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Effect of growth regulators and explant types on callus induction in *Telfairia occidentalis* Hook F

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Different concentrations of growth regulators and three types of explants were investigated for their efficiency on callus induction in *Telfairia occidentalis* with a view of providing baseline information for the development of a callus initiation protocol. Three concentrations of kinetin (KN) (0.1, 3.3 and 5.0 mg/L) in combination with two concentrations of 2,4-dichlorophenoxy acetic acid (2,4-D) (2.0 and 5.0 mg/L) and two concentrations of naphthalene acetic acid (NAA) (0.25 and 0.5 mg/L) in combination with 0.25 mg/L benzyl adenine (BA) were tested for their effect on callus induction from stem, leaf and nodal explants collected from field-grown *Telfairia* plants. Media supplemented with 2,4-D in combination with kinetin gave the highest cumulative percent callus induction. With regards to cumulative percentage callus induction and total callus produced, media supplemented with BA alone was better than media supplemented with kinetin alone. Irrespective of the growth regulator type, percent callus induction was not significantly different among explant types. The study concluded that, 2,4-D is a better auxin for high callus induction in *T. occidentalis* explants as compared to NAA. However, there is still a need to test the effect of 2,4-D in combination with BA on callus induction.

Key words: *Telfairia occidentalis,* callus initiation, 2,4-D, BA, kinetin.

INTRODUCTION

Telfairia occidentalis Hook F. commonly called fluted pumpkin is one of the native vegetables of Nigeria and found in the moist coastal areas of West Africa (Ajayi et al., 2007; Odiaka et al., 2008). It belongs to the family Cucurbitaceae. The edible leaves and young shoots have a high nutritional and medicinal value. The leaves are rich in protein (29%), fat (18%) as well as minerals and vitamins (20%) (Akanbi et al., 2007). The leaves are also rich in iron as a result of which fresh leaf concoction is used as a health tonic for the treatment of anaemia (Akoroda, 1990; Schippers, 2000). Other parts of the plant have several important uses in the production of marmalade,

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Abbreviations: 2,4-D, 2, 4-Dichlorophenoxy acetic acid; **BA,** benzyl adenine; **NAA,** naphthalene acetic acid; **KN,** kinetin.

fodder (Egbekun et al., 1998; Odiaka and Schippers, 2004); food, fats and oil for soap making, cooking, margarine production, and also in production of drying oil for paints and varnishes (Badifu and Ogunsua, 1991; Giami and Isichei, 1999; Akwaowo et al., 2000; Fashina et al., 2002; Steinkraus, 2002; Giami et al., 2003; Agatemor, 2006; Akanbi et al., 2007) among other uses. Many biological constraints have become potent threats to the existence of *T. occidentalis* necessitating an urgent need to collect and conserve the existing narrow genetic diversity (Ajayi et al., 2006b). However, conservation by seed storage in spite of itsrelatively low cost is impossible because the seed is recalcitrant (seeds lose viability due to desiccation when stored for a considerable length of time) (Odiaka and Schippers, 2004; Ajayi et al., 2006b). The availability of seeds for planting is also limited by its recalcitrance and the fact that seeds are utilized for a variety of purposes.

Micropropagation, the propagation of plants *in vitro*, is an alternative means of vegetative propagation. According to Debeaujon and Branchard (1993), strategies based on the application of biotechnologies to crop improvement programs generally require regeneration of whole plants from cells or tissues cultivated *in vitro*. In order to maximize the potential benefits of micropropagation, protocols have to be developed for different plants and often times explants from different parts of the same plant to suit their peculiar needs. According to Pierik (1999), there are great differences in cell division and regenerative capacity between plants even within a single species.

Earlier attempts on tissue culture and micropropagation of *T. occidentalis* were carried out using shoot tips, nodal cuttings and roots (Balogun et al., 2002; Ajayi et al., 2006a; Balogun et al., 2007; Sanusi et al., 2008) but the possibility of somatic embryogenesis has not been explored. As a first step towards this, callus initiation protocol has to be in place for the supply of callus for induction of somatic embryos. The present study was initiated to investigate the influence of different concentrations of auxins and cytokinins on callus induction from different explants.

MATERIALS AND METHODS

Fruits of *T. occidentalis* landraces were collected from the area around Ile-Ife, Osun state, Nigeria (Latitude: 7° 467' 0 N, Longitude: 4° 567 ' 0 E) and plants were raised in nursery. Leaf, stem and nodal explants were obtained from nursery grown *Telfairia* plants. They were washed under running tap water to remove dirt and reduce microbial load. The explants were then surface sterilized for 10 min with 10% (v/v) sodium hypochlorite solution to which two drops of Tween 20 was added as a surfactant. The explants were then rinsed three times in sterile distilled water. The leaves were thereafter trimmed into pieces of about 2 cm^2 and the stems were cut into about 2 cm long pieces before inoculation. Five replicates of leaf, nodal and stem explants were used per treatment. All protocols were carried out under the laminar airflow chamber.

Murashige and Skoog's (1962) (MS) medium supplemented with 3% (w/v) sucrose was used as the basal medium in all the experiments. The media was solidified with 0.8% (w/v) agar and the pH adjusted to 5.7 ± 0.1. The media was dispensed into McCartney bottles which were sealed with cotton wool and thereafter wrapped with aluminum foil before autoclaving at 121°C and 15 lb/in^2 for 15 min. Cultures were maintained at 25°C ± 2°C in the dark. The following concentrations of growth regulators were tested for their effect on callus initiation and embryogenic callus induction from stem, leaf and nodal explants: 5 mg/L 2,4-D + 3.3 mg/L kinetin; 2 mg/L 2,4-D + 5 mg/L kinetin; 5 mg/L 2,4-D + 0.1 mg/L kinetin; 0.25 mg/L BA + 0.25 mg/L NAA; 0.25 mg/L BA + 0.5 mg/L NAA; 0.5 mg/L BA; 0.5 mg/L kinetin.

The cultures were monitored weekly for the effect of each treatment on the induction of callus or organogenesis. The explants were visually observed for the presence and type of callus (Remotti and Loffler, 1995; Mencuccini and Rugini, 1993). Responses of explants to callus induction were expressed as percentages (%) of induced explants. Where applicable data collected were subjected to analysis of variance and means were separated with Duncan's multiple range test (DMRT), using system analysis software (SAS) version 9.2.

RESULTS

Callus was induced by all the growth regulator treatments. However, not all explants produced callus on the different growth regulator treatments. For example, callus was not induced on leaf explants inoculated on media supplemented with 5.0 mg/L 2,4-D and 3.3 mg/L kinetin but, there was 60% callus induction on leaf explants inoculated on media supplemented with 2.0 mg/L 2,4-D in combination with 5.0 mg/L kinetin (Figure 1A). Percentage callus induction of stem explants was highest on media supplemented with 0.25 mg/L BA and 0.25 mg/L NAA.

For nodal explants, however, the highest percentage callus induction occurred on media supplemented with 5.0 mg/L 2,4-D and 3.3 mg/L kinetin. Leaf explants produced highest percentage callus induction on media supplemented with 2.0 mg/L 2,4-D in combination with 5.0 mg/L kinetin and on media supplemented with 5.0 mg/L 2,4-D in combination with 0.1 mg/L kinetin (Figure 1A).

Stem and leaf explants generated biggest callus size on media supplemented with 5.0 mg/L 2,4-D and 0.1 mg/L kinetin. Nodal explants on the other hand, generated the largest callus on media supplemented with 2.0 mg/L 2,4-D and 5.0 mg/L kinetin (Figure 1B). Averaged over all treatments, media supplemented with 5.0 mg/L 2,4-D in combination with 0.1 mg/L kinetin gave the best results for callus induction and size of callus (Figure 2A and B).

Stem and leaf explants generated more callus than nodal explants (Figure 3). With regards to growth regulator type, 2,4-D in combination with kinetin was more effective than BA in combination with NAA for callus induction and callogenesis (Figure 4A and B). Effect of different growth regulators and explants on morphology

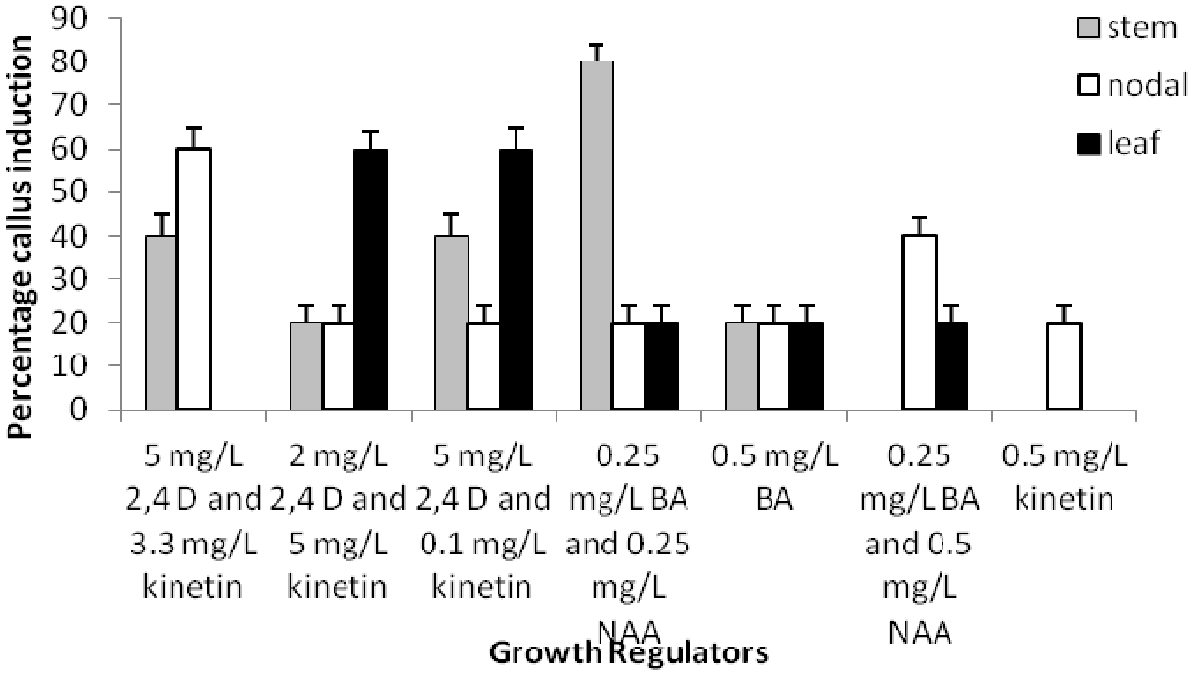

Figure 1A. Effect of different concentrations of growth regulators on percentage callus induction from different explants of *T. occidentalis*.

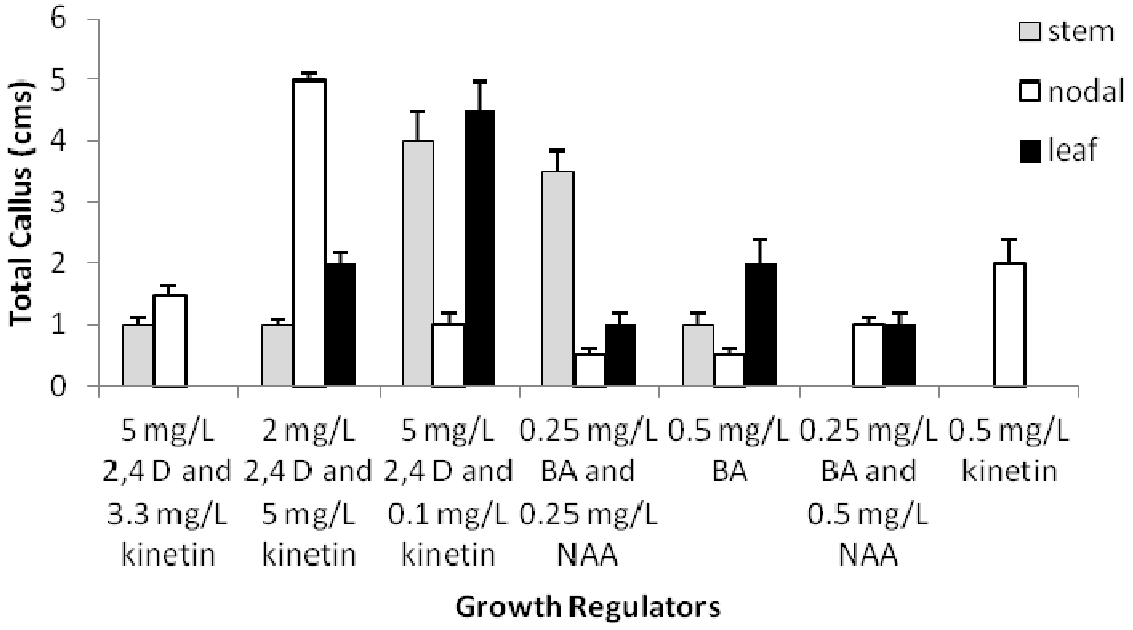

Figure 1B. Effect of different concentrations of growth regulators on total callus produced on different explants of *T. occidentalis*. **Note:** Vertical bars represent mean ± SE.

of callus is presented in Plate 1. The calli induced from leaf were soft, creamy, and nodular both on medium supplemented with 2,4-D + kinetin and BA + NAA; stem calli were cream and loose on medium supplemented with BA + NAA and greenish on 2,4-D + kinetin while calli from nodal explants were white and fuzzy.

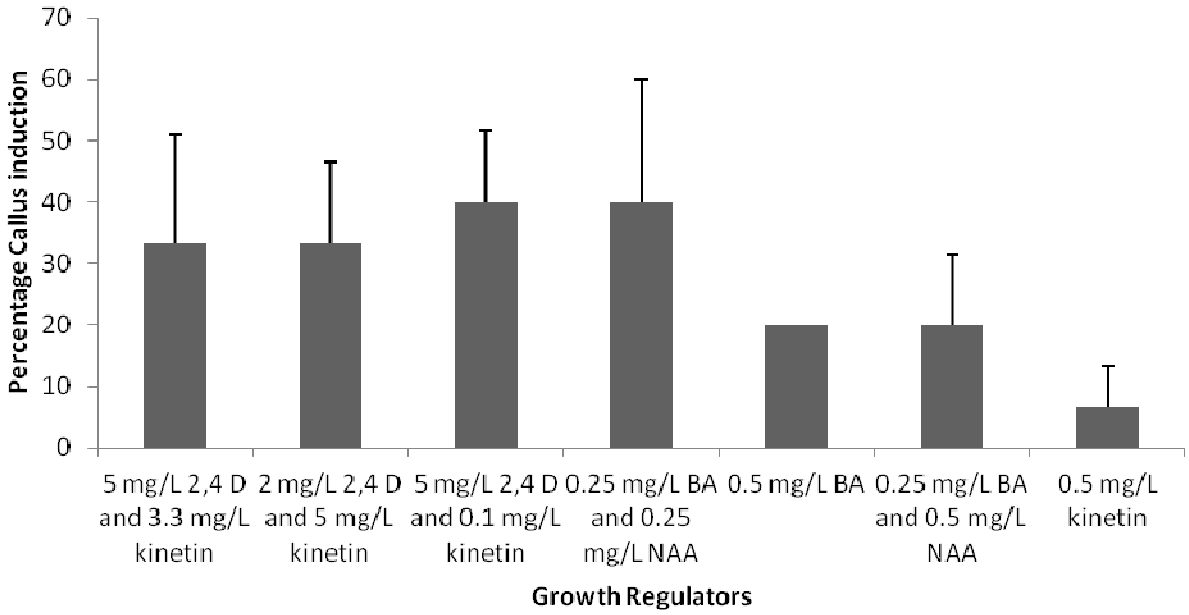

Figure 2A. Effect of different concentrations of growth regulators on percentage callus induction from a combination of all explant types.

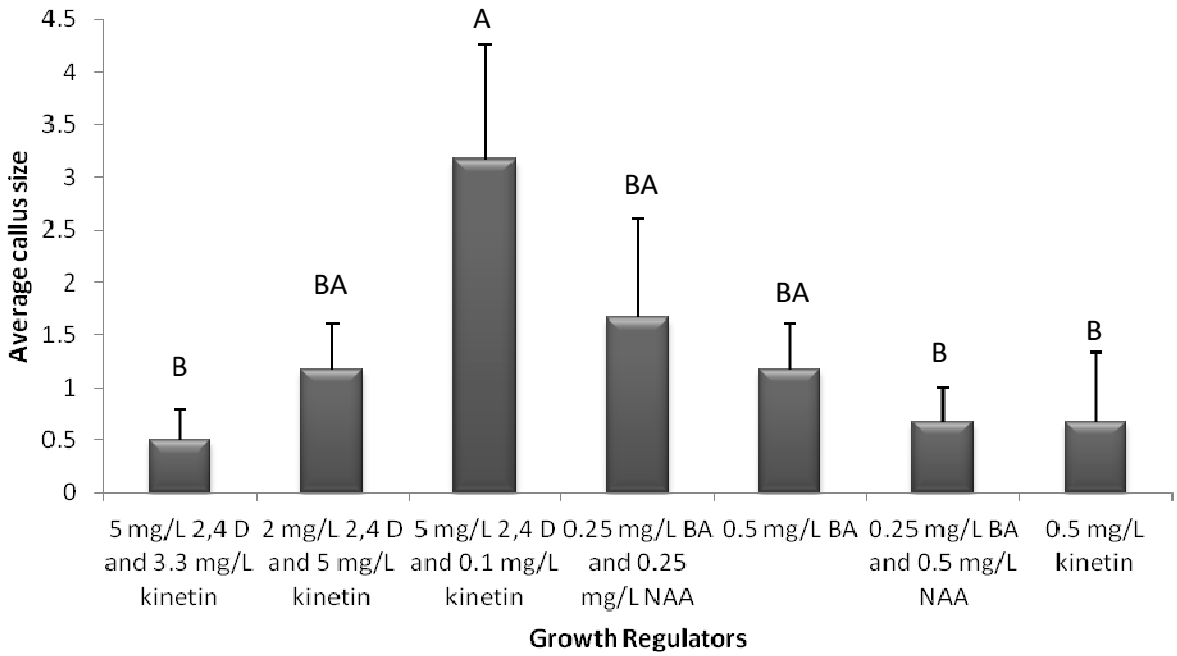

Figure 2B. Effect of different concentrations of growth regulators on callus size from a combination of all explants types. **Note:** Vertical bars represent mean ± SE, and values with the same superscript letters are not significantly different at P< 0.05.

DISCUSSION

For a given growth regulator to be useful, it must not only induce callus but, the degree of callusing must also be appreciable. In this study, highest callus induction and degree of callusing occurred on medium supplemented with 5 mg/L 2,4-D and 0.1 mg/L kinetin.

Liu et al. (2006) reported that auxin 2,4-D by itself or in combination with cytokinins has been widely used to enhance callus induction and maintenance.

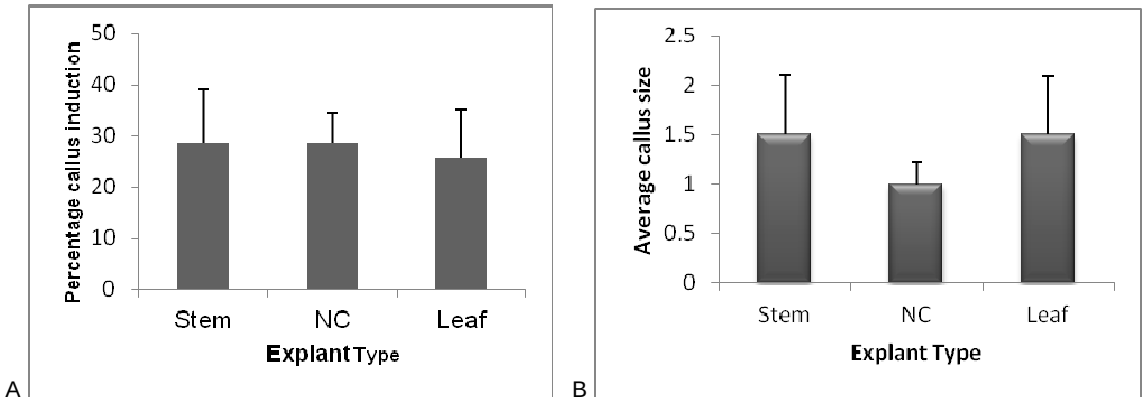

Figure 3. Effect of different explants types on (A) percentage callus induction and (B) callus size. **Note:** Vertical bars represent mean± SE, and the unit of measurement was cm.

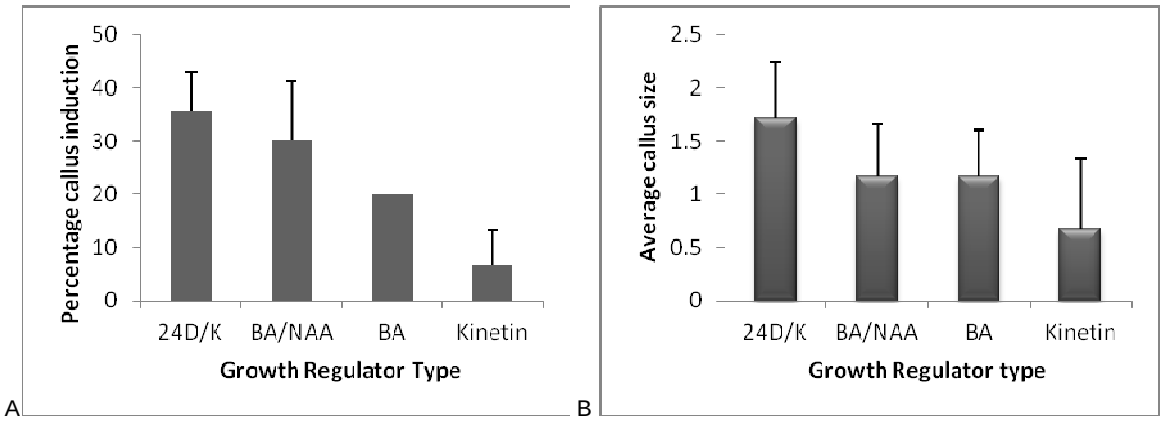

Figure 4. Effect of different growth regulator types on (A) percentage callus induction and (B) callus size. **Note:** Vertical bars represent mean± SE, and the unit of growth regulators was mg/L.

Moreover, many researchers have reported the use of 2,4-D for callus induction in other Cucurbits (Selvaraj et al., 2006; Usman et al., 2011; Thiruvengadam et al., 2012). Overall, among different explants, the highest callus induction was observed on MS medium containing 2,4-D for different explants from cucumber cultivars explored for callus induction in response to different media (Usman et al., 2011). Pal et al. (2007) also reported that for *Cucurbita pepo*, callus was induced on medium containing 2,4-D from both explants utilized, but not on 2,4-D-free medium. Optimum level of callus from *Trichosanthes dioica* was likewise found on medium supplemented with 1.0 mg/l of 2, 4-D (Malek et al., 2010).

Initiation of callus using cytokinin alone has not been promising in this study. Abd Elaleem et al., (2009) working on *Solanum tuberosum* reported that BA alone was not efficient for callus initiation except in combination with 2,4-D. Arivalagan et al. (2012) also reported that when kinetin alone was used as media supplement, callus was not induced.

Cytokinins, such as BA and kinetin, at low concentrations, in combination with auxins have been reported (Chai and Mariam, 1998; Arivalagan et al., 2012) to be frequently used in plant species to promote callus initiation. Efficient callus was induced from leaf and stem explants of *Citrullus colocynthis* on MS medium containing 1.5 mg/l 2 4-D + 1.0 mg/l BAP and 2.0 mg/l 2,4-D + 1.0 mg/l BAP (Savitha et al., 2010). According to Fellner and Lebeda (1998), a combination of growth regulators such as 2,4-D, BAP or Kinetin seems to be necessary for the formation and differentiation of calli from *Cucumis sativus* and *Cucumis melo* explants. Zouzou et al. (2008) also reported that auxin and cytokinin combination is suitable to obtain more vigorous and friable callus and that 0.1 mg/l 2,4-D and 0.5 mg/l kinetin was the proper combination of hormones to induce callus in cotton (*Gossypium hirsutum* L.). Media supplemented with BA alone significantly induced more callus on *T.*

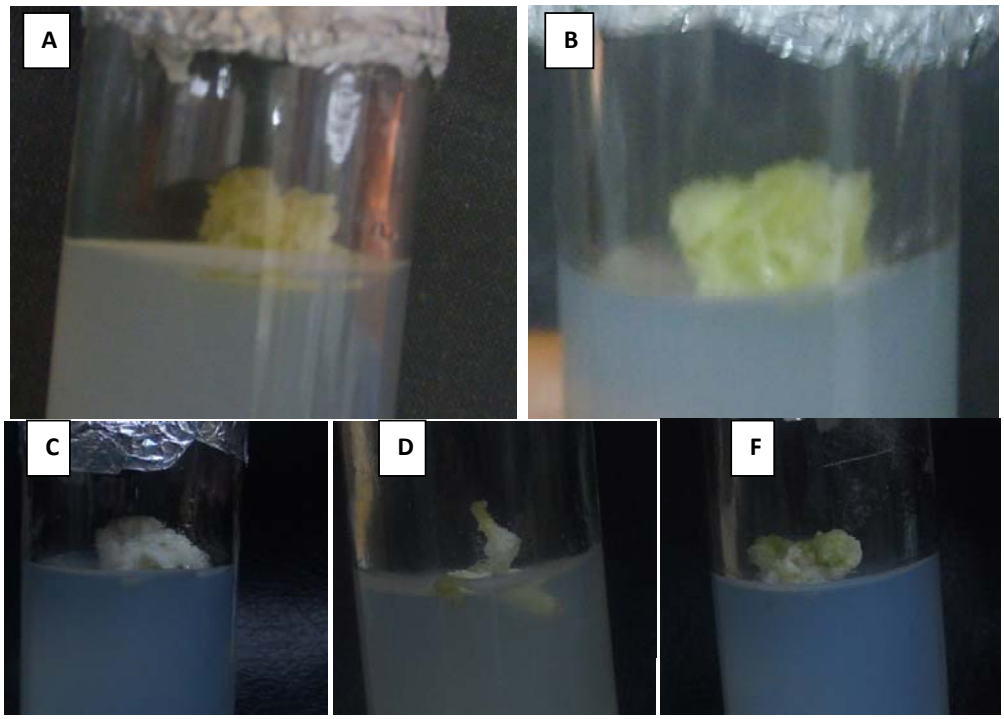

Plate 1. Effect of different concentrations of 2,4-D, Kinetin, NAA and BAP on callus morphology in *T. occidentalis*.
Note: (A) Yellowish callus on stem explant of *T. occidentalis* inoculated on 5 mg/L 2,4-D + 0.1 mg/L kinetin; (B) Yellowish green callus on stem explant of *T. occidentalis* inoculated on 5 mg/L 2,4-D + 0.1 mg/L kinetin; (C) Cream callus on stem explant of *T. occidentalis* inoculated on 0.25 mg/L BA +0.25 mg/L NAA; (D) White callus on nodal explant of *T. occidentalis* inoculated on 0.25 mg/L BA + 0.25 mg/L NAA; (E) Nodular callus on leaf explant of *T. occidentalis* inoculated on 0.5 mg/L BA

occidentalis explants than media supplemented with kinetin alone. However, there was more significant increase in callus induction and proliferation with the addition of kinetin to 2,4-D than with the addition of BA to NAA. This could be due to the resulting ratios of auxin to cytokinins in each situation; the endogenous hormones are present within the explants and the sensitivity of the explants to the hormones. Arivalagan et al. (2012) in their study of the effect of growth hormones on callus induction of *Sauropus androgynous* observed that when auxins alone were used as media supplements, callus was not induced. Introduction of kinetin along with any of the auxins used however, promoted induction of callus.

Equal concentrations of BA and NAA elicited more callus formation than BA in combination with higher concentration of NAA. This finding is supported by the observations of Malek et al. (2010) where he reported that the highest amount of callus from *Trichosanthes dioica* was observed in combination of 0.5 mg/l BAP + 0.5 mg/l NAA when leaf or inter-node explants was cultured in the medium. In this study, low concentration of Kinetin in the medium, in combination with 2,4-D, stimulated the induction and proliferation of callus in *T. occidentalis*. For cotton, low concentration of 2,4-D and high concentration of Kinetin stimulated the proliferation of callus (Zouzou et al., 2008).

The callus formed in *T. occidentalis* was different amongst the explants, with stem and leaf explants generating more callus than nodal explants. These results are in agreement with other results published by several authors who showed that stem (internode) explants are more callogenic (Lou and Kako, 1994; Savitha et al., 2010; Malek et al., 2010). Leaf and internode explants have also been used to induce callus in other cucurbits (Savitha et al., 2010; Malek et al., 2010; Lou and Kako, 1994). According to Zouzou et al. (2008), variation in callus forming ability of different explants types has been reported in many other plants and callogenesis specificity of explants type could be explained by their differential reactivity to media components. The calli color observed in this study were similar to those obtained by Lou and Kako (1994) where they reported that the first leaf- and cotyledon-derived calli of *C. sativus* were yellowish and whitish and the internode-derived callus was larger and greenish.

The results reported in this research work are expected to contribute to the scientific baseline data necessary for the callus initiation of *T. occidentalis*; a *sine qua non* for

its somatic embryogenesis and conservation. Pumpkin cells *(C. pepo* L.) synthesize a tetracylic terpenoid – *cucurbitacin* (Lavie and Glotter, 1971) that could be of interest to the pharmaceutical industry (Katavic and Jelaska, 1991). This terpenoid or its equivalent may be present in *T. occidentalis* and this study provides preliminary information on optimization of media content and hormonal concentration that may provide desired source of pharmacologically active plant constituents through callus culture.

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