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Short Communication Report

EFFECT OF VARYING CONCENTRATIONS OF AUXIN (2,4-D) ON *IN VITRO* CALLUS INITIATION USING LEAF OF *ARTEMISIA ANNUA* (L)

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ABSTRACT
Study was carried out to determine the effect of varying concentrations of auxin on callus initiation using the leaves *Artemisia annua* as explants which were sterilized and inoculated into Murashig and Skoog basal medium supplemented with varying concentrations of 2,4-Dichlorophenoxy acetic acid (2,4-D) and incubated in the growth chamber for 4 weeks at 27ºc. Best results were obtained with 1.0 µm/l and 1.5 µm/l concentrations. Analysis of variance (ANOVA) indicated significant difference among all the treatments ($P<0.05$) on the parameters studied compared with the control. Consequently, 1.0 µm/l and 1.5 µm/l concentrations of 2,4-D are ideal for callus initiation in *A. annua.* This provides the means to mass propagation of *A. annua* through callus initiation and subsequent provision of raw materials required for artemisinin extraction.

Keywords: *Artemisia annua,* Callus, Auxin, *In vitro*, 2,4-Dichlorophenoxy acetic acid (2,4-D)

INTRODUCTION
Artemisia annua commonly known as wormwood or "vilayati afsanteen" is a perennial herb belonging to the family Asteraceae. It is native to China, where it is known as qinghao (green herb) and has been used for over 2000 years to treat symptoms associated with fever and malaria. It is known in the United States as sweet Annie, annual or sweet wormwood (Ferreira *et al.*, 1997). *A. annua* is traditionally used because of its antihelminthic, insecticidal, anticancerous, antiseptic and febrifuge properties. It's oil has been found to repel fleas, mosquitoes and killed house flies(Morton 1981).In antiquity, plants of the genus *Artemisia* were also used to control the pangs of childbirth, regulate women's menstrual disorders, and as an abortifacient. In 1969, the Chinese screened their medicinal plants in search of an effective antimalarial compound. Consequently, a diethyl ether extract of *Artemisia annua* was found to be effective against plasmodium species and in 1972 the active ingredient, artemisinin, was isolated and identified (Riddle and Esters, 1992).

Auxins such as 2,4-Dichlorophenoxy acetic acid are generally used in plant cell culture at varying concentration ranges and were reported to influence a multitude of physiological processes, and the morphological appearance of cultured tissues. (Baskaran and Jayabalan, 2005; Mannan *et al,* 2012).Similarly, when added in appropriate concentrations they regulate cell division and elongation, tissue swelling, formation of adventitious roots, inhibition of adventitious and axillary shoot formation, callus initiation, growth and induction of embryogenesis (PhytoTechnology Laboratories, 2011).
Most callus cells are totipotent, being able to regenerate a whole, and true to type plant. Propagation of plants through callus initiation has the ultimate advantages of rapid shoot multiplication rate hence mass propagation and a high regeneration percentage using leaves, petioles, internodes and cotyledons as explants. (Hamish and Sue, 1998; Razdan, 2002).This study was designed to establish an effective protocol for callus initiation in *Artemisia annua* leaf using varying concentrations of 2,4-Dichlorophenoxy acetic acid.

The experiment was conducted in the laboratory of Biological Science Department of Kaduna State University, latitude 10.52ºc N and 7.44ºc E longitude 614 m elevation above the sea level, Kaduna, Nigeria. Young and fresh leaves of *Artemisia annua* were collected from the Institute for Agricultural Research (IAR). Ahmadu Bello University, Zaria.

Leave explants were removed from *Artemisia annua* plant by cutting with sharp knife. Surface sterilization of the explants was carried out in the following steps:

i. washed with household soap and rinsed on running tap water.
ii. washed again with soap and rinsed with double distilled water in the laminar flow hood.
iii. dipped in 70% ethanol for 3 minutes and rinsed with double distilled water.
iv. soaked in 3% sodium hypochlorite (Naocl) for 5 minutes.
v. rinsed 3-4 times with sterile double distilled water.(Hamish and Sue,1998)

Exactly 300ml MS (1962) basal media supplemented with different concentrations of 2,4-D (0.0 µm/l, 0.6 µm/l, 0.8 µm/l, 1.0 µm/l, 1.5 µm/l) was prepared. The media was solidified with 4.5g Agar after adjusting pH to 5.8±1. The media was autoclaved at 121◦c for 15 minutes. 40mls was dispensed into each bottle and was labelled according to the concentration of 2,4-D used and was sealed with cling film.

Sterilized leaves were inoculated aseptically into the media under the laminar flow hood, four leaves was inoculated into each bottle .Explants were kept on the growth chamber at 26±27◦c under a 16 hours photo period under the fluorescent light and monitored for callus initiation.

Three parameters were studied: Vigour was determined based on morphological appearance adopting the procedure of (Gibson, 1980). A scale of 1-5 was used where 1 = very high vigour and 5 =

very low vigour. Days to callus formation (DCF) was determined by observing the number of days to the commencement of callus initiation after inoculation of the explants. Percentage regeneration (PR) was calculated according to Wiese and Binning (1987) Where Gr = (number germination since n-1)/n. where: Gr = germination (regeneration) rate; n= the days of incubation. Callus initiation commenced 2 weeks after incubation and data on vigour, days to callus formation and percentage regeneration were observed and recorded. The experiment was carried out using Completely Randomised Design (CRD). Data generated from the study was analyzed using Analysis of Variance (ANOVA). To further test for significant difference, the data was also subjected to Duncan Multiple Range Test (DMRT) using SAS,2002. (9.0 version).

Swelling of the explants was observed a week after inoculation (Fig.1a). However, callus initiation was observed 2 weeks after inoculation. It was observed that callus initiated at the cut edges of the explants and developed into a full grown callus. Based on the morphological appearance, the callus formed was yellowish, whitish, compact and nodular. Callus initiation reached its bloom 4 weeks after incubation (Fig.1b).

With respect to vigor, there is significant difference among the treatments concentration. A 2,4-D concentration of 1.5 µm/l had the excellent vigor followed by1.0 µm/l and 0.8 µm/l respectively (Table1). Based on days to callus formation, there is no significant difference among treatments (26 days)compared with the control (Table1). However, a significant difference was observed among the treatments with respect to percentage regeneration. 2,4-D concentration of 1.5 µm/l has the highest % regeneration (100%) followed by 1.0 µm/l (75%) and 0.8 µm/l (58%)(Table 1). The least result (41.7%) was observed with 0.6 µm/l concentration (Table 1).

Callus initiation from cut edges brought about repeated cell division which enabled the explants tissue to produce double its original weight as a callus tissue.(Hamish and Sue, 1998; Razdan, 2002). The formation of whitish, compact and nodular callus was in accordance with the work. However, the best vigor observed with 2,4-D concentration of 1.5mg/l suggests that medium supplemented with high level of 2,4-D supports efficient plant morphological appearance. Similar results were obtained by Tahir *et al (*2011), who initiated callus using sugarcane explants. The poor regeneration capacity observed at lower concentration of 2,4-D may be attributed to incomplete differentiation of the cells of the Artemisia explants (Hamish and Sue 1998; Razdan 2002).

The development of callus from immature leaf explants is directly related to the presence of 2,4-D and its effectiveness in callus initiation in leaf explants. Similar results were observed with callus initiation using leaf of sugarcane as explants. (Mamum *et al,* 2004; Tahir *et al,* 2011). Also, young leaves contain higher population of actively dividing cells therefore more responsive to callus initiation (Hamish and Sue, 1998; Razdan, 2002).

Callus regeneration percent was observed to increase with increase in the concentration of 2,4-D indicating that callogenesis in Artemisia was influenced by 2,4-D. (Baskaran and Jayabalan, 2005; PhytoTechnology Laboratories, 2011; Mannan *et al,* 2012). Because of the high demand for *Artemisia annua* and its natural products, results of this study indicated the significance of 2,4-D as a viable hormone for large scale propagation of the plant via embryogenesis. And this may ultimately be a source of artemisinin which is a raw material needed for the provision of Artemisnin Combined Therapy (ACT) as a recommended drug by World Health Organization (WHO) towards eradication of malaria fever in the world.

Table1: Effects of Varying Concentrations of 2,4-D on Callus Initiation

Treatment (µm/l)	Vigor	Days to Callus Formation	(%)Regeneration
0.6	2^{c}	26^{a}	41.7^{dc}
0.8	2^{c}	26^{a}	58^{bc}
1.0	3^{b}	26^{a}	75^{ab}
1.5	5^{a}	26a	100^{a}
Control	2^{c}	20a	25^{b}

Means with the same letter are not significantly different.

Figure 1: Some stages of callus formation
[A, Swelling of the leaf explants; B, White, friable and embryonic callus]

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