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In vitro propagation of *Tacca leontopetaloides* (L.) Kuntze in Nigeria

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Abstract

This study was carried out to assess the propagation of *Tacca leontopetaloides* (Taccaceae) *in vitro* using seed embryos. The seeds were cultured on Murashige & Skoog basal media augmented with various concentrations of single or combined growth hormones to obtain a suitable protocol for plantlet regeneration. Seed embryo germination of 57% was obtained, as against 20% for germination under conventional sowing. The best shoot proliferation was obtained using media fortified with 0.1 mg/L 6-benzyl amino purine and 0.01 mg/L naphthalene acetic acid, while the best rooting was obtained with MS media supplemented with 0.1 mg/L indole butyric acid. Callus formation was not observed on plantlets *in vitro*, indicating much easier and less challenging *in vitro* plant propagation.

Keywords: tissue culture, micro-propagation, regeneration, recalcitrance

Introduction

Tacca leontopetaloides (L.) Kuntze is a wild perennial herb belonging to the family Taccaceae. *Tacca* is the only genus in the family, relatively recently separated from the family Dioscoreaceae, but closely related (Caddick *et al.* 2002). The plant is native to the Old World tropics, from western Africa through southern Asia to northern Australia. Because of its wide distribution, the plant has numerous common names, but Polynesian arrowroot appears to be the most widely used. In Nigeria, the plant is widespread in the middle belt of Nigeria (Manek *et al.* 2005) and in the southwestern states, suggesting its ecogeographical distribution within the tropical humid forests of the western parts of Nigeria, up to the Sudan savannah region of the northern parts of Nigeria. It is common as solitary plants in open fields, under the shade of trees or on hilltops. It is found as a plant only in the rainy season, because from September through the dry season the shoot dies off and the tubers become dormant; new shoots sprout and emerge at the onset of rains the following year. It is interesting to note that this plant produces fleshy sweet-tasting fruits, dispersed by birds and mammals that eat the fruit (Drenth 1972).

In Northern Nigeria, the tubers are considered a food, especially during the time other staple foods are scarce (Kay 1987). On many Polynesian islands the bitter raw tubers are used to treat stomach ailments, mainly diarrhoea and dysentery (Kay 1987), as well as guinea worm infection, hepatitis and an antidote for snake bite. The root starch is used to stiffen fabrics on some islands (Ukpabi *et al.* 2009), and has great potential for making alcohol. In Nigeria, the tubers are prepared and eaten, especially by the Tiv, while the Hausa find it useful in traditional ceremonies and ancestor worship.

The plant remains a wild and under-utilized plant in Nigeria, and has not been given adequate scientific attention. The seeds have shown poor germination; vegetative propagation by tubers appears to be the most widely used method, especially in northern Nigeria. Since it is the same tubers that are eaten, there is a need to develop other methods of cultivating the plant other than from the tubers. Therefore this study was conducted to develop a protocol for the *in vitro* propagation of embryos for induced shooting and root formation.

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Materials & Methods

The study was carried out in the Biotechnology Laboratory of the National Centre for Genetic Resources & Biotechnology, Moor Plantation, Ibadan, Nigeria. The seeds of *Tacca leontopetaloides* used in the embryo culture were collected from an open field along the Eruwa-Olokemeji road, Eruwa, Oyo state, Nigeria. Glasswares and dissecting tools were sterilized for 30 min by autoclaving at 121 ºC and 1.06 kg cm^{-2} pressure. The seed explants were washed thoroughly using household liquid soap, after which they were rinsed for 15 min under running tap water. Subsequent sterilization was carried out in a laminar flow cabinet under aseptic conditions. The explants were surface-sterilized in 70% ethanol for 5 min, followed by 20% v/v sodium hypochlorite (NaOCl) solution for 20 min, and then rinsed three times with sterile distilled water.

Murashige & Skoog (1962) basal medium was used in all cultures. In various treatments, the basal medium was supplemented with growth regulators including 6-benzyl amino purine, naphthalene acetic acid and indole butyric acid. Treatments were prepared in stock solutions and used to initiate cultures, form and proliferate buds and to induce rooting. 300 sterilized seeds of *T. leontopetaloides* embryos were inserted into the culture initiation medium. The embryo of the seeds could not be removed because the seeds are tiny. The cultures were placed in a growth room at 27 ± 1 ^{0}C with photoperiod of 16 hr light (2000 lux) and 8 hr darkness.

The germinated seeds were transferred to treatment media made up of the basal medium supplemented with varying concentrations of growth regulators for shoot bud proliferation. The experiment was replicated three times. Germination rate, shoot length, number of shoots and nodes were observed. Regenerated shoots were then implanted in different treatment media under continuous daylight to induce rooting. The experiment was replicated three times, and the number of roots and root length of the plantlets measured.

The data were subjected to one-way analysis of variance (ANOVA) using SPSS version 9.0.1 (SPSS Inc, 1999).

Results

The medium into which the seed explants were implanted supported germination of the embryos: they germinated within between three to four weeks, with 172 of the 300 seeds germinating, a germination percentage of 57%. This compares favourably with less than 20% germination observed for *in vivo* sowing in trays. Plate 1a-c illustrates the germination of *T. leontopetaloides* seeds.

Plate 1 A–C: Photographs showing *T. leontopetaloides* seed germination in different stages

The germinated plantlets were divided into a 10 groups of 15 plantlets each and inoculated on media supplemented with various concentrations of 6-benzyl amino purine and naphthalene acetic acid + 6-benzyl amino purine. Table 1 shows that plantlets treated with 0.1 mg/L naphthalene acetic acid + 0.01 mg/L 6-benzyl amino purine have the highest shoot proliferation (6 per plantlet), shoot length (5.0 cm) and number of nodes (4). The number of shoots, shoot length and number of nodes were significantly different among treatments. Plate 2a shows the shoot proliferation of *T. leontopetaloides* plantlets.

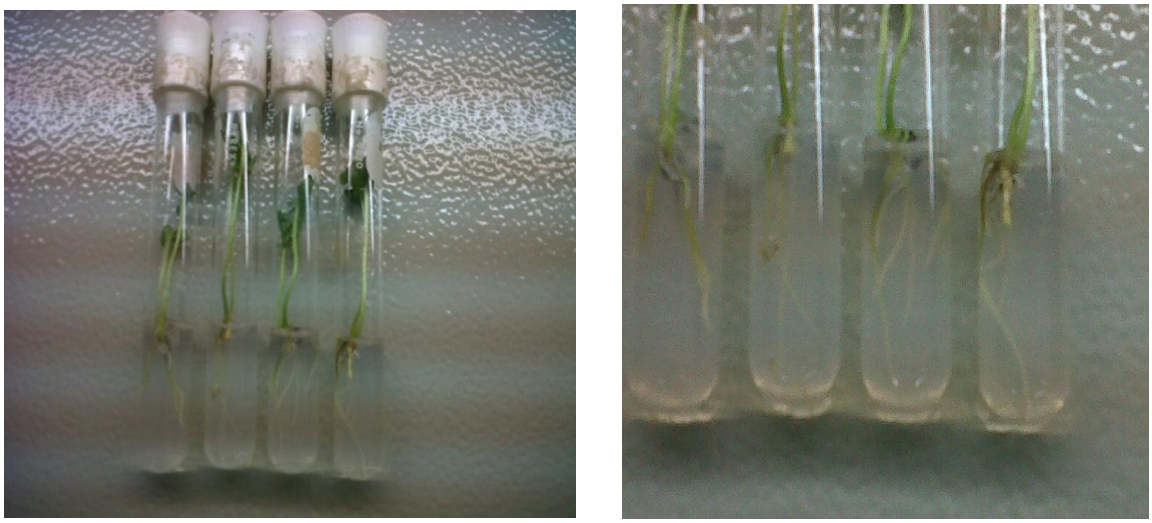

Plate 2: Photographs showing shoot proliferation and rooting of *T. leontopetaloides* plantlets. A: shoot proliferation; B: roots showing root fibres

A total of 80 plantlets were used for the rooting studies. The regenerated shoots were rescued aseptically from the cultured tubes, separated from each other and again cultured on freshly-prepared root induction medium containing medium supplemented with various concentrations of naphthalene acetic acid and indole butyric acid to induce rooting. Rooting was successful for about 45 of the 80 plantlets (Table 2). Medium supplemented with 0.01 mg/L indole butyric acid gave the highest number and longest roots. Plate 2b illustrates one of the *T. leontopetaloides* plantlets with developed roots.

Treatment	**Shoot number**	**Shoot length (cm)**	**Node number**
medium only	1 ± 0.0^{a}	1 ± 0.10^{d}	1 ± 0.0^{b}
medium + 0.025 mg/L BAP	1 ± 0.0^{a}	1.3 ± 0.17^{cd}	1 ± 0.0^{b}
medium + 0.05 mg/L BAP	1 ± 0.0^{a}	1.7 ± 0.19^{bc}	1 ± 0.0^{b}
medium + 0.075 mg/L BAP	1 ± 0.0^{a}	2.0 ± 0.10^{b}	2 ± 0.44^{a}
medium + 0.1 mg/L BAP	1 ± 0.0^{a}	2.7 ± 0.18^{a}	2 ± 0.32^{a}
medium only	0 ± 0.0^{c}	0.5 ± 0.11^{d}	1 ± 0.0^{c}
medium + 0.025 mg/L BAP + 0.01 mg/L NAA	2 ± 0.0^{b}	2.7 ± 0.08^{c}	1 ± 0.0^{c}
medium + 0.05 mg/L BAP + 0.01 mg/L NAA	2 ± 0.0^{b}	3.1 ± 0.11^{bc}	2 ± 0.0^{b}
medium + 0.075 mg/L BAP + 0.01 mg/L NAA	2 ± 0.0^{b}	3.4 ± 0.1^{b}	2 ± 0.031^{b}
medium + 0.1 mg/L BAP + 0.01 mg/L NAA	6 ± 0.71^{a}	5.0 ± 0.31^{a}	4 ± 0.54^{a}

Table 1: Two experiments on effects of 6-benzyl amino purine (BAP) and naphthalene acetic acid (NAA) concentrations shoot proliferation of *T. leontopetaloides* plantlets. Means ± s.e. Within each experiment, means with different superscript letters are significantly different (multiple range test).

When the plantlets grew up to 10 cm high with sufficient root system, they were taken out from the tubes. Medium attached to the roots were gently washed out with running tap water. The plantlets were then weaned in the acclimitization chamber in a medium of the mixture of top soil, coconut fibre and stone dust in 5:2:5 ratio for 3 weeks, after which they were successfully established on the soil in the screen house. Plate 3a-b shows the photographs of the young *T. leontopetaloides* in the screen house.

Treatment	Root length (cm)	Root number
medium only	0.0 ± 0.0^{d}	0.0 ± 0.0^{d}
medium + 0.025 mg/L naphthalene acetic acid	0.8 ± 0.06^{c}	0.0 ± 0.0^{d}
medium + 0.05 mg/L naphthalene acetic acid	1.7 ± 0.25^{b}	0.0 ± 0.0^{c}
medium + 0.075 mg/L naphthalene acetic acid	2.1 ± 0.21^{b}	1.0 ± 0.0^{c}
medium + 0.1 mg/L naphthalene acetic acid	3.8 ± 0.16^{a}	1.0 ± 0.0^{c}
medium only	0.9 ± 0.19^{c}	0.0 ± 0.0^{c}
medium + 0.025 mg/L indole butyric acid	3.4 ± 0.74^{b}	2.0 ± 0.31^{b}
medium + 0.05 mg/L indole butyric acid	5.0 ± 0.83^{ab}	2.0 ± 0.0^{b}
medium + 0.075 mg/L indole butyric acid	5.8 ± 0.80^{a}	5.0 ± 0.55^{a}
medium + 0.1 mg/L indole butyric acid	6.6 ± 0.92^{a}	6.0 ± 0.63^{a}

Table 2: Two experiments on the effects of the concentration of two growth regulators on root induction in *T. leontopetaloides* plantlets. Means ± s.e. Within each experiment, means with different superscript letters are significantly different (multiple range test).

Plate 3 a–b: Photographs showing plant acclimatization

Discussion

Considering the fact that the tubers of *T. leontopetaloides* are edible and these same tubers are used for propagation, the only alternative is the use of seeds for propagation. However, seed germination is very poor. Therefore this study investigated the *in vitro* propagation of *T. leontopetaloides* using seed embryos. The results indicate that *in vitro* culture was more effective in the germination of seeds than conventional sowing into soil. It is pertinent to note that no callus was formed during embryo culture, another indication of the suitability of

propagation of this plant by *in vitro* methods. Shoot proliferation increased with increases in growth hormone concentration, as did rooting.

The results from this study indicated that *in vitro* propagation of *T. leontopetaloides* seed was successful with media supplemented with growth hormones for germination, shoot proliferation and rooting. Progress made on regenerating whole plantlets from mature seed will enhance cultivation and *in vitro* conservation of the plant, remedying the inability to store the seeds in genebanks and poor seed germination due to recalcitrance to germination. Since the plant is only actively growing in the rainy season, *in vitro* propagation ensures cultivation and multiplication in the off-season, thereby increasing yield potential and breaking the limitation of the planting period so that all-year-round planting becomes possible. Englemann (1991) noted that *in vitro* propagation of plants enhances germplasm diversity, provides stocks for micrografting and promotes breeding for improvement. Further studies are required on the micro-propagation of the nodal cuttings of this plant under wider range of growth hormones.

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الملخص العربي

إنبات نبات تاكا ليونتوبنتالويدس (*Tacca leontopetaloides* (L.) Kuntze) معملياً بدولة نيجيريا

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الملخص العربي

أجريت هذه الدراسة لتقييم عملية إنبات نبات تاكا ليونتوبنتالويدس (*Tacca leontopetaloides*) معملياً باستخدام أجنة البذور. تم إنبات البذور في بيئة موراشيج وسكوج (Murashige & Skoog) ودُعِمت بتركيزات مختلفة من هرمونات النمو الأحادية أو المختلطة للحصول على بروتوكول مناسب لنمو الشتلات. ولقد تم الحصول على نسبة إنبات لأجنة البذور تبلغ 57 ٪ مقابل 20 ٪ للإنبات في الظروف التقليدية. كما لوحظ أن أفضل نمو للمجموع الخضري تم عند إضافة 0.1 ملجم / لتر من مادة 6-بنزيل أمينو بيورين و 0.01 ملجم / لتر من مادة حمض النفثالين أسيتيك للبيئة المستخدمة، في حين أنه تم الحصول على أفضل نمو للجذر عند تزويد البيئة بنسبة 0.1 ملجم / لتر من حامض بيوريتيك الإندول. ولم يلاحظ تصلب أيٍ من أجزاء الشتلات في المعمل مما يدل على أن نمو النبات معملياً يتم بصورة أسهل وبتحدى قليل.