Asexual propagation of worm wood (*Artemisia annua* L) using stem cuttings

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ABSTRACT

Malaria is a global health issue that continues to claim lives and causes serious economic loss. The most effective drugs currently available for malaria treatment contain the anti-malarial compound, artemisinin, derived from *Artemisia annua*. The most effective concentration of some plant growth hormones and length of stem cuttings on the regeneration of *A. annua* was investigated in the Botanical Garden of the Department of Biology, Kaduna State University Kaduna. *Artemisia* stem cuttings of varying lengths 8, 12 and 16 cm were treated with NAA and GA$_3$ at concentrations of 0.01, 0.02 and 0.03 mgL$^{-1}$, respectively for two hours each with a control. The experiment was laid down on a Completely Randomized Design (CRD) with three replications. Results of Analysis of Variance (ANOVA) indicated significant difference ($p < 0.05$) between the treatments with respect to days to regeneration, % regeneration, number of leaves, plant height and vigor. Least days to regeneration of 6 days, highest plant height of 19 cm, best vigor of 1 and highest percentage regeneration of 100% were obtained with 16 cm stem cuttings treated with 0.03 mgL$^{-1}$ GA$_3$ concentration. Similarly, GA$_3$ concentration of 0.03 mg L$^{-1}$ gave the best result in the regeneration and seedling characteristics of *A. annua*. Therefore, the aforementioned concentration is suitable for the asexual propagation of *A. annua* and related members of the family using stem cuttings.

Keywords: *Artemisia*, asexual, propagation stem- cuttings.

INTRODUCTION

*A. annua* L (Wormwood) is a member of the family Asteraceae. One of the prominent characteristic of the plant is the extreme bitterness of all its parts (Tripathi et al., 2000, 2001; Ferreira and Janick, 2009). Its cultivation has spread from its center of origin (China) to Africa, mainly Kenya, Tanzania and Nigeria in response to the call by the World Health Organization for the use of Artemisinin-Combination Therapies (ACT) for treating malaria fever (Ferreira et al., 2005; Brisibe, 2006). Artesunate, Artemether and Artemisinin are the three common derivatives found in *A. annua* (Muhammad et al., 2014). Artemisinin also shows a promise as a potential therapeutic agent for other parasitic and viral diseases as well as, for the treatment of certain cancers and the reduction of angiogenesis (Lai et al., 2013).

Micro propagation and seed multiplication common practices for the propagation of *A. annua* are not always suitable to all conditions. As a matter of fact, the first method is expensive and time consuming in order to obtain a good adaptation from *in vitro* culture to open field conditions (Saranga and Cameron, 2006), whereas the second is characterized by high genetic variability and scarcely homogeneous production. Hence, propagation by cuttings remains an appropriate method that gives a rapid propagation with reduced costs.

Rooting capacity in cuttings is influenced by internal factors, such as genotype, nutritional status or phonological stage and external factors, like temperature and light intensity (Hartmann et al., 1997; Agbo and Obi 2008; Priadjati et al., 2001). Internal factors are strictly related to
the amount of plant growth regulators that are physiologically necessary for the rooting phase (Guo et al., 2009; Amri et al., 2010; Zobolo, 2010). Exogenous auxins are commonly used to improve natural rooting efficiency in root and stem cuttings, but it was demonstrated in various plant species that relatively high auxin concentrations are required only during the induction phase, while during development these plant growth regulators become inhibitory (Hartmann et al., 1997). Rooting formation of cuttings is also affected by physical and chemical characteristics of rooting substrate such as bulk density, porosity, water-holding capacity and pH, etc which promotes or inhibits root growth (Hartmann et al., 1997).

Moreover, adventitious rooting is often related to the season in which cuttings are collected as the availability of internal auxin as well as, nutrients content of plant tissue may determine significant variations in root capacity of cuttings (Rosier et al., 2004).

Malaria is a global health issue that will continue to spread with the increase in mosquito populations caused by the rise in global temperatures. The plasmodium falciparum has increased resistance to anti-malarial drugs such as chloroquine. The most effective drugs currently available for malaria treatment in which plasmodium parasite is yet to develop resistance contains the compound, Artemisinin, which is derived from A. annua plant (Muhammad et al., 2014). In Nigeria, productive Artemisia seed is expensive and not readily available. Successful and quantitative production of biomass is an important step towards maximizing Artemisinin content in Artemisia for the treatment of malaria fever. However, this has been hindered by several biotic and abiotic factors such as pest, diseases and climatic constraints. Consequently, these have led to poor performance and hence, decrease in yields and are characterized by high genetic variability and scarcely homogeneous production.

In Nigeria, there is insufficient agro-technological information about ideal planting dates, seed density, harvesting system and post-harvesting coupled with harsh weather. Therefore, the determination of an effective method of propagation of Artemisia, which will be rapid, provides large biomass materials and is inexpensive. This creates job to growers and provides raw materials to pharmaceutical industries for the extraction of artemisinin which eventually will be used for the synthesis of malaria drugs for fight against the disease. Therefore, propagation by stem cuttings remains an appropriate method that provides rapid propagation with reduced costs.

**Aim and objectives**

The aim of this study is to examine the effect of plant growth regulators and length of stem cuttings in the asexual propagation of A. annua.

The objectives of this work include:

1) To determine the effect of length of stem cuttings on the propagation of A. annua;
2) To determine the suitable concentrations of auxin (NAA) on the asexual propagation of A. annua;
3) To determine the suitable concentrations of Gibberellic acid (GA$_3$) on the asexual propagation of A. annua.

**MATERIALS AND METHODS**

**Study area**

The experiment was conducted in the botanical garden of the Department of Biology Kaduna State University, which is located at latitude 10°31 North, and longitude 7°26 East and 6.14 m above sea level.

**Soil analysis**

Soil for the experiment was randomly collected from a depth of 0 to 15 cm, 15 to 20 cm and 30 to 45 cm and was thoroughly mixed. The soil was then taken to the soil laboratory in KEPA for analysis; the result of the analysis reveals that the soil used for the experiment was sandy loamy soil with neutral pH of 6.5. The organic carbon content of the soil was 0.2990 (w/w). The soil had 127 kg/ha available nitrogen, 24 kg/ha available phosphorus and 11.9 kg/ha available potassium.

**Treatments**

**Hormone treatment**

The cuttings were soaked in three different NAA and GA$_3$ concentrations (0.1, 0.2 and 0.3%) respectively with a control (0%) for 2 h each.

**Planting**

The Artemisia stems of the following lengths; 8, 12 and 16 cm were planted in polythene bags containing a mixture of sandy loamy soil and cow dung in the ration of 3:1 and monitored for regeneration.

**Observation and data collection**

Data were obtained and recorded at week interval for eight weeks on the following parameters:

- Days to leaf sprouting determined by number of days to regenerate per cutting after planting;
- % regeneration was calculated according to Wiese and
Binning (1987) where \( Gr = \frac{\text{number germinating since } n-1}{n} \). Where: \( Gr \) = germination (regeneration) rate; \( n \) = the days of incubation;
- Vigor was determined based on morphological appearance, leaf emergence and early percentage regeneration adopting the procedure of Gibson (1980). A scale of 1 to 5 was used, where 1 = very low vigor and 5 = very high vigor;
- Plant height was determined by spreading a thread against the length of a plantlet which was then placed on a tape to measure the height in centimeter;
- Number of leaves was determined by counting the leaves on each of the established seedling.

**Experimental design**

The experiment was laid down using a completely randomized design (CRD). There were nine treatments with three replications. Sharp knife was used to excise the material from a mother plant of three months old. The cuttings with buds and of varying length from 8cm, 12cm and 16cm which were treated and planted within 24 hours after collection. Leaves were removed from each cutting.

**Statistical analysis**

The data generated from this work was analyzed using analysis of variance (ANOVA), SAS (2002) statistical package. Least significant difference (LSD) was also used to compare treatment means (\( p < 0.05 \)).

**RESULTS**

After planting, the stem cuttings in the polythene bags were placed under the shade to minimize the rate of evaporation from the stem which in turn prevents drying of the stem cuttings. This process enhanced the regeneration process of *A. annua* and is very crucial during asexual propagation *A. annua* (Figure 1).

**Days to regeneration**

All stem cuttings treated with NAA did not regenerate and dried off sixteen days after planting. Using GA\(_3\) treatment, stems with 16 cm/0.03 mgL\(^{-1}\) regenerated in six days (6 days) after planting, this was followed by 16 cm/0.02 mgL\(^{-1}\) which regenerated in nine (9) days after planting as compared with the control which regenerated in twelve (12) days after planting. The 8 cm stem cuttings treated with GA\(_3\) concentration of 0.01, 0.02 and 0.03 mgL\(^{-1}\) did not regenerate and dried off in 10 to 14 days after planting (Tables 1 and 2).

**Percentage regeneration**

GA\(_3\) concentration of 0.03 mgL\(^{-1}\) and 16 cm stem cuttings
had the highest percentage regeneration of 100% as compared to the control which had 33.33%. This was followed by 16 cm/0.02 mgL⁻¹ which had 66.66% and lastly 12 cm/0.03 mgL⁻¹ which had 33.33% (Figure 2, Tables 1 and 2).

**Plant height**

GA₃ concentration of 0.03 mgL⁻¹ and 16 cm stem cuttings produced the highest seedling length compared to control. This was followed by 16 cm/0.02 mgL⁻¹ and lastly 12 cm/0.03 mgL⁻¹ which had the least average seedling height (Figure 2, Tables 1 and 2).

**Number of leaves**

Concentration of 0.02 mgL⁻¹ of GA₃ and 16 cm stem cuttings had the highest number of leaves (76), followed by 16 cm/0.03 mgL⁻¹ (41) compared to the control which had 37 number of leaves; least number of leaves were obtained with 12 cm/0.03 mgL⁻¹ (Tables 1 and 2).

**Plant vigor**

Best plant vigor of 2 was produced by 0.03 and 0.02 mgL⁻¹ concentration of GA₃ and 16 cm stem cuttings compared to the control. They were followed by 0.03 mgL⁻¹ concentration of GA₃ and 12 cm stem cuttings which had 4 (Tables 1 and 2).

### DISCUSSION

The significant effect observed using different concentrations of GA₃ on the regeneration and phenotypic characteristics of stem of *A. annua* producing the least days to regeneration, plant height, vigor and average number of leaves is similar to the findings of Jennifer (2010) who studied the effect of GA₃, salicylic acid and methyl jasmonate on *A. annua* in hydroponic system and found that the cuttings treated with GA₃ showed the highest growth rate and seedling characteristics.

Similarly, results of this study indicated that the higher the concentration of GA₃ the greater the rate of regeneration and other seedling characteristics. This is contrary to the findings of Brian (1958) who described comparing the effect of range of GA₃ concentration on *Pisum sativum* and found that low concentration led to a greater increase in internode extension and other morphological characteristics.

The stem cuttings length was also an effective factor where higher regeneration percentage was obtained with 16 cm stem cuttings. The higher regeneration capacity resulted from the higher nutrition and humidity content of the 16 cm cuttings compared to the shorter cuttings. 16 cm stem cuttings gave the best performance in all the parameters studied which was followed by 12 cm cuttings as compared to control. 8 cm stem cuttings did not survive and dried off within 10 to 14 days after planting.

All the stem cuttings treated with NAA did not survive, turned brown and dried off within 10 to 14 days after planting. The poor performance of the auxin, NAA treated cuttings on comparison with GA₃ treatment and control.

### Table 1: The effects of varying concentration of GA₃ on asexual propagation of *A. annua*.

<table>
<thead>
<tr>
<th>GA₃ Treatment</th>
<th>DR (days)</th>
<th>PR (%)</th>
<th>PH (cm)</th>
<th>NL</th>
<th>VG</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.01 mgL⁻¹</td>
<td>0.00&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.00&lt;sup&gt;bb&lt;/sup&gt;</td>
<td>0.00&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.00&lt;sup&gt;bb&lt;/sup&gt;</td>
<td>0.00&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>0.02 mgL⁻¹</td>
<td>2.97&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>33.33&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.95&lt;sup&gt;aa&lt;/sup&gt;</td>
<td>25.33&lt;sup&gt;aa&lt;/sup&gt;</td>
<td>0.88&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>0.03 mgL⁻¹</td>
<td>4.00&lt;sup&gt;aa&lt;/sup&gt;</td>
<td>37.03&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7.25&lt;sup&gt;aa&lt;/sup&gt;</td>
<td>25.22&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.55&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Control</td>
<td>2.55&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>14.81&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.55&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>8.22&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.22&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>LSD</td>
<td>3.66</td>
<td>14.91</td>
<td>3.94</td>
<td>11.59</td>
<td>0.85</td>
</tr>
</tbody>
</table>

Means within a column followed by the same letter along columns are not significantly different (p=0.05). Key: DR = Days to regeneration, PR = Percentage regeneration, PH = Plant Height, NL = Number of Leaves, VG = Vigor.

### Table 2: The effect of length of stem cuttings on asexual propagation of *A. annua*.

<table>
<thead>
<tr>
<th>Treatment (cm)</th>
<th>DR (days)</th>
<th>PR (%)</th>
<th>PH(cm)</th>
<th>NL</th>
<th>VG</th>
</tr>
</thead>
<tbody>
<tr>
<td>16</td>
<td>4.91&lt;sup&gt;aa&lt;/sup&gt;</td>
<td>55.55&lt;sup&gt;a&lt;/sup&gt;</td>
<td>10.25&lt;sup&gt;a&lt;/sup&gt;</td>
<td>40.33&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.83&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>12</td>
<td>2.25&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>8.33&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>2.18&lt;sup&gt;bb&lt;/sup&gt;</td>
<td>3.75&lt;sup&gt;bb&lt;/sup&gt;</td>
<td>0.17&lt;sup&gt;bb&lt;/sup&gt;</td>
</tr>
<tr>
<td>8</td>
<td>0.00&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.00&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.00&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.00&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.00&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>LSD</td>
<td>3.26</td>
<td>12.91</td>
<td>3.33</td>
<td>10.03</td>
<td>0.41</td>
</tr>
</tbody>
</table>

Means within a column followed by the same letter along columns are not significantly different (p=0.05). Key: DR = Days to regeneration, PR = Percentage regeneration, PH = Plant Height, NL = Number of Leaves and VG = Vigor.
could be indicative that this auxin produced an inhibitory effect on initiation of rooting and shooting of *A. annua*. This was observed by Rao et al. (2010) who reported 86% rooting in *Tinospora cordifolia* without hormone treatments. Low concentrations of IBA were able to increase rooting. However, with increasing concentrations of hormones, rooting percent was observed to decline. The poor performance of auxin treated cuttings on comparison with the control could be indicative that these chemicals, which would otherwise act as stimulants of rooting, could have had an inhibitory effect on initiation of rooting.

**Conclusion**

Pre-treatment of *A. annua* stem cuttings with GA3 at concentrations of 0.03 and 0.02 mgL\(^{-1}\), respectively increased *A. annua* when growth was compared to control. The overall growth performance parameters indicated that 16 cm stem cuttings were significantly superior to all other stem cuttings length for the asexual propagation of *A. annua*.

Asexual propagation *A. annua* is a cost effective method for propagation of *A. annua*. This in turn provides raw materials for extraction of artemisinin for the synthesis of anti-malarial medication as cure for malaria fever most especially in the tropical countries where there is increase in malaria incidence.

Based on the findings of this study, the following recommendations are made:

1) The use of approximately 16 cm long shoot cuttings as planting material for the asexual propagation of *A. annua* is recommended;
2) Pre-treatment of the stem cuttings with GA3 at concentration of 0.03 mgL\(^{-1}\) is recommended;
3) Placing the stem cuttings under shade with little light penetration after planting is also recommended;
4) Further research should be conducted on the asexual propagation of *A. annua* using different parts of the plant such as leaves and root cuttings.

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