in a solution of 70% ethanol for 5minutes, rinsed in sterile distilled water and then placed in a solution of 35% commercial bleach for 20minutes. Thereafter they were immersed in a solution of 17% commercial bleach for 10minutes and then rinsed thoroughly with several changes of distilled water. Each seed was planted in previously prepared sterile soil contained in vials; the soils were watered adequately to support sprouting and germination. The vials were properly covered with their lids sealed with paraffin nylon and labeled accordingly, then kept inside the laboratory for germination. This procedure was carried out inside the lamina air flow hood. Five weeks after planting, the plantlets were ready to be harvested for shoot tip and meristem culture.

Plant Growth Regulators: The basic nutrients solution of ½ N6 (Modified by Welander, 1988) was used as the basal medium and was supplemented with different combinations of plant growth hormones and a control medium having no plant growth hormones. These different combinations represent the treatments for the two genotypes. The combinations are as follows;

- Benzylaminopurine (BAP) 2mg/litre.
- Benzylaminopurine (BAP) 2mg/litre + Idole acetic acid (IAA) 2mg/litre.
- Benzylaminopurine (BAP) 2mg/litre+ Kinetin (Kin) 2mg/l.
- Kinetin 2mg/litre.
- Control: no plant growth hormones.

Culture media: The basic nutrients solution of ½ N6 (Modified by Welander, 1988) medium was supplemented with 15g sucrose, 0.025g myo-inositol per litre, pH of the medium used was adjusted to 5.7 using a pH meter with NaOH and HCl. Agar was added as gelling agent to the medium at a concentration of 3.5 g/l before the medium was sterilized at 121^{o}C for 15 min. At the end of the sterilization, the medium was allowed to cool. It was aseptically poured into sterile test tubes in the laminar flow hood and allowed to set for 24 h.

Experiment I: Shoot induction, Elongation and Multiplication via Shoot tip Culture: Shoot tips of between 5.0- 8.0mm were aseptically excised from the apex of the 5 weeks growing vine of fluted pumpkin and were placed on test tubes containing approximately 10ml of medium (½ N6) supplemented with the different plant growth regulators stated above.

Experiment II: Shoot Induction via Meristem Culture: Five weeks after planting, shoot apical meristems consisting of the apical dome with one or two leaf primordia were isolated using sterile hypodermic needle and scalpel under a dissecting microscope (Olympus) as described by (Alam *et al,* 2004). Isolated meristems (0.3-0.5 mm) were transferred quickly into test tubes containing 10ml sterilized ½ N6 medium supplemented with different plant growth regulators stated above. Only plantlets that are not contaminated were used for *in vitro* culture. These same experiments were carried out on the second genotype of the fluted pumpkin.

Data Collection and Statistical Analysis: Experiments were set up in a completely randomized design under factorial arrangement. For regeneration experiments at least fifteen test tubes were used for each treatment. Callus formation, shoot length, number of shoots formed per explant, number of leaves formed per explant and number of nodes formed per explants were recorded. Data were analyzed using the statistical software SAS. Analysis of variance (ANOVA) was used to test the statistical significance, and the significance of differences among means was carried out using (Duncan,1955) multiple range tests at $p< 0.05$.

RESULTS

Effect of Genotypes on Shoot Induction/Regeneration: The two genotypes used in this work showed different regeneration response although regeneration of shoots occurred in both of them. Independently, the medium (PGR) and explants type used, when only the genotypes are considered, Genotype I presented on the average significantly higher performance than Genotype II (Figure 1). A close comparison between both genotypes even if all factors are put into consideration also revealed that the culture of explants from Genotype I showed higher mean number of shoots per explants (1.0), number of nodes (1.5) and percentage of explants producing shoot than Genotype II in most cases (not illustrated).

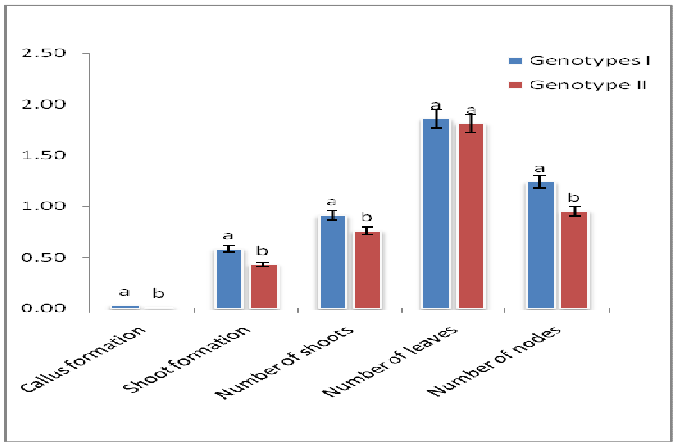

Figure1: Effects of Genotypes on regeneration parameters. Y- axis represents mean values (in numbers) of regeneration parameters *Means with the same letter are not significantly different at p<0.05

Effect of Explant Type: The response of the two different types and sizes of the explants used in this study for shoot induction, elongation and multiplication varied greatly (Figure 2). Average number of leaves was 3.0 for shoot tip explants and 0.5 for meristem explants. Shoot tip explants initiated shoots (Plate 1A) out of which some were further elongated, some to a considerable length of 6cm (Plate 1B) while others produced multiple shoots (Plate 1C). The meristem explants initiated shoots also but as at the 9 weeks after culture initiation these shoots had not elongated beyond 1.0cm (Plate 2A). Overall performance of shoot tip explants was significantly higher than meristem explants (P<0.05).

Effect of Plant Growth Regulators (PGR) on Shoot tip Culture: For shoot induction/initiation, elongation and multiplication, ½ N6 medium supplemented with different combinations of PGR at 2.0mg/l were used. Shoot tip explants cultured in all the media formulations induced shoots. Explants in medium supplemented with BAP2.0mg/l+ IAA2.0mg/l developed multiple shoots (1.6) (Table 1, Figure 5). Table 1 also shows that the effect of BAP and KIN alone were similar to the control as shoots were induced. However Among the different hormonal combinations, BAP2.0mg/l+ IAA2.0mg/l were found best and the result was significantly higher than that of BAP2.0mg/l which had the least response (0.9) (Figure 3). The control medium induced shoots and tiny roots also (Plate 1D). Figure 5 also shows that Kinetin alone gave the best response in terms of shoot number for meristem explants although they were not significantly different from other hormonal treatments.

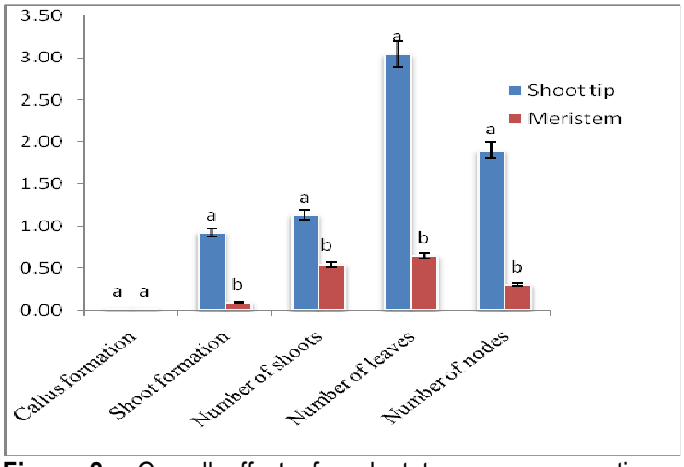

Figure 2: Overall effect of explant type on regeneration parameters. Y- axis represents the mean values of regeneration parameters.*Means with the same letter are not significantly different at p<0.05.

Effect of PGRs on Shoot Multiplication: The *in vitro* multiplication of shoots was strongly influenced by the cytokinin employed (i.e. BAP). ½ N6 medium supplemented with 2mg/lBAP and 2mg/l IAA in combination showed a better efficiency of shoot proliferation and multiplication (Table1). A maximum of 8 new shoots (multiple shoots) were developed from a single explant cultured in the medium containing BAP+IAA (Plate1C), while others cultured in other media either formed less number of shoots or none at all.

Effect of PGR on Meristem Culture: Initial growth of cultured meristem started within 10- 16 days as evidenced by increase in size. All treatments induced shoots from explants and leaf formation was also seen in all treatments including the control. However, explants cultured in BAP+IAA and BAP+Kin supplemented medium formed calli as well. (Table 2). Growth continued rather slowly with the development of tiny leaves (Plate 2A.). ½ N6 supplemented with BAP2.0mg/l showed the highest response in all parameters measured (Figure 4). A high degree of callus formation instead of shoot formation was observed in the medium supplemented with BAP2.0mg/l+IAA2.0mg/l (Plate 2B).

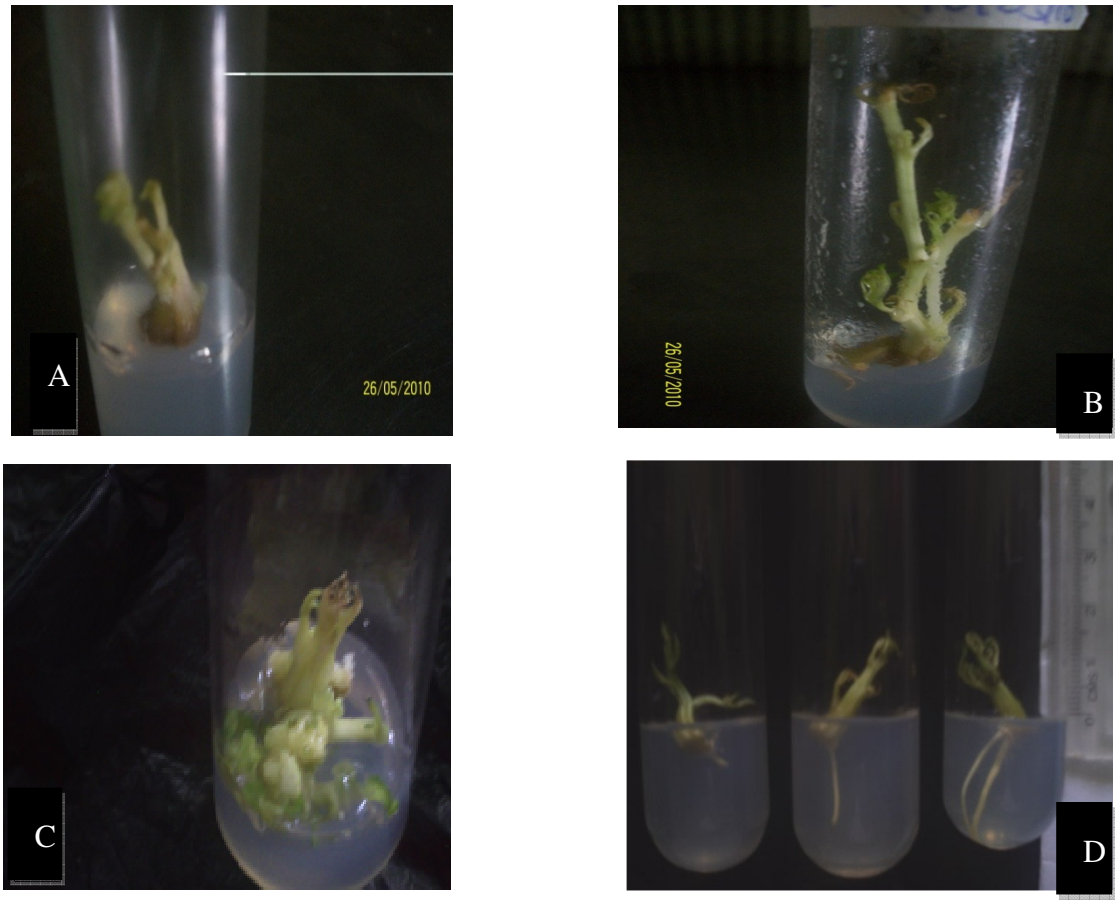

Plate1: Regeneration of *Telfairia occidentalis* in Shoot tip Culture Showing (A), shoot induction, (B), shoot elongation, (C), shoot multiplication, and (D), explants in control medium.

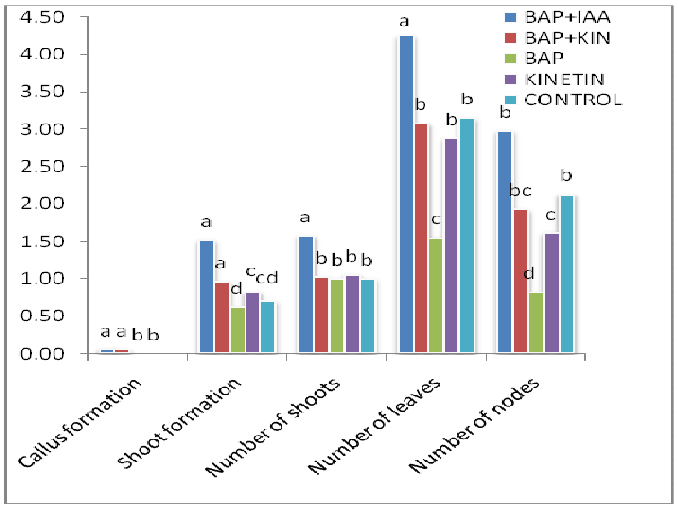

Figure 3: Effect of plant growth regulators (All PGR are at a concentration of 2.0mg/l) on Shoot tip Culture response. Y- axis represents the mean value (in numbers) of regeneration parameters*Means with the same letter are not significantly different at p<0.05.

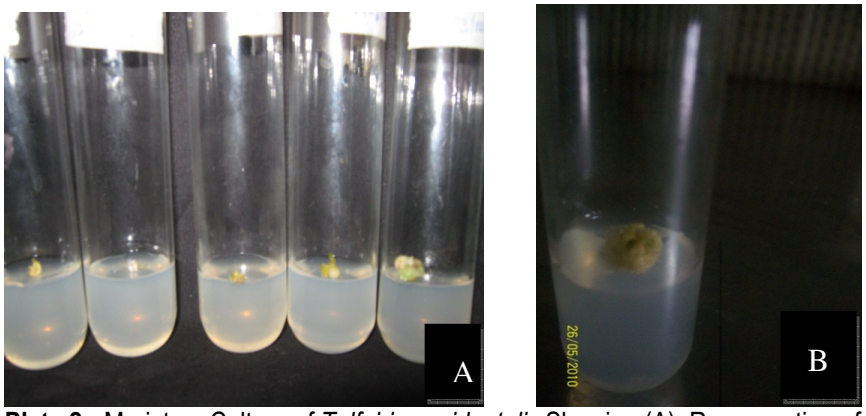

Plate 2: Meristem Culture of *Telfairia occidentalis* Showing (A), Regeneration of meristem culture. (B), callus formation on medium supplemented with BAP+IAA

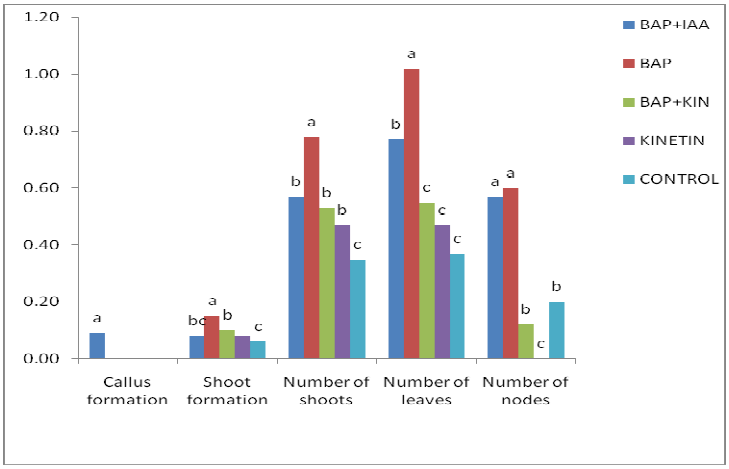

Figure 4: Effect of plant growth regulators (All PGRs are at a concentration of 2.0mg/litre) on Meristem Culture response. Y- axis represents the mean value (in numbers) of regeneration parameters
*Means with the same letter are not significantly different at p<0.05.

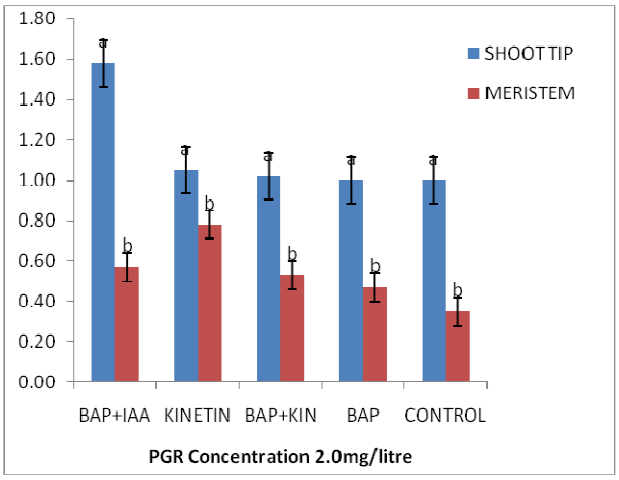

Figure 5: Effect of Plant Growth Regulators on Number of Shoots formed in shoot tip and meristem cultures. Y- axis represents the mean number of shoots.
Values with same letters are not significantly different at $p<0.05$.

Table 1: Effect of plant hormones on shoot induction, leaf and callus formation in Shoot tip culture of *T. occidentalis*

Treatments	Shoot induction	Multiple shoot formation	Callus formation
BAP	+	+	-
KIN	+	+	-
BAP+IAA	+	*+	+
BAP+KIN	+	+	+
Control	+	+	-

+ = organ present, - = organ absent, * = organ formation significantly high ($p< 0.05$)

Table 2: Effect of plant hormones on shoot induction, leaf and callus formation in Meristem Culture of *T. occidentalis*

Treatments	Shoot induction	Leaf formation	Callus formation
BAP	+	+	-
KIN	+	+	-
BAP+IAA	+	+	+
BAP+KIN	+	+	+
Control	+	+	-

+ = organ present, - = organ absent * = organ formation significantly high ($p< 0.05$)

DISCUSSION

Results of shoot tip induction, elongation and multiplication experiments suggest that explants obtained from shoot tips after five weeks of culture did better than those obtained from meristem explants of *Telfairia occidentalis*. It was observed that both explants induced shoots but those from shoot tip developed earlier and responded better than those from meristem. This is one of the advantages shoot tip explants have over those of the meristem, and it is largely due to the fact that shoot tip explants are larger in size than meristem. However, the fact remains that meristem explants have the advantage of producing plantlets that are virus free which however, cannot be guaranteed for plantlets produced from shoot tip or any other part of the parent plant. This is similar to the work of (Nyla *et al,* 2005) who reported that in almost all the

cultivars used for *in vitro* shoot regeneration of tomato (*Lycopersicon esculentum*), a maximum regeneration was observed from shoot tips, while leaf disc showed poor response. Though the use of meristem explants of *Telfairia occidentalis* has not been reported in producing plantlets that are virus free, its use in other plants have been reported by some researchers. (Malaurie *et al,* 1995) studied the influence of meristem-tip size and location on morphological development in *Dioscorea cayenensis - D. rotundata* complex 'Grosse Caille' and one genotype of *D. praehensilis. (*Saeed *et al,* 1997) successfully regenerated complete plantlets from meristem explants of cotton plants. These reports confirm that plantlets could eventually be obtained if shoots induced from meristem culture in this study are elongated and rooted by further experimentation with various concentrations and combinations of auxins, cytokinetins and other growth additives.

In this study, the effect of PGR on shoot induction, elongation and multiplication was investigated. Culture medium has been shown to be a critical factor for induction of organogenesis in plant tissue culture. In the current work we used five different media to induce shoots from *Telfairia* shoot tip and meristem explants. These media, with various combinations of cytokinins and auxin, triggered direct shoot organogenesis from the explants, where the medium supplemented with BAP+IAA proved to be the best in terms of shoot elongation and multiplication. New shoots of about 6cm and up to 8 new shoots were developed from a single explant 9 weeks after inoculation. This is in line with the report of (Thangavel *et al*, 2008), who noted that the *in vitro* shoot multiplication through axillary bud culture of *Talinum portulacifolium* L. were strongly influenced by the cytokinin employed (BAP) in when MS medium was supplemented with 6µM BAP and 2µM indole-acetic acid (IAA). They also reported that maximum of 8 new shoots were developed from a single explant after 3 subcultures. (Xu *et al,* 2008) similarly reported that BAP containing medium had significant positive effect on in vitro shoot regeneration of leafy spurge (*Euphorbia esula*). (Mhatre *et al*. 2000) reported that IAA not only induced roots but also eliminated tufted shoots and calli in *Vitis vinifera*; they also reported that MS medium supplemented with 2µM IAA along with BAP was able to yield maximum number of fresh shoots. (Sai *et al,* 2006), were also able to induce multiple shoot from shoot tip/meristem explants in sorghum. The importance of cytokinin for organogenesis has been studied in many plant species (Pereira *et al,* 2000; Guo *et al,* 2005; Zhang *et al,* 2008), and was considered as an exclusive element for shoot formation. Our results show that in meristem culture, media supplemented with BAP alone had the highest regeneration response while that supplemented with BAP+IAA formed callus. High callus formation from a combination of cytokinin (BAP) and auxin (NAA) in *Telfairia occidentalis* has also been reported (Balogun *et al,* 2007). They have also reported that basal medium supplemented with BAP produced better regeneration response from nodal explants of *Telfairia occidentalis* cultured *in vitro.*

The production of callus or direct organogenesis could be due to the cytokinin-auxin balance as the cytokinin-auxin balance strongly promotes organogenesis and development of callus tissues. (Marks and Simpson, 1994) suggested that callus formation may be due to the action of accumulated auxins at the basal cut ends which stimulates cell proliferation, especially in the presence of cytokines. This hypothesis seems to hold true for *G. africanum*, where callus was initiated with a combination of a cytokine and an auxin. (Preece *et al,* 1991) also reported that formation of callus at the basal cut explant on cytokines-enriched medium is frequent in species with strong apical dominance.

In comparison, results obtained from the two genotypes used in this work revealed that there were genotypic variations in response of the explants to growth hormones. For most of the parameters measured, Genotype I had significantly higher values than Genotype II. A similar type of comparison for genotype and explant type selection was also reported earlier (Llorentea and Apóstoloa 1998; Gubis *et al,* 2003 ;). They found considerable differences among clones in response to growth regulators at all stages of *in vitro* propagation in the effect of different growth regulators and genotype on *in vitro* propagation of jojoba. Significant differences among clones were observed in wax quality and quantity and in seed yield, in salt tolerance (Mills and Benzioni 1992), in range of chill requirement (Dunstone, 1996).

In conclusion, this present study shows regeneration of *Telfairia occidentalis* through *in vitro* shoot and meristem explants was successfully achieved, although the efficiency was affected by different factors. However, shoot multiplication was minimal but occurred most in medium supplemented with BAP+IAA. Shoot tip explants responded better than meristem explants, this was expected because they are larger in size. Explants from genotype I responded better than those of

genotype II in most of the culture media. The *in vitro* protocol reported in this study could be used for clonal *in vitro* propagation of *Telfairia occidentalis* through either slow growth or cryopreservation and to obtain competent target tissue for genetic modification and transformation.

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