

## Potential of *Launea taraxacifolia* (Willd) Amin Ex. C. Jeffrey for *in Vitro* Regeneration

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### Abstract

This study investigated the potential of *Launea taraxacifolia* for *in vitro* regeneration. Stem and leaf explants were inoculated on full strength Murashige and Skoog (MS) medium supplemented with varying concentrations of 2, 4-dichlorophenoxyacetic acid (2,4-D). Leaf explants responded to all concentrations of 2,4-D used while stem explants responded to only two of the 2, 4-D concentrations suggesting that leaf explants might be a better source of explants. Leaf explants generated shoots on medium supplemented with 0.5 mg/l kinetin and 0.1 mg/l 2, 4-D. This study is the first report on *in vitro* regeneration of *Launea taraxacifolia*.

**Keywords:** 2,4-D, "Efo yanrin", kinetin, micropropagation, vegetable, wild lettuce

### Introduction

*Launea taraxacifolia* (Willd) Amin., Ex. C. Jeffrey also known as wild lettuce, is an erect rhizomatous annual or perennial herb with greyish blue bloom (Akobundu and Agyakwa, 1998). It was formerly referred to as *Lactuca taraxacifolia* Ex. Hornemann and is known as "Efo yanrin" by the Yoruba people of Southern Nigeria. It is generally collected from the wild in Nigeria and other parts of Africa, and has been suggested to originate from the Ethiopian highlands from where it spreads as weed to other parts of the world.

Adegbite (1987) reported that it is used as common vegetables by the African people; the leaves can be eaten fresh as a salad or cooked in soups and sauces. The leaves are fed to nursing cattle particularly in northern Nigeria where it is claimed to increase milk production and induce multiple births (Burkill, 1985). Medicinally, the leaves, when rubbed on limbs have been reported to enhance walking in children, the Yoruba people of southern Nigeria mix the leaves of *L. taraxacifolia* with local black soap for curing skin diseases. It is also reported to have therapeutic uses for respiratory problems and chest congestion and the milky latex can be used to cure the popular eye disease called conjunctivitis (Adegbite, 1987; Ayensu, 1978; Dalziel, 1948; Schipper, 2000).

*L. taraxacifolia* is one of the undervalued reservoirs of diversity of African leafy vegetables which are extremely important for food security, nutrition and poverty alleviation throughout the continent. Apart from being displaced in many areas, many local vegetable species that are used have been neglected by science and development (Chwenya and Eyzaguirre, 1999; Opabode and Adebooye, 2005). Adebooye *et al.* (2003) also reported that most of these African leafy vegetables are not easily available as

farmers now gather them with great drudgery and difficulty from the few stands that are left in the wild. 'Yanrin' is one of the traditional leafy vegetables that are important especially in Nigeria. It is known to have low seed viability (Adegbite, 1987) and reproduces asexually by sprouting vigorously from roots. According to Adebisi (2004), selection of plants with tender leaves of low bitterness would increase its popularity.

However, Sakpere and Aremu (2008) reported that, a limitation to cultivation and domestication of this plant is a lack of variability imposed by vegetative mode of propagation. Since the plant is propagated mainly by vegetatively propagation, conventional breeding is inefficient. Molecular breeding will therefore be a more efficient method for genetic improvement of the plant and *in vitro* propagation methods are essential for molecular breeding.

Variation differences are the basis of plant breeding and in recent times many new techniques have become available for breeders, many of which take advantage of the ability to regenerate whole plants from single cells. *In vitro* cultured cells can differentiate into whole plants through organogenesis or somatic embryogenesis. Plants regenerated in this way are known to exhibit variation called 'somaclonal variation' (Larkin and Scowcroft, 1981). One of the prerequisites for a successful application of *in vitro* selection is the presence of *in vitro* regeneration techniques. However, *in vitro* propagation protocols for *L. taraxacifolia* have not been reported even though Opabode and Adebooye (2005) suggested the establishment of an *in vitro* propagation protocol for the plant. Therefore, a micro propagation study was initiated to test the potential of the plant for *in vitro* regeneration and to establish benchmarks for the development of protocols for *in vitro* propagation of the plant. This study, to our knowledge is the first report on the *in vitro* propagation of *L. taraxacifolia*.

## Materials and methods

### Plant materials

Twenty five roots of *L. taraxacifolia* plants growing near the Department of Botany, Obafemi Awolowo University, Ile-Ife, Osun State, Nigeria, between latitude: 7°46'7"N, longitude: 4°56'7"E were collected and buried in six plastic pots filled with top soil. Holes of about 1-2cm diameter were bored at the base of the pots in order to facilitate drainage. The pots were left outside under ambient conditions and watered daily in order to ensure good establishment and rapid sprouting of plants. The plants generated from these roots were used as source of explants.

Explants collected from healthy *L. taraxacifolia* plants raised as above were washed under running tap water to remove dirt and reduce microbial population. The explants were then sterilized with a solution of 5% (v/v) sodium hypochlorite (NaOCl) and two drops of 'Tween 20' for 10 minutes. The explants were rinsed in three changes of sterile distilled water. The leaves were then wounded by trimming their edges and then cutting them into about 2 cm<sup>2</sup> pieces. Stem explants were cut into 1 cm long pieces. The stem and leaf explants were cultured separately. All protocols were carried out under the laminar airflow chamber. Each experimental unit was repeated five times.

Full strength Murashige and Skoog (MS) medium (Murashige and Skoog, 1962) supplemented with varying concentrations of 2, 4-dichlorophenoxyacetic acid (2, 4-D) (0.1, 0.4 and 0.8 mg/l) and Kinetin (K) (0.5 or 1.0 mg/l) was used in all experiments. The pH of the media was adjusted to 5.7±0.1 and gelled with 0.8 g agar prior to autoclaving at 121°C and 108 KPa (15 psi) pressure for 15 minutes. The cultures were incubated in the dark at 25°C±2°C. MS medium without growth regulators served as the control. The explants were evaluated visually for the presence and type of callus and then scored (Remotti and Loffler, 1995). Results were observed at regular intervals and data collected were presented as percentages.

## Results and discussion

Leaf explants on MS medium without plant growth regulators (PGR) produced callus within 2 weeks, some developed roots in addition, but no shoot. This could be as a result of high level of endogenous PGR's, particularly auxins, in the leaf explants. It has been reported that callus formation can be correlated with high endogenous auxin concentration in leaves and might have a potential for triggering induction of somatic embryogenesis (Mashayekhi *et al.*, 2008; Welander and Snygg, 1987). Using cold treated capitula explants, Sands *et al.* (2003) reported that PGR-free MS was the better medium for shooting compared to all other treatments in the micropropagation of *Calocephalus lacteus*. Callus was not formed on stem explants inoculated on PGR free MS medium.

Roots were generated on leaf explants in cultures containing MS media supplemented with 0.1 mg/l 2,4-D in 1 week and in cultures supplemented with 0.4 mg/l 2,4-D in 5 weeks. However, in cultures containing MS medium supplemented with 0.8 mg/l 2,4-D, cream coloured callus was produced on leaf explants after 7 weeks. This may suggest inhibition of organogenesis at concentrations above 0.4 mg/l 2, 4-D.

For stem explants, root formation was initiated after 6½ weeks on MS medium supplemented with 0.1 mg/l 2,4-D only; callus was initiated on stem explants inoculated on medium supplemented with 0.4 mg/l 2,4-D, while stem explants cultured on MS medium supplemented with 0.8 mg/l 2,4-D showed no response. The highest percentage response was obtained from leaf explants (Tab. 1 and Fig. 1) suggesting that leaf explants are a better choice for micropropagation of *L. taraxacifolia*.

The choice of explants is of cardinal importance and makes an absolute difference between success and failure in inducing regeneration *in vitro*. This is because different explants and even callus do not respond in the same way to different growth regulators. Callus initiated on different explants showed varying textures and colour (Tab. 2).

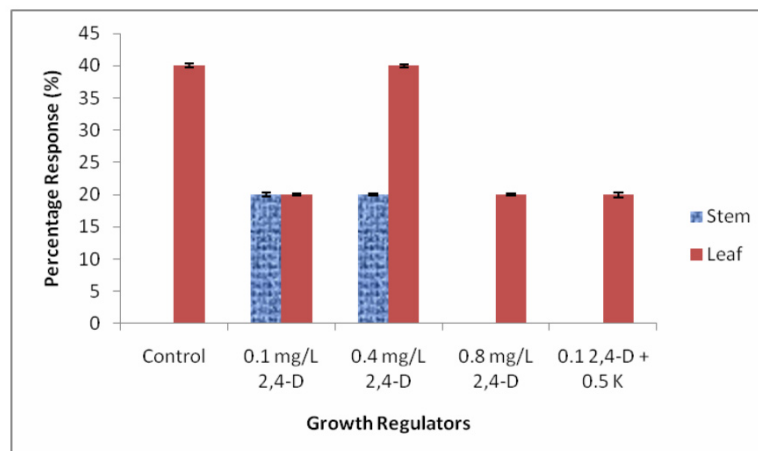


Fig. 1. Response of *Launea taraxacifolia* explants to MS medium supplemented with 2,4-D alone or in combination with Kinetin

Tab. 1. Types of responses induced on stem and leaf explants of *Launea taraxacifolia* grown on MS medium supplemented with 2,4-D alone or in combination with Kinetin

Explant	Total Response from explants (%)	Type of Response (%)				
		C	C/R	R	R/S	C/R/S
Stem	10	5	5	-	-	-
Leaf	28	8	4	12	-	4

Key: C = Callus only; C/R = Callus/Root; R = Root only; R/S = Root/Shoot; C/R/S = Callus/Root/Shoot

Leaf explants that had generated roots on medium supplemented with 0.1 mg/l 2,4-D sub-cultured unto a medium supplemented with 0.1 mg/l 2,4-D and 1.0 mg/l Kinetin generated callus while leaf explants inoculated on medium supplemented with 0.5 mg/l Kinetin and 0.1 mg/l 2,4-D, generated compact, dark brown callus after which shoots emerged from the explant followed by more roots (Plate 1B). According to Stojakowska and Malarz (2004), the tendency to indirect organogenesis was diminished when Kinetin was used in cultures.

However, in the *in vitro* propagation of *Vernonia amygdalina*, Khalafalla et al. (2007) reported that Benzyl

adenine (BA) alone or in combination with Naphthalene acetic acid (NAA) was more effective for shoot multiplication than Kinetin alone or in combination with 2,4-D, mainly because explants grown on the medium containing Kinetin alone or in combination with 2,4-D formed excessive callus and callus growth on explant have been reported to interfere with the propagation process (Thiem, 2003).

In the tissue culture of *Lactuca sativa*, Pink and Carter (1987) reported that medium supplemented with 0.5 mg/l Kinetin gave poor shoot growth while 1.0 mg/l Kinetin promoted shoot growth. However, in the present study, addition of 0.5 mg/l Kinetin to the culture medium promoted shoot development while the addition of 1.0 mg/l Kinetin promoted callus formation. There have been reported differences in tissue sensitivity to plant growth regulators (Lisowska and Wysokinska, 2000). Depending on the ultimate aim of the experiment, different plant growth regulators are employed.

Media containing Benzyl adenine (BA) together with NAA have been reported to be preferably used for multiple shoot induction and maintenance in *Asteraceae* plants (Stojakowska and Malarz, 2004). According to Trejgell et



Plate 1. Responses of *L. taraxacifolia* leaf explants to MS medium supplemented with 2,4-D alone or in combination with Kinetin: A: leaf inoculated on MS+ 0.4mg/l 2,4-D showing roots; B: Plantlet generated on leaf explants inoculated on MS+ 0.5 mg l<sup>-1</sup> kinetin and 0.1 mg l<sup>-1</sup> 2,4-D; C: Roots on leaf explants inoculated on MS+ 0.1mg/l 2,4-D and D: Roots on leaf explants inoculated on MS medium without growth regulators

Tab. 2. Effect of MS medium supplemented with 2,4-D alone or in combination with Kinetin on type of callus induced on *Launea taraxacifolia* explants

	Explant	Callus size (Cms)	Colour	Texture	Induction time (weeks)
Control	Leaf	≤ 1	Beige	Soft friable	2
0.1 mg/l 2,4-D	Stem	≤ 1	Cream	Pulpy	2.5
0.4 mg/l 2,4-D	Stem	≤ 1	Off white	Loose	5
0.8 mg/l 2,4-D	Leaf	≤ 1	Cream	Pulpy	7
0.1 mg/l 2,4-D + 0.5 mg/l K	Leaf	≤ 1	Dark Brown	Compact	5

al. (2009), the caulogenic role of BA has been shown in other plants of the *Asteraceae* family.

This needs to be tested for *Launea taraxacifolia* since it is known that different species can respond differently to the same growth regulators. The effect of higher concentrations of Kinetin also needs to be investigated.

### Conclusions

In conclusion, this study has established the potential for *in vitro* regeneration of *Launea taraxacifolia*. Further research in order to develop an efficient micropropagation system for the species and to compare the effects of BA and NAA and 2, 4-D and kinetin is ongoing.

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