Propagation of Allamanda (*Allamanda cathartica* L.) using *in-vitro* Techniques and Some Conventional Methods

By

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Propagation of Allamanda (Allamanda cathartica L.) using in- vitro Techniques and some Conventional Methods

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Date of Examination: April/2014
Dedication

To

my family
my friends

Khalid
ACKNOWLEDGEMENTS

Praise be to Allah the Almighty God who gave me health, strength and patience to accomplish this work.

I would like to thank Prof. Mohamed Ahmed Ali the main supervisor for his guidance and useful suggestion.

Appreciation is extended to Dr. Igbal Abdelgader Abdellatif the co-supervisor and deep thanks to University of Gezira faculty of Agricultural Sciences department of Horticultural sciences.

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My deep thanks to my family for encouragement and patience.
Propagation of Allamanda (*Allamanda cathartica* L.) Using In Vitro Techniques and some Conventional Methods

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Abstract

Allamanda is a popular ornamental plant with any uses in the garden. Propagation of Allamanda by conventional methods is slow and difficult. The main objective of this study was the improvement of *in vitro* and conventional methods for Allamanda. The effects of cytokinins (BAP and kinetin) were tested for the induction of shoot morphogenesis on *in vitro* cultures of nodal explants. Also, the effects of charcoal, different rooting media, type of cuttings and growth regulators were tested for the improvement of conventional propagation, during the years 2011-2013. BAP at 0.5mg/l was the best concentration for shoot regeneration, while kinetin had no significant effect. Addition of charcoal to the water medium had no significant effect on the success of cuttings. The best growth was obtained on loamy soil and peat moss. Shoot tip was better than stem cutting in the conventional propagation of Allamanda. Radicante (growth regulator) treatment in water medium for 90 minutes was the best time for root regeneration. It was recommend that Allamanda can be successfully propagated through *in vitro* method using MS media with 0.5 mg/l BAP and conventionally by using shoot tip, loamy soil and peat moss or in water medium supplied with Radicante.
باشر استخدام تقنيات 
التكاثر النسيجي وبعض الطرق التقليدية

خالد محمد علي يوسف

ملخص الدراسة

نبات الالمندا من نباتات الزينة المرغوبة ولله عدة استخدامات في الحدائق، إثارة الالمندا بالطرق التقليدية بعد بطيئا وصعبا. الهدف من هذا البحث هو تطوير طرق التكاثر التقليدي والنيسيجي للألماندا. تم اختبار اثر الفحم النيائي موض ومعدات الدعم والسيك النسيجي والنيسيجي للألماندا. تم اختبار اثر السيكابكيين (kin) و BAP على تحسين النمو الخضري ص-messages التكاثر المزروعات نسيجا خلال الفترة من 2011 إلى 2013. كانت لإضافة 0.5 ملليل/لتر من هرمون البيريتوك اسيد (PAB) إلى الوسط مورشيجو وسكوك اثر معنوي في تغيير النمو الخضري. بينما لم يكن هناك اثر للهرمون الكايتكين عند إضافة الوسط الغذائي مورشيجو وسكوك. إضافة الفحم النيائي والصناعي إلى الماء لم تعطي اثر معنوي. أثبت التجارب أن أفضل وسط غذائي هو القرير والبيت موس. وكانت القمة الدامية أفضل مقارنة بالعقل الخشبي في إثارة الفحم النيائي للألماندا. أفضل زمن للتجذير كان 90 دقيقة عند إضافة بذرة من النمو الراديكات للماء كوسط غذائي. أوصت الدراسة بأثاث الألماندا عن طريق التكاثر النهائي باستخدام الوسط مورشيجو وسكوك والإضافة 0.5 من BAP. أخيراً اثاثا علاج الطرق التقليدية باستخدام العقلة الفحلية وأن أفضل ثري هي القرير والبيت موس كما أوصت الدراسة باستخدام الماء كوسط غذائي مضافا إليه هرمون النمو الراديكات.
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CHAPTER ONE
INTRODUCTION

Ornamental plants are classified into different groups according to their uses such as herbaceous flowering plant, shrubs, trees, cacti and succulent plants, indoor plants and many others. On the other hand, shrubs have many uses in landscape such as foundation planting, fill some of the space of the garden counterbalancing the flat areas give privacy and provide a back drop for smaller plants. More over Shrubs are best planted in combination to create pleasing decorative effect and provide variety of interest.

Among different shrubs, Allamanda is considered to be as an exceptionally beautiful shrubs that can fulfill all the above mentioned uses beside being used as single specimen in a lawn area where its special quality are put on show. Climbing Allamanda is used to decorate walls and fences while woody one make use of vertical space that is often wasted (Niki philps, 2014). Allamanda is cultivated as an ornamental plant for its long frequent flowers, its vine that require a trellis or fence for support and it can be pruned into a shrub form. If not pruned it can sprawl to a height of 20 feet (Anonymous, 2006).

Allamanda is used to be propagated conventionally by seeds and stem cuttings. Seed setting is never been seen under Sudan condition and hence the vegetative propagation through stem cutting is the only adapted method. Furthermore, the milk sap found in this plant makes the propagation by stem cutting more difficult and slow. Accordingly the objectives of this study were:

1. To test the effect of cutting types, soil type and rooting hormones on conventional propagation of Allamanda cathartica.
2. To study the effects of different growth regulators on nodal segments morphogenesis to establish a protocols for micropropagation.
CHAPTER TWO
LITREATURE REVIEW

2.1. Classification
Allamanda belongs to the Kingdom plantae, Phylum Magnoliophta, Class Magnoliopsida, sub class Asteridae, Order Gentianales, Family Apocynaceae, sub family Rauvolfoideae, Tribe plumerieae, Genus allamanda, variety Allamanda cathartica (Liogier, 1995; Howard, 1989).

2.2. Apocynaceae family

Apocynaceae is the most important family within this order with 5000 species distributed worldwide. Seventy percent of the genus and half of the species distributed in the neotropical region are found in the native Brazilian flora (Koch et al., 2012). Today four subfamilies are described for the Apocynaceae family: Rauvolfoideae, Apocynoideae, Scamonoideae and Asclepiadoideae (Rapini, 2002). The latter is the major subfamily of Apocynaceae and comprises approximately 3000 species divided into 172 genera, distributed mainly in Neotropical areas of South America, such as Brazil, where the highest diversity of the species has been found. The iridoids class has significant distribution within A pocynaceae family but is concentrated in just a few genera. The most representative of these are Plumeria, Himatantha, Allamanda and Gerbera. According to the traditional classification the family comprises approximately 87 iridoids the main ones being plumieride, plumericin and isoplumericin.

2.3. General Description of Allamanda
Allamanda a prolific bloomer is scrambling perennial shrub or vine up to 4.6m tall. It is a fast grower. The big yellow funnel shaped flowers are arranged in rather long racemes mostly at the end of the branches. The leaves are smooth thick and grow in pairs or in whorls of three or four along the stem. The fruits are capsules, splitting to release winged seed. Yellow Allamanda has white milky sap in all parts (Liogier 1995, Howard 1989).
2.4. Vegetative propagation

Vegetative or asexual propagation has been established for reproduction of plants which exhibit desirable characteristics. Asexual method of propagation were used different plant parts such as roots, stems, or leaves of stock plants for grafting, tissue culture, division or cutting propagation (Davidson et al; 2000). Generally propagation by stem cutting has numerous advantages, many plants can be grown in high density trays from a limited amount of stock plants as compared to other a sexual means of reproduction. It is typically less expensive, quicker, relatively simple (Hartmann et al., 2002). Stem cutting production involves removing shoot tip from mother plant and planting it in growing substrate to root. This method allows for retention of foliar flowering habit a characteristics that may not be carried over via seed production. Successful propagation of ornamental plants by rooting of vegetative stem cuttings depends on several factors including physiological state us of stem cuttings, the propagation environment, fertility management, and growth regulator treatments, whether applied to the stock plant prior to harvesting or exogenously applied rooting hormones to stem cutting (Atzmon et al., 1997; Hartmann et al; 2002).

2.5. Factors affecting the regeneration of plants from cuttings

Great differences exist among species and among cultivars in the rooting ability of cutting taken from them. Stem cutting of some cultivars root so readily. On the other hand cuttings of many cultivars or species have yet to be rooted, cuttings of other cultivars can be rooted only if various influencing factors are taken into consideration and maintained at the optimum condition. The environmental factors are of great importance to the last group, and the attention given to them makes the difference between success and failure in obtaining satisfactory rooting (Hartmann and Kester, 1975).

2.5.1. Physiological condition of stock plant

The physiological age of plant material harvested for propagation has been documented as an important factor in some tropical species. Terminal stem or tip cuttings includeing the stem apex or shoot tip, developing immature leaves, and
one or more mature leaves. Generally produce more efficient crop, by effectively rooting in short time (Dole and Wilkins, 1999). Older stem tissue is slow to root than young tissue, but generally cuttings with thick stems and short internodes produce best plants. Bougainvillea (Bougainvillea glabra choisy) roots better from semi-hardwood to hardwood cuttings depending on temperature (Schoellhorn and Alvarez, 2002) location (Auld, 1987) and time of year (Chakraverty, 1970). In plants that are difficult to root the age of the stock plants can be very important factor. Either stem or root cuttings taken from young seedlings plants (in the juvenile growth phase) will root more readily than those taken from older plants (in adult growth phase). Juvenility in relation to rooting may possibly be explained by the increasing production of rooting inhibitors as the plant grows older. Stem cuttings taken from young seedlings of number of Eucalyptus species root easily but as the stock plants become older rooting decreases dramatically (Hartmann and Kester, 1979).

There is considerable evidence that the nutritional status of the mother plant affects the development of roots and shoots from cuttings taken from such plants. Many internal factors such as auxin levels and carbohydrate storage can of course influence root initiation on cuttings (Hartmann and Kester, 1975). The capacity for stem to root has been shown to be due to an interaction of inherent factors present in the stem cells as well as to transportable substances such as auxins, carbohydrates, nitrogenous compounds, vitamins and various unidentified. Substances that interact with auxins to affect rooting may be referred to as rooting co-factor (Janick, 1979).

2.5.2. Mineral nutrition

Mineral nutrition is one of many factors influencing formation of adventitious roots in cuttings. Rooting stages can be generalized into two categories root initiation and root growth and development (Blazich, 1988). Therefore when considering the influence of various mineral on adventitious root formation one should consider the function of these elements during each stage of development. Root initiation involved dedifferentiation of specific cells leading to the formation of root meristems and is dependent on the presence of auxin whether endogenous or artificially applied (Blazich, 1988). Most studies have not given clear understanding of the importance of
specific nutrients in the initiation of adventitious roots, however, one could argue that any nutrient essential for root initiation is also necessary for plant growth and development (Hartmann et al., 2002).

2.5.3. Rooting hormone treatments

Countless studies have reported on the stimulatory influence of auxin on the propagation of cuttings of difficult to root species (Cerveny, 2006). Treating cuttings with auxin increases rooting percentage hastens root formation and increases uniformity of rooting (Davis et al., 1988). Indole acetic acid (IAA) is the naturally occurring auxin found in plants and has been documented in nearly every aspect of plant growth and development. Some of the processes regulated by IAA included induction of cell division, stem elongation, apical dominance, induction of rooting and vascular tissue differentiation (Srivastava, 2002).

2.5.4. Growing Environment

Propagation success also depends on the level to which a suitable growing environment is offered. The environmental factors such as temperature, ambient air temperature and relative humidity levels should be fully monitored during propagation. Whether the plant material is herbaceous, softwood, semi hardwood, or hardwood. Rooting is affected by temperature as well as the plant. In general the air temperature must be less than that of the rooting media or soil. That means callus formation is more faster in warm climate specially in herbaceous plants (Elgaitani, 1990). Day time air temperature of 21 - 27°C with night temperature about 15°C are satisfactory for rooting cutting of most species (Hartmann and Kester, 1975). Tropical Plants such as Bougainvillea benefit from bottom soil temperatures of 30 °C (86 F) (Singh et al., 1976). If bottom heat is provided, soil thermometers or remote recording sensors should be installed within the root zone area and checked frequently. Excessively high temperatures in substrate even for a short time are likely to resulted in damage to basal tissue. In addition to temperature it is important to maintain high humidity levels while rooting vegetative stem cuttings in order to reduce water loss due to transpiration (Hartman et al., 2002). Intermittent mist should be used to maintain humidity levels. But unfortunately
prolonged periods of mist can leach mineral nutrients (Hartman et al., 2002). In most cases cuttings benefit from either a top dressing of slow release fertilizer or a low level application of liquid fertilizer after roots begin to emerge from the cutting base, because some tropical plants often require extensive rooting time in propagation (Howard, 1994). Callus formation on the base of cutting is a result of cambium activity which leads to respiration of tissues and for this it is important to use rooting media that allow for good aeration (Elgaitani, 1990).

2.5.5. Season

The season can have in some instances dramatic influence on the results obtained in rooting of cuttings and may provide the key to highly successful rooting. Season of the year in which the cuttings are taken is very important for some species. However, for others it makes no differences. Excellent rooting of leafy olive cuttings under mist can be obtained during late spring and summer, whereas rooting drops almost to zero with similar cuttings taken in winter (Hartmann and Kester, 1975).

2.6. Application of tissue culture in Allamanda cathartica

Propagation of herbaceous plants and trees, whether ornamentals, fruit crops or forest trees by tissue culture techniques has been described as having potential merit to rapidly increase clones with specific growth characteristics in large number necessary for plantation condition. The application of these techniques to Allamanda may provide solution to mass production of elite clones with desirable ornamental traits.

2.6.1. Different factors affecting in vitro propagation of plants

2.6.1.1. Type of explant

Explant choice and the time of excision are important for culture success. Healthy and vigorously growing plants will render suitable explant. Origin and size of explants tissue determine the development of established culture. Physiological status of parenchyma cells surrounding the cut surface amount of callus, explanted cell, tissue and organ as well as their environmental condition must be sterilized (Tweeddle et al., 1984).
2.6.1.2. Medium composition

One of the most important factors affecting the growth and morphogenesis of plant tissue in culture is the composition of culture medium. The basic nutrient requirements of cultured plant cell are similar to those of whole plants. Plant tissue media include the major and minor nutrient, carbohydrates, trace amount of certain organic compounds, like vitamin, amino acids and plant growth regulators and other components (George and Sherrington, 1984). There are different formulations of media that have been used in tissue culture such as B5 (Gamborge et al., 1968) and Nitsch and Nitsch (1969) but the formulation developed by Murashige and Skooge (MS) (Murashige and Skooge, 1962) is the most common medium employed for plant tissue culture (George and Sherrington, 1984). A pH of the culture medium of 5.7-5.8 is suitable for maintaining all the nutrients in soluble form. Gelling agent should be dissolved before the adjustment of the pH (Murashige and Skooge, 1962).

2.6.1.3. Humidity

The humidity of cultured tissues is always maintained to prevent drying of the cultures. The relative humidity in growth rooms should be kept at 70%, while for inside culture vessels it should be greater. Ibrahim (2006) reported that high humidity (98% RH) in side culture vessels appeared to assist in the formation of shoot verification on carnation.

2.6.1.4. Light

Light plays an important role in inducing morphogenesis in tissue culture (Murashige, 1974). George and Sherrington (1984) showed that the rate of photosynthesis by most cultured tissue was relatively low and cultures were mainly dependent of external supply of sucrose under low light density. They also found that wavelength light intensity and photoperiod influenced growth and morphogenesis of cultured tissues. Moreover light quality has been reported to affect in vitro cultures in both the red and blue ends of the visible spectrum. The blue light in the region of 467 nm was effective in inducing bud formation from tobacco callus (George and Sherrington, 1984).
2.6.1.5. Temperature

Most tissue cultures are maintained in incubation rooms at constant temperature day and night. The average temperature employed in most experimental reports was found to be 25°C. However there were some plant species that have certain temperature requirement for growth and development. For example *Begonia rex* produced the highest number of shoots at 18°C (Fonnedhbech, 1974), while shoot tip culture of *Flouribunda* rose grew best at 27°C (Abbas, 2008).

2.6.1.6. Genotype

The growth of cultured tissues or organ and in vitro morphogenesis are probably more influenced by genotype than by any other factor (George and Sherrington, 1984). The culture environments and media need to be changed from one genus or species to another.

2.7. Uses of *Allamanda cathartica*

2.7.1. Vase life of cut flowers of Allamanda

The greatest desire of a florist is to prolong the vase life of flowers. The appearance of bent neck (slight bending of the floral axis), wilting of outer petals and yellowing leaves indicate the end of useful vase life of cut flowers (Ketsa and Narkbua, 2001; Reid, 2002). Water and food supplies are major factors that contribute to the performance of cut flowers such as roses (Ketsa et al., 1993). A high concentration of carbohydrates in the leaves of harvested cut flowers is a prerequisite for long vase life (Marissen, 1995).

The vase life of many flowers can be extended by the application of different chemicals. Hormones such as cytokinins have been shown to prolong the vase life of cut Flowers (Mor et al., 1985). Coconut milk as a source of cytokinins has been shown to delay the senescence of cut Allamanda flowers (Fredrick, 1995). Gibberellic acid has also been shown to increase the vase life of cut flowers by preventing leaves from yellowing early (Funnel and Heins, 1998).
2.7.2. Medicinal uses

Medicinal plants form the backbone of traditional medicine in the last few decades with intense pharmacological studies. They are considered as potential sources of new compounds of therapeutic value and as sources of lead compounds in drug development. In developing countries it is estimated that about 80% of the population depends on traditional medicine for their primary healthcare (Hassan et al., 2007). The latex of Aallamanda is employed as purgative and for relieving colics. It has also been implicated in the treatment of malaria and Jaundice (Nayak et al., 2006).
CHAPTER THREE
MATERIAL AND METHODS

This study was carried out under green and lath house condition and the plant tissue culture laboratory of the Agricultural Research Corporation (ARC) Wad medani Sudan during the period 2011-2013.

3.1. Plants materials

Stock plants of Allamanda cathratica were collected from different nurseries in Wad medani, Sudan. The plants were cultured in pots containing a mixture of silt and oil (1:2). Plant were fertilized monthly with urea. Cuttings of 15cm long were taken for the experimental work.

3.2. Vegetative Propagation

3.2.1. Effect of type of cutting on vegetative propagation of Allamanda

In this experiment the response of two types of cuttings (shoot tip and nodal hard wood) were tested in ten replications. They were cultured in pots containing sandy soil and covered with plastic sheets. The plastic cover was removed after three weeks and data were collected.

3.2.2. Effect of charcoal on vegetative propagation of Allamanda

In this experiment the effect of two types of charcoal (Locally made charcoal and activated Charcoal) was tested. Water was used as control. Five grams from each type of charcoal were placed in 100ml of water, which represented a replication. Eight replication per treatment and 4 plants per replication were used. The water was changed every 3 days.

3.2.3. Effect of type of media on propagation of Allamanda

The effect of three types of media (sand, clay and peat moss) was tested on the propagation of Allamanda shoot tips in ten replications and 4 cuttings per replication.
3.2.4. Effect of application time of Radicante on vegetative propagation of Allamanda

Four times (0, 30, 60, 90 minute) of applications of rooting hormone (radicante) were tested on propagation of Allamanda. Nine gram from the powder hormone were dissolved in one liter of distilled water and distributed in 4 replications per treatment and 10 plants per replication.

3.2.5. Data collection and experimental design

Data on percentage of cuttings with shoots, number of shoots per cutting and number of leaves were collected after three weeks. Completely randomized design was used. Data were analyzed by MSTATC program and means separation was done by Duncan Multiple Range test. Data were transformed to square root before analysis when ever necessary.

3.3. Propagation of Allamanda cathratica by tissue culture

3.3.1. Preparation and sterilization of explants

Plant material used in this study was nodal explants of Allamanda. Explants were first washed in running tap water for 30 minute to remove all surface dust and then sterilized by dipping in 70% ethanol for 2 seconds followed by 15% chlorox with three drops of tween 20 per 100 ml, as wetting agent for 30 minutes. Explants were washed three times with sterilized distilled water to remove all residues of the disinfectant.

3.3.2. Basal nutrient medium

The basal nutrient medium as Murashige and Skoog (1962). The meduim was solidified using agar at 0.8% and then the pH was adjusted to 5.7± 0.1 with NaOH or HCL before autoclaving.

3.3.3. Sterilization

Medium were sterilized by autoclaving at 121ºc under pressure of 1.05Kg/cm for 15 minutes. Forceps and dissecting blades were dipped in 95% ethanol followed
by flaming, after every use they were again dipped and reffamed. The laminar air flow cabinet was surface sterilized by ethanol (70%). It was switched on for 15 minutes before using. The UV lamp was switched over night to disinfect the transfer room.

3.4. Research work
3.4.1. Effect of different concentrations of kinetin
Murashige and Skoog (MS) (1962). Medium was supplemented with kinetin at concentrations of 0, 0.5 ,1 and 2mg/l. Each treatment was replicated 10 times and incubated for 8 weeks. The percentage of explant with shoot, number of leaves and callus size were observed weekly.

3.4.2. Effect of different levels of BAP on in vitro morphogenesis of nodal explant of Allamanda
Morphogenesis of nodal explant was investigated on MS medium was supplemented with different concentrations of BAP (0, 0.5, 1, 2 mg/l ) each treatment was replicated 10 times and 4 explants treatment were incubated for 8 weeks. The percentage of explant with shoot, number of leaves, shoot percentage were observed weekly.

3.4.3. Incubation conditions
All cultures were maintained in culture room at 25 ±2 °C , 16 hours light and 8 hours dark using fluorescent lamps (1000 lux).

3.4.4 Data analysis
The experimental design used was the completely randomized design (CRD) and LSD was used for means separation.
CHAPTER FOUR  
RESULTS AND DISCUSSION

4.1. Vegetative Propagation

4.1.1. Effect of type of cutting on vegetative propagation of Allamanda

The effect of shoot tip and hard cutting on the percentage of explants with shoots were comparable after three weeks from propagation (Table 1). The shoot tips of Allamanda cathartica gave significantly higher number of leaves compared to hard cutting. This result might be due to the fact that shoot tip cutting generally produce a new plant faster since a well developed shoot is already present as reported by Gary (1982). Our result agreed with those of Hartmann and Kester (1975) who reported that the presence of leaves on cutting exerts strong stimulating influence on root. Similarly Ismail (2011) found that the shoot tip cutting of Dieffenbachia species gave high percentage of rooted compared to cane cutting.

4.1.2. Effect of charcoal on vegetative propagation of Allamanda

Addition of the two types of charcoal to water did not affect the percentage of regenerated shoots and number of leaves of Allamanda in comparison with water alone (Table 2). Water dilute the phenolic oxidates and charcoal adsorps the phenolic complex an and Staden, 1998). Altayeb (2008) reported that the addition of charcoal at 2.0 g/l gave better shoot length, number of nodes, number of leaves and vigor of Acacia seyal. Abdalla (2013) reported that the addition of charcoal (3g/l) promoted shoot elongation, enhanced root induction and root elongation of Rose cv. Sara in the presence of a cytokinin (BAP). The charcoal was added for three days after that charcoal was replaced by water to the end of experiment. Also the frequent change of water diluted the phenolic compounds excreted by explants.
Table (1): Effect of type of cutting on vegetative propagation of Allamanda after 3 weeks

<table>
<thead>
<tr>
<th>Type of cutting</th>
<th>% of shoot</th>
<th>Number of leaves per cutting</th>
</tr>
</thead>
<tbody>
<tr>
<td>Shoot tip</td>
<td>100</td>
<td>4.9a</td>
</tr>
<tr>
<td>Hard cutting</td>
<td>100</td>
<td>2.95b</td>
</tr>
<tr>
<td>Sig</td>
<td></td>
<td>*</td>
</tr>
</tbody>
</table>

Means having the same letters in columns are not significantly different according to Student T-test
Plate (1) plant regenerated from stem cuttings of Allamanda covered with plastic sheets after 3 weeks
Plate (2) plant regenerated from shoot tip of Allamanda covered with plastic sheets after 3 weeks
Table (2): Effect of charcoal on vegetative propagation of Allamanda after 3 weeks

<table>
<thead>
<tr>
<th>media</th>
<th>% of shoot</th>
<th>Number of leaves per cutting</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water + charcoal</td>
<td>68.8</td>
<td>4.7</td>
</tr>
<tr>
<td>Water + AC</td>
<td>68.8</td>
<td>4.1</td>
</tr>
<tr>
<td>Water</td>
<td>68.8</td>
<td>4.3</td>
</tr>
<tr>
<td>SE±</td>
<td>3.1</td>
<td>0.28</td>
</tr>
<tr>
<td>CV%</td>
<td>23.1</td>
<td>32.5</td>
</tr>
</tbody>
</table>
Plate (3) shoot and root initiation on water
4.1.3. Effect of type of media on propagation of Allamanda

All Allamanda cuttings were successfully propagated on all media (Table 3). These results showed that the best numbers of leaves were obtained on loamy soil and peat moss, which were comparable, but significantly different from that of sand. This result might be due to the fact that the loamy soil is more fertile and the physical properties of these medium are better than sandy soil. Our results agreed with those of Adams et al. (1984) who found that the mixture of loamy soil and fine sand was very productive horticultural soil due to the good water holding capacity and hence increased growth and development. These results were confirmed by Mustafa (2008) who found that the highest plant height and number of leaves of Ashoka tree (Polyalthia longifolia) were induced on silty soil. Similarly our results are consistent with those of Ismail (2011) who reported that the highest number of leaves per plant of two varieties of Diffenbachia (Amoeno and Tropic snow) were obtained on loamy soil and peat moss.

4.1.4. Effect of application time of Radicante on vegetative propagation of Allamanda

Table (4) showed the effect of water in comparison with rooting hormone (Radicante) on vegetative propagation of Allamanda cathartica. All cutting regenerated shoot and roots on water and Radicante application after 4 weeks. However, the best time for Radicante application was 30 minutes, which was significantly better in number of roots compared to water. There were no significant differences in the number of leaves per cutting for the different application times. Results obtained has suggested that some tropical species such as oleander (Nerium Oleander L.) and bougainvillea benefited from applications of 3,000 mg.L-1 IBA, and 4,000 to 16,000 mg.L-1 IBA respectively (Hartmann et al., 2002). In other difficult-to-root tropical species, an evaluation of commercially available rooting hormones should be conducted to determine the optimum concentration for obtaining the highest rooting percentage. Auxin containing rooting hormones can be applied to the base of cuttings as talc based powder or dipped in a liquid solution containing 50% ethanol or isopropyl alcohol and 50% water. Cuttings are dipped for a period of time from a few seconds to 12 hours (Dole and
Wilkins, 1999). Liquid treatments usually provide the most consistent results because application is more uniform than with powder.
Table (3): Effect of type of media on propagation of Allamanda after 3 weeks

<table>
<thead>
<tr>
<th>Type of soil</th>
<th>% of cuttings with shoots</th>
<th>Number of leaves per plant</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sand</td>
<td>100</td>
<td>3.8b</td>
</tr>
<tr>
<td>Loamy</td>
<td>100</td>
<td>5.1a</td>
</tr>
<tr>
<td>Peatmoss</td>
<td>100</td>
<td>5.1a</td>
</tr>
<tr>
<td>CV%</td>
<td></td>
<td>19.9</td>
</tr>
<tr>
<td>SE±</td>
<td></td>
<td>0.29</td>
</tr>
</tbody>
</table>

Means in columns with the same letter are not significantly different at P≤0.05 according to Duncan's Multiple Range Test.
Plate (4) Propagation of Allamanda on loamy soil after 3 weeks
Plate (5) Induction of shoot and root regeneration on loamy soil.

Table (4): Effect of application time of Radicante on vegetative propagation of Allamanda after 4 weeks
<table>
<thead>
<tr>
<th>Time (minute)</th>
<th>% of rooted per cuttings</th>
<th>Number of root per cutting</th>
<th>% of shoot per cutting</th>
<th>Number of leaves per cutting</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>100</td>
<td>(1.3) b 1.1</td>
<td>(87.5) 9.3</td>
<td>(4.4) 2.1</td>
</tr>
<tr>
<td>30</td>
<td>100</td>
<td>(4.1) a 2.3</td>
<td>(100) 10</td>
<td>(3.5) 1.8</td>
</tr>
<tr>
<td>60</td>
<td>100</td>
<td>(3.6) a 1.8</td>
<td>(87.5) 9.3</td>
<td>(3.1) 1.7</td>
</tr>
<tr>
<td>90</td>
<td>100</td>
<td>(3.6) a 1.8</td>
<td>(100) 10</td>
<td>(3.1) 1.7</td>
</tr>
<tr>
<td>CV%</td>
<td></td>
<td>(30.6) 16.1</td>
<td>(18.9) 10.8</td>
<td>(29.0) 14.94</td>
</tr>
<tr>
<td>Sig.</td>
<td>**</td>
<td>NS</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>SE±</td>
<td>(0.5) 0.14</td>
<td>(8.8) 0.5</td>
<td>(0.5) 0.14</td>
<td></td>
</tr>
</tbody>
</table>

Means within columns with the same letter are not significantly different at P≤0.05 according to Duncan’s Multiple Range Test.

** and NS indicates significant at P≤0.01 , 0.001 and not significant, respectively.
4.2. Propagation by Tissue Culture

4.2.1. Morphogenesis of nodal explants of *Allamanda cathartica* on MS medium supplemented with different concentrations of kinetin after 4 weeks

Percentages of explants with shoots and number of leaves per explants were not significantly different on the different concentrations (0.0, 0.5, 1 and 2mg/l) of kinetin after 4 weeks. These results agreed with those of Abdalla (2012) who found that nodal explant of *Plumbago auriculata* did not give any shoot morphogenesis on different concentrations of kinetin after 4 weeks. These results agreed with those of Ibrahim (2006) who stated that *Lantana camara* L. on different levels of kinetin showed no significant differences with respect to shoot proliferation and number of shoot. However number of leaves was slightly higher in explants cultured on MS supplemented with 1.0 mg/l kinetin compared to the other concentrations and was significant with all treatments.

4.2.2. Morphogenesis of nodal explant of *Allamanda cathartica* cultured on MS medium supplemented with different concentrations of BAP after 4 week.

Table (6) showed the effect of BAP on morphogenesis of nodal explants of *Allamanda cathartica* after 4 weeks. All explants regenerated shoot, on MS medium supplemented with different concentrations (0.0, 0.5, 1.0 and 2.0 mg/l) of BAP after 4 weeks. The number of shoots per explants was significantly higher on 2.0mg/l BAP compared with those on MS medium devoted from BAP. There were no significant differences among the numbers of shoots regenerated on BAP at 0.5, 1.0 and 2.0 mg/l concentration. Number of leaves per explant for the different concentration of BAP was not differ significantly. The best concentration for shoot regeneration was 0.5 mg/l BAP. This result agreed with that of Ibrahim (2006) who found that the highest proliferation rate was obtained on 0.8 mg/l BAP on *lantana camara* L. Moreover, Abbas (2008) found that the best number of shoots induced on Floribunda rose cultivar "Sara" culture was on 0.2 mg/l BAP. Wong *et al.* (2012) disagreed with these result by finding that the best shoot multiplication of *Allamanda cathartica* from nodal explant was induced on MS supplemented with BAP at 5.0 mg/l BAP. These results are in consistent with those of Abdalla (2012) who found higher percentage of explants with shoot morphogenesis of
*Plumbago auriculata* in BAP at concentration 0.02 mg/l. But after 4 weeks internal bacterial contamination was observed on all cultures, which suppressed the growth of plantlets in both experiments.
Table (5): Morphogenesis of nodal explants of *Allamanda cathartica* on MS medium supplemented with different concentrations of Kinetin after 4 weeks

<table>
<thead>
<tr>
<th>Kin conc. (mg/l)</th>
<th>% of explants with shoot</th>
<th>No. of Shoot per explants</th>
<th>No. of Leaves per explants</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0</td>
<td>100</td>
<td>(1.5) 1.2</td>
<td>(3.5) 1.9</td>
</tr>
<tr>
<td>0.5</td>
<td>100</td>
<td>(2.2) 1.5</td>
<td>(3.5) 1.9</td>
</tr>
<tr>
<td>1.0</td>
<td>100</td>
<td>(2.5) 1.6</td>
<td>(4.4) 2.1</td>
</tr>
<tr>
<td>2.0</td>
<td>100</td>
<td>(2.3) 1.5</td>
<td>(3.3) 1.8</td>
</tr>
<tr>
<td>Sig</td>
<td></td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>CV%</td>
<td></td>
<td>25.6</td>
<td>29.75</td>
</tr>
<tr>
<td>SE±</td>
<td></td>
<td>0.11</td>
<td>0.18</td>
</tr>
</tbody>
</table>

Means in columns followed by the same letter are not significant at P≤0.05 according to Duncan’s Multiple Range Test.

NS = Not significantly different.
Table (6): Morphogenesis of nodal explants of *Allamanda cathartica* on MS medium supplemented with different concentrations of BAP after 4 weeks

<table>
<thead>
<tr>
<th>BAP (mg/l)</th>
<th>% of explant with shoot</th>
<th>No. of shoot per explants</th>
<th>No. of leaves per explant</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0</td>
<td>100</td>
<td>(1.4) 1.1 b</td>
<td>(2.6) 1.5</td>
</tr>
<tr>
<td>0.5</td>
<td>100</td>
<td>(2.4) 1.5 ab</td>
<td>(2.6) 1.5</td>
</tr>
<tr>
<td>1.0</td>
<td>100</td>
<td>(1.6) 1.2 ab</td>
<td>(3.8) 1.8</td>
</tr>
<tr>
<td>2.0</td>
<td>100</td>
<td>(3.0) 1.7 a</td>
<td>(5.2) 2.2</td>
</tr>
<tr>
<td>SE±</td>
<td></td>
<td>(0.45) 0.16</td>
<td>(0.87) 0.24</td>
</tr>
<tr>
<td>Sig.</td>
<td></td>
<td>*</td>
<td>NS</td>
</tr>
<tr>
<td>CV%</td>
<td></td>
<td>(49.9)25.81</td>
<td>(54.6) 29.93</td>
</tr>
</tbody>
</table>

Means within columns with the same letter are not significantly different at P≤0.05 according to Duncan’s Multiple Range Test.
* and NS indicates significance at P≤0.01, 0.001 and not significant, respectively.
CONCLUSIONS AND FUTURE WORK

1. *Allamanda cathratica* can be conventionally propagated by Shoot tip.
2. Application of Radicante for 30 minutes improved rooting of *Allamanda cathratica* stem cuttings.
3. Charcoal, wood or activated in liquid media did not improve rooting success of *Allamanda cathratica*
4. *Allamanda cathratica* can be propagated successfully by tissue culture using nodal explant and 0.5 mg/l BAP.
5. Further study is needed to investigate other conventional methods of propagation, growth regulators, rooting media and other genotypes of *Allamanda*.
6. Improvement of *in vitro* propagation technique is needed for mass propagation, especially the problem of contamination.
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