IN VITRO NODE CULTURE OF *ROSA INDICA* L.

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Abstract

Rosaceae is a large plant family, which has hundreds of genera and over thousands of species including shrubs, herbs and trees. Rose is a very important plant from various aspects. Nodes from matured plants were sterilized and cultured for 60 days under on MS (Musrashige and Skoog, 1962) medium supplemented with various concentrations of plant growth regulator IAA. Five treatments of IAA various concentrations (0,0.5mg/l, 1mg/l, 1.5mg/l and 2mg/l) were used in this experiment and each on solid medium. The results showed that the best growth of callus was obtained from 1.5 mg/l of IAA as compared with control and 0.5mg/l and 1mg/l of IAA showed the lowest growth of callus. The best growth of roots was obtained from 1 mg/l of IAA and 1.5 mg/l of IAA showed the lowest growth of roots.

INTRODUCTION

Rose is a woody perennial flowering plant of the genus Rosa, in the family Rosaceae. There are over three hundred species and thousands of cultivars. They belong to the Rosaceae and are grown worldwide as cut flower and potted plants and in home gardens. They form a group of plants that can erect shrubs, climbing, or trailing with stems that are often armed with sharp prickles. Flowers vary inside and shape and are usually large and showy in colors ranging from white through yellows and reds. Roses can be propagated by seeds, cutting, layering and grafting. Seed propagation often results in variation while other methods of rose propagation are low and time consuming. So, there is need to introduce efficient methods for faster propagation of roses (Shabbir *et al*., 2009).

Roses are one of the most important ornamentals and are most often used for ornamental, medicinal, and aromatic purposes (Kanchanapooma, 2010).

Most species are native to Asia with smaller numbers native to Europe, North America and Northwestern Africa. Species, cultivars and hybrids are all widely grown for their beauty and often are fragrant. Rose perfumes are made from rose oil which is a mixture of volatile essential oils obtained by steam distilling the crushed petals of roses. An associated product is rose water which is used for cooking, cosmetics, medicine and religious practices (Horn, 1992).

Rosa indica L. is used in the treatment of tuberculosis, asthma, leucorrhea, inflammation of mouth and pharynx. Rose tip lighters menstrual pains and bud used for the treatment of kidney stones (Rizk and Nowaihi, 1989). Rose flowers is used for promoting good health in women (Zhang, 2010). Rose water shows anticollagenase, antielastase, and antioxidant activities (Thring, 2009).

Plant tissue culture is the science of culturing isolated plant cells, tissues or organs on artificial media. The term plant tissue culture is an *in vitro* culture of any part of a plant, whether an organ, a tissue, a single cell or a protoplast under aseptic conditions and well formulated media to produce an entire plant (http://www.researchgate. net). German botanist G. Haberlandt is the father of tissue culture. Tissue culture system in rose has been established (Kim *et al*, 2003). When the node cultured in proper medium, it would produce rhizome. Some of the callus formation in Domingo and Vicken by using IAA (Wit *et al*., 1990). It is the important technique for the production of disease-free and high-quality plants within a short period of time. The techniques of tissue culture have been utilized extensively in research laboratories all over the world (Kim *et al*, 2003).

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Tissue culture is the growth of tissues or cells in an artificial medium separate from the organism. This is typically facilitated via use of a liquid, semi-solid or solid growth medium, such as broth or agar. Tissue culture commonly refers to the culture of animal cells and tissues, with the more specific term plant tissue culture being used for plants. Some of the propagation of commercial cultivars are difficult conventionally. Although the vegetative way is a predominant technique in roses propagation, but it does not ensure healthy and disease-free plants. Moreover, in conventional propagation, major limiting factors depend on season and slow multiplication rates(Bhojwani,1996).

In vitro culture, technique is an alternative method for plant propagation. Micropropagation of roses was reported by various researchers using cultures of axillary buds and apical meristems (Pati *et al*. 2006).

There are five types of plant hormones. They are auxins, cytokinins, gibberellins, ethylene and abscissic acids. Auxins were the first plant hormones discovered. The Greek word "auxein," which means "to increase" or "to grow" and Indole-3-acetic acid (IAA) is the most common plant hormone of the auxin class. It regulates various aspects of plant growth and development (Teale *et al*, 2006). As all auxins, IAA has many different effects, such as inducing cell elongation and cell division with all subsequent results for plant growth and development. Auxins promote both cell division and cell growth. The most important naturally occurring auxin is IAA (indole-3-acetic acid). In plant cell culture media, the use of auxin is limited because it is unstable to both heat and light Auxins that are widely used in plant propagation and tissue culture. In tissue culture, auxins are balanced with cytokinins for full morphogenesis. The potent natural auxin, indole-3-acetic acid (IAA), is available in both acid and salt form. Naturally occurring phenylacetic acid is weaker than IAA, but may be more abundant in nature. Synthetic auxins, such as 1-naphthaleneacetic acid (NAA) and 2-4-dichlorophenoxyacetic acid (2,4 -D) are potent and less expensive alternatives to IAA. 2,4-D is very often the auxin of choice in inducing callus and in keeping cells in the dedifferentiated state. Most of our auxins are available as acids or as salts (www.aladdin-e.com).

Auxin

Auxin, IAA (indole-3-acetic acid) are powerful growth hormone produced naturally by plants. They are found in shoot and root tips and promote cell division, stem and root growth and cell elongation. Auxins are the most commonly use as the growth hormones in culture medium prepared for tissue culture. Auxins promote root initiation and Auxin induces both growth of pre-existing roots and adventitious roots formation (Chambers, 1999).

Agar

Agar solidifies the medium and acts as a substratum. It doesn't have nutritive importance. It is obtained from sea weeds and is used to provide solid surface for growth. When using agar, within any growth medium, it is important to know that the solidification of the agar is pH-dependent. The optimal range for solidification is between 5.4 - 5.7.

The major commercial use of tissue culture techniques in vegetative propagation of roses is the combination of rapid multiplication and regeneration. In the *in vitro* techniques, the small quantity of material source has promoted the plant propagation research process. Roses with different hormones have been done in tissue culture for callus initiation, its maintenance and regeneration of shoots and roots from callus and direct from nodal segments (Ali *et al*., 1993).

Tissue culture has largely been integrated into biotechnology as a research tool for callus formation, proliferation, and regeneration of plantlets. Callus is a wound tissue composed of highly vacuum. Callus is an undifferentiated mass of tissue which appears on explants within a few weeks of transfer onto growth medium with suitable hormones (Bhojwani and Razdan, 1996). According to the above facts, the study was aimed to find out the effect of various concentration of auxin on the *Rosa indica* L. nodes in *in vitro* culture and to observe the rapid growth and emergence of the new roots and callus within short time.

MATERIALS AND METHODS

Plant material collections

The plants of *Rosa indica* L. Rose (Hinn-Si) were collected near the University of Yangon, Kamayut Township in Yangon Region. The experiment was carried in Tissue Culture Lab of Botany Department in Dagon University from July to December 2019.

Surface Sterilization

Firstly, the explants were washed by sterile distilled water (Running tap water) for 10 seconds. And remove the leaves and then, recut into 3-4 cm long internodal segments. Those nodal segments were sterilized by 15% sodium hypochlorite for 15 minutes constant shaking. Finally, they were washed with ethanol, sterilized distilled water, put into filter paper and recut 1cm long internodal segments for node culture preparation

Culture vessels

Plastic boxes with the capacity of 250 ml, each containing 30 ml of solid medium were used.

Culture medium

MS (Culture medium) media was used as a basal medium for the experiment. The basal medium with supplemented various levels of concentrations of IAA. According to the Murashige and Skoog (1962), method of 1000ml medium preparation applied in the present study is as follow; 300ml distilled water was poured into 3000ml beaker. Proper amount of stock solution were added. Sugar 30g was added and stirred. Agar powder 8g was slowly added and stirred. The pH value was measured and adjusted (5.8) with 0.1N NaOH and 0.1N HCl. The volume of solution was made up to 1000 ml and the level was marked. The solution was gently heated until it started to boil. Then, medium was heated and stirred until agar was completely dissolved and became amber-colored. The medium was dispensed into culture plastic boxes. The culture plastic boxes were covered and autoclaved. Then, the plastic boxes were cooled and used. One node was cultured for each treatment. The culture plastic boxes were covered with plastic tape for convention of evaporation from the media and laboratory mites.

Table 1. Nodal segments of *Rosa indica* L. were cultured in MS medium containing various concentrations of Auxin.

Treatments	Basal Medium (solid)	Various concentrations of Auxin (mg/l)
T_0	MS	Control
T_1	MS	0.5
T_2	MS	1.0
T_3	MS	1.5
T_4	MS	2.0

Culture conditions

Cultures were maintained under 8 hours daily lights at 22 ± 2˚C and 35-38 % of relative humidity.

Data collections

Growth of callus and rooting formation, was recorded every 15 days. The length of the roots and root number, was recorded at 60 days after culture. The fresh and dry weight were recorded at 45 days and 60 days after culture.

Intact Plant | Excision of Explant | Washed by running tap water | Recut into 3-4cm long internodal segments

Figure 1. Cut the inter nodal segments (8–10 cm long) from *Rosa indica* L. plant near the University of Yangon, Kamaryut Township in Yangon Region.

15% Sodium hypochlorite for 15 mins constant shaking | Washed with ethanol, Sterilized distilled water and put into filter paper

Figure 2. Preparation for node culture sterilized and put into filter paper

pH meter

MS (Culture medium) media was used as a basal medium for the experiment. The basal medium with supplemented various level of concentration of IAA. All media were supplemented with 30 gl^{-1} sucrose and solidified with 8 gl^{-1} agar. Media were adjusted to pH 5.8 with 1N NaOH or 1N HCl.

30 ml of the media were added into 250 ml plastic boxes

Culture medium is sterilized in Autoclave

Figure 3. Preparation for culture media for aseptic condition

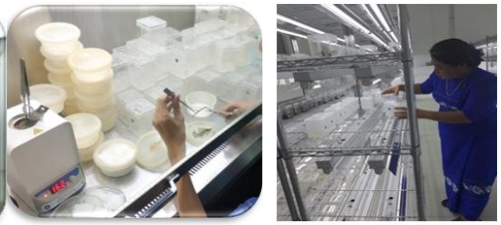

Figure. 4. Inoculation of explants in the Culture medium under aseptic conditions

RESULTS

Morphological characters of *Rosa indica* L.

Scientific Name – *Rosa indica* L.
Family – Rosaceae
English Name – Rose
Myanmar Name – Hnin-Si

Rosa indica L. plant has erect shrubs with sharp prickles that are opposite to each other. Flowers are white, yellow, or red in color. Leaves are borne alternately long, and pinnate with 3-13 leaflets. Flowers have five or more petals and 4-5 sepals. Rose hip is an aggregated fruit and berry-like structure.

Effect of hormone and its concentrations on callus and root formation

The results showed that the effect of various concentration of IAA with MS basal medium was studied. The highest callus formation (100%) were observed in 1.5 mg/l IAA and the lowest callus formation (60%) were observed in 0.5mg/l and 1mg/l and the other treatments 0 mg/l and 2mg/l have no callus formation (Table 2, Figure 5).

The highest root formation (80%) were observed in 1mg/l IAA and the lowest root formation (40 %) were observed in 1.5mg/l and 2 mg/l have (60%) and the other treatments have no root formation (Table 3, Figure 6).

The growth value showed that the response of nodal segment on various levels of auxin which were added to MS medium during 60 days in culture *Rosa indica* L. were observed in 0.5mg/l IAA had 3.4 and 2mg/l had 1.1 (Table 4,Figure 7,8).

Table 2. Effect of various concentrations of IAA hormones on callus formation

Treatments	15Days	30Days	45Days	60Days	Total	Percentage (%)
	Callus	Callus	Callus	Callus	Callus	Callus
T_0	-	-	-	-	-	-
T_1	1	2	-	-	3	60
T_2	-	3	-	-	3	60
T_3	2	3	-	-	5	100
T_4	-	-	-	-	-	-

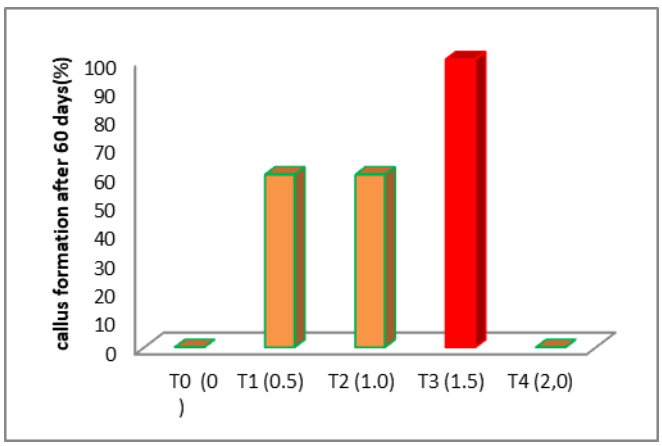

Figure 5. Effect of various concentrations of IAA in callus formation

Table 3. Effect of various concentrations of IAA hormones on root formation.

Treatments	15Days	30Days	45Days	60Days	Total	Percentage (%)
	Root	Root	Root	Root	Root	Root
T_0	-	-	-	-	-	-
T_1	-	-	-	-	-	-
T_2	1	-	3	-	4	80
T_3	-	-	2	-	2	40
T_4	1	-	2	-	3	60

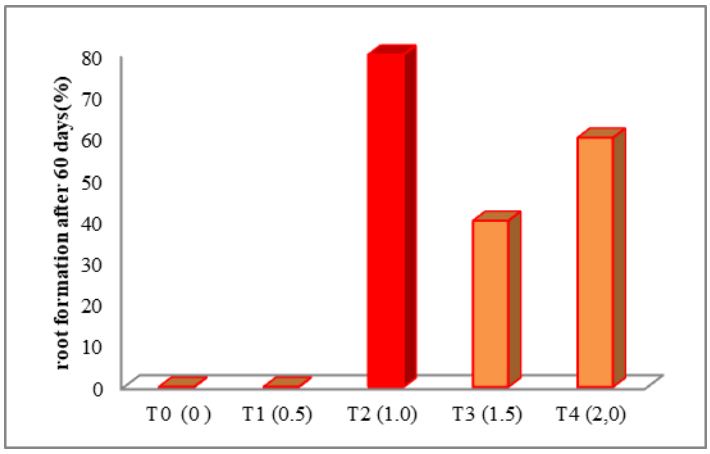

Figure 6. Effect of various concentrations of IAA in root formation

Table 4. The response of nodal segment on various levels of Auxin which were added to MS medium during 60 days in culture *Rosa indica* L.

Treatment	Fresh Weight(mg)		Dry Weight(mg)		Root number	Root Length (cm)	Growth value
	Initial	Final	Initial	Final	Final	Final	Final
T0	-	-	-	-	-	-	-
T1	110	370	40	150	-	-	3.4
T2	110	230	20	90	1	0.5	2.1
T3	150	300	50	140	1	0.6	2.0
T4	170	180	30	60	3	0.7	1.1

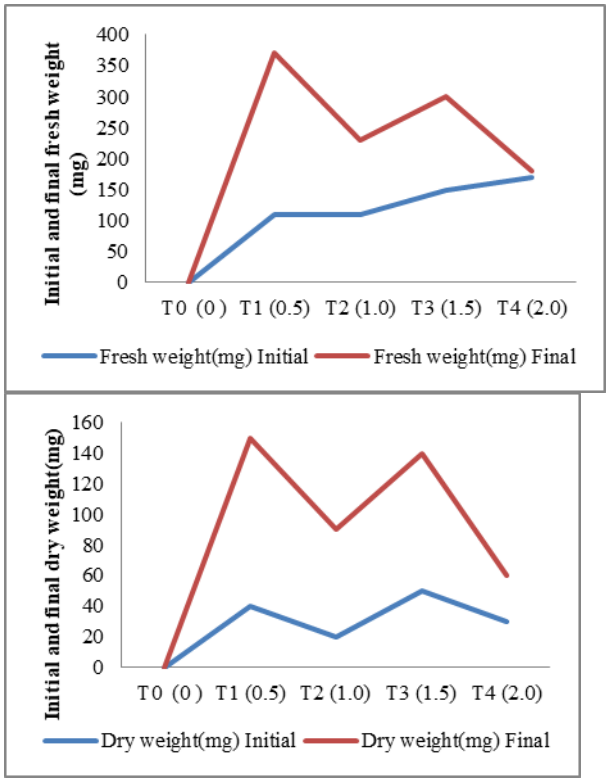

Figure 7. Growth Value of fresh weight and dry weight of *Rosa indica* L.

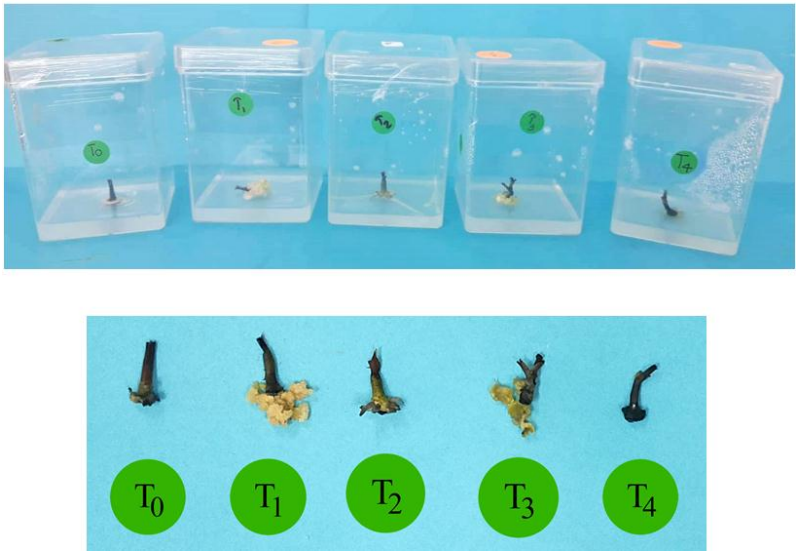

Figure 8. Fresh and Dry weight on MS medium supplemented with various concentrations IAA after 60days of culture period

DISCUSSION AND CONCLUSION

The nodal segments were cultured in the various concentration of IAA media (0, 0.5, 1, 1.5 and 2 mg/l) for *in vitro* propagation of *Rosa indica* L.

In this experiment, Murashige and Skoog's (1962) medium (MS) was used for rose propagation. Davies (1980) reported that the standard MS medium induced the best rates of the cell proliferation in different rose cultivars. The best results were obtained from Murashige and Skoog (MS) basal medium supplemented with 1.5 mg/l gave the best callus formation growth and 1mg/l gave the best root formation. Every

treatment has no root and callus formation. Callus formation was observed from the nodal segment explants when cultured on auxins alone in various concentrations. It is generally believed that auxin is the most active plant growth regulators in stimulating root multiplication, but Khosh-Khui and Sink (1982) demonstrated that combinations of auxins were more effective for rooting than using each auxin PGR alone.

In the present study, nodal explants were found to be the best responding explant producing maximum number of roots and callus formation. It is an agreement with Nontaswatsri and Fukai (2007) who reported that the successful plant regeneration from nodal segments of *D. caryophyllus* prooduce root and callus formation. Pati *et al* (2001) reported that friable callus was initiated from nodal segments of *R. damascena* and *R. bourboniana* cultured on MS medium supplemented with varying concentrations of PGR. Chambers,1999 reported that auxins promote root initiation and Auxin induces both growth of pre-existing roots and adventitious roots formation.

In conclusion, *Rosa indica* L. was established by manipulating the cultural condition and growth regulators. This study will help for conservation and mass propagation of roses for horticulture.

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