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# *In Vitro* Propagation of *Ficus lyrata*

S.Z. EL-AGAMY and M. EL-F. ZEAWAIL
*Faculty of Meteorology, Environment and Arid Land Agriculture, King Abdulaziz University, Jeddah, Saudi Arabia*

ABSTRACT. Survival of *Ficus lyrata* shoot tips was relatively high (82.22%) when they were sterilized by ethanol and calcium hypochlorite and cultured on Murashige and Skoog (MS) basal medium supplemented with (mg per liter) 0.02 Indolebutyric acid (IBA), 0.1 kinetin and 1.0 gibberellic acid ($GA_3$). Proliferation rate varied – according to cytokinin type and concentration – from 1.25 to 6.33 shoot/explant (overall average 3.62 shoot/explant). Proliferation occurred on MS medium supplemented with 0.5, 1.0 and 2.0 mg/L of either of kinetin or benzylaminopurine (BA). Rooting was easily (100%) obtained on MS medium supplemented with 0.5 mg/L IBA. Number of leaves, roots and average length of plantlets and roots were also recorded.

## Introduction

The manipulation of tissue techniques in ornamental plants has been found to introduce great potentials of propagation, breeding and elimination of pathogens (Murashige 1974, Harney and Knap 1979, Muriithi *et al.* 1982). Mass production of ornamental plants could be easily achieved through the proliferation of shoot tips and the differentiation of calli (Debergh and Dewael 1977, Cooke 1977, Khosh-Khui and Sink 1982, Arafa 1987). Debergh and Dewael (1977) and Debergh and Maene (1981) had developed *in vitro* propagation of some ornamental plants including *Ficus spp, Dracaena spp* and *Dieffenbachia* through shoot tips and callus regeneration.

Murashige and Skoog (1962) medium was proven to be a basic medium for enormous pieces of research. Growth hormones, *i.e.*, auxins, cytokinins and gibberellins were extensively used as great tools to direct and control growth towards a particular

way. Different forms of cytokinins are widely used either alone or combined with auxins in some specific combination to induce proliferation of aspectic shoot (Hill 1967, Yang and Close 1974, and Narayanswamy 1977). Auxins are the classic root inducer used in cuttings and proliferated shoots.

The objective of this work was to develop a technique for rapid clonal propagation of *Ficus lyrata* can be used in the local mass production of such ornamental plant in the western region of Saudi Arabia.

## Material and Methods

Planting materials of *Ficus lyrata* were brought from Hada Al-Sham Experiment Station, Faculty of Meteorology, Environment and Arid Land Agriculture, King Abdulaziz University. Shoot tips were cleaned and scales removed. Tips were excised aseptically at 4 to 6 mm in length and were subjected to sterilization procedure. Shoot tips were immersed in 70% ethyl alcohol solution for 2 minutes and then transferred into 5% calcium hypochlorite solution for 15 minutes. Explants were then rinsed 3 times in redistilled sterile water. Murashige and Skoog (MS) (1962) prepared basal medium was supplemented with (mg per liter); 0.02 Indolebutyric acid (IBA), 0.1 kinetin and 1.0 Gibberellic acid ($GA_3$). Medium pH was adjusted to 5.6-5.8 and agar was added as 7.0 g per liter. Medium sterilization was then proceeded in the autoclave at 120°C and 1.5 kg/cm$^2$ pressure for 40 minutes. Sterile explants were cultured aseptically on the sterilized medium under laminar flow hood. Cultures were maintained in culture room with 24 ± 1°C and a 16-hr photoperiod of cool-white fluorescent lamp.

Survival percentages were recorded 6 weeks after culturing. Survived explants were then transferred onto shooting media. Two types of cytokinins; *i.e.*, kinetin and benzylaminopurine (BA) at 3 levels each (0.5, 1.0 and 2.0 mg/L) were added to MS media in addition to control treatment. Number of proliferated shoots per tube was recorded after 4 weeks of culturing the survived explants. Proliferated shoots were cultured on MS medium supplemented with 0.5 mg/L IBA for rooting. Rooting percentages of proliferated shoots were also recorded. Other data such as number of roots and leaves per plantlet and the average length of roots and plantlet's height were also included.

Proliferation data in response to cytokinins application were statistically analyzed by the analysis of variance. Treatments means were separated for significance by the Duncan's multiple range test at 5% level (Steel and Torrie 1980).

## Results and Discussion

Disinfestation of explant by dipping in alcohol followed by calcium hypochlorite treatment proved to be efficient in eliminating sources of pathogen in cultured explants. Six weeks after explant culturing and incubation, survival percentages of each run was recorded. Percentages survival of shoot tips of *Ficus lyrata* cultured on MS medium supplemented with 0.02 mg/L IBA, 0.1 mg/L kinetin and 1.0 mg/L $GA_3$ ranged from 61.11 to 100 with an average of 82.22% survival (Table 1 and Fig. 1).

TABLE 1. Survival of *Ficus lyrata* shoot tips cultured on Murashige and Skoog medium (MS) with supplements*.

| Run | Cultured flasks (no.) | Survived explant | |
|---|---|---|---|
| | | (no.) | (%) |
| 1 | 18 | 18 | 100.00 |
| 2 | 18 | 16 | 88.89 |
| 3 | 18 | 16 | 88.89 |
| 4 | 18 | 13 | 72.22 |
| 5 | 18 | 11 | 61.11 |
| Average | 18 | 14.80 | 82.22 |

* Supplements were: mg per liter; 0.02 IBA, 0.1 kinetin and 1.0 $GA_3$.

FIG. 1. Aseptic establishment of *Ficus lyrata* shoot tip.

Proliferation of survived explant in response to cytokinin type and concentration was also studied (Fig. 2). In general, cytokinins proved to induce more shoots when added to the medium compared to control. For instance, proliferation rate of control treatment was 1.24 shoot/explant while it was 4.02 shoot/explant when cytokinin was added to the medium (overall average). Benzylaminopurine (BA) tended to be more effective in inducing shoots compared to kinetin. Average shoot number produced per explant was 4.48 when BA was added to the medium while it was 3.55 when kinetin was used (Table 2). Differences within and/or among cytokinin treatments were

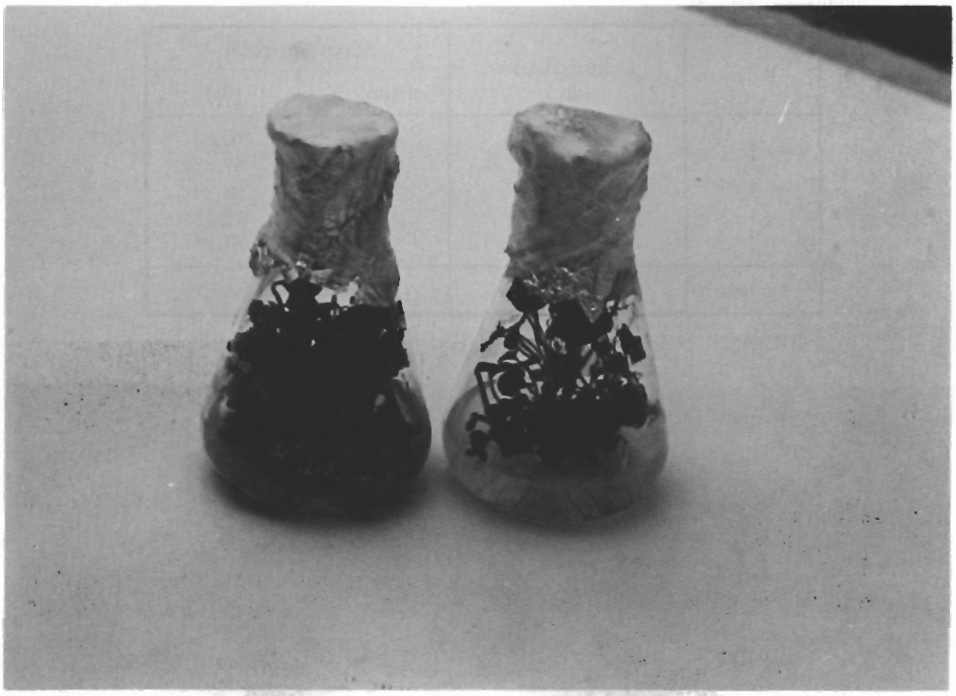

FIG. 2. Shoot proliferation of *Ficus lyrata* after 6 weeks of culturing.

TABLE 2. Proliferation rate of *Ficus lyrata* on MS medium supplemented with different cytokinin types and levels.

| Treatment | Cultured explant (no.) | Shoots produced (no.) | Proliferation rate (shoot / explant) |
|---|---|---|---|
| Control | 25 | 31 | 1.24 d* |
| 0.5 mg/l kinetin | 25 | 156 | 6.24 a |
| 1.0 mg/l kinetin | 25 | 68 | 2.72 c |
| 2.0 mg/l kinetin | 25 | 42 | 1.68 d |
| | | | ave. 3.55 |
| 0.5 mg/l BA | 25 | 116 | 4.64 b |
| 1.0 mg/l BA | 25 | 62 | 2.48 c |
| 2.0 mg/l BA | 25 | 158 | 6.32 a |
| | | | ave. 4.48 |
| | | Cytokinin average | 4.02 |
| | | Overall average | 3.62 |

* Means were separated for significance by the Duncan's multiple range test (1 and 5%).

statistically significant. Benzylaminopurine (BA) at 2.0 mg/l concentration and kinetin at 0.5 mg/l had the highest proliferation rates (6.32 and 6.24 shoot/explant respectively) followed by BA (0.5 mg/l) treatment with proliferation rate of 4.64 shoot/explant. Addition of one milligram per liter of either cytokinin to the medium had induced slightly lower proliferation rate of *Ficus lyrata* (2.72 and 2.48 shoot/explant for kinetin and benzylaminopurine respectively) but at least it was twice as much as control treatment produced (1.24 shoot/explant).

Proliferated shoots were successfully rooted (100%) when they were cultured on Murashige and Skoog medium supplemented with 0.5 mg/l indolebutyric acid (Fig. 3). It was also noticed that proliferated shoots had been rooted on such proposed medium just two weeks after culturing.

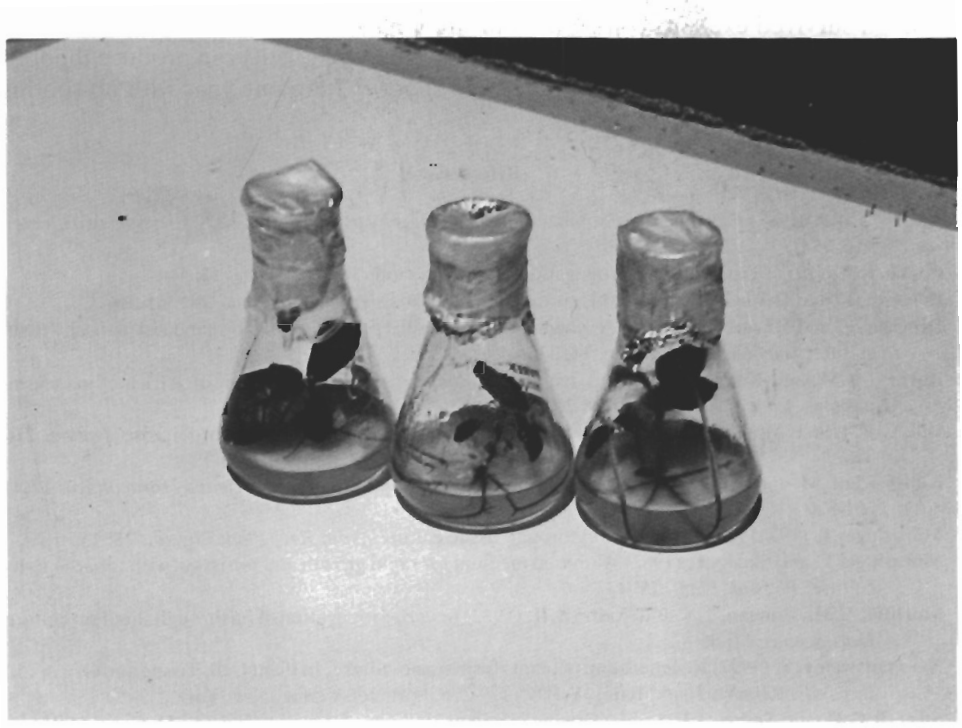

FIG. 3. *In vitro* adventitious root formation on proliferated shoots of *Ficus lyrata* (after two weeks).

Other morphological data including plantlet height, number of leaves per plantlet, number of roots per proliferated shoot and root length were taken four weeks after culturing on rooting medium. Average plantlet height was 3.13 cm (ranged from 1.6-5.2 cm) (Table 3). Number of leaves per plantlet varied from 3.0 to 7.0 with an average of 5.29 leaves per plantlet. Adventitious roots formed on proliferated shoots varied from 3.0 to 13.0 root/plantlet with an overall average of 7.38 root/plantlet. Such roots were about 2.34 cm in length as an average with a range from 1.2 to 3.4 cm.

TABLE 3. Averages number of roots and leaves per proliferated shoot and averages root length and plantlet height after one month of culturing on rooting medium*.

| Character | Range | Mean |
|---|---|---|
| Avg. number of roots/proliferated shoot | 3.0 – 13.0 | 7.38 |
| Avg. root length (cm) | 1.2 – 3.4 | 2.34 |
| Avg. number of leaves/proliferated shoot | 3.0 – 7.0 | 5.29 |
| Avg. plantlet height (cm) | 1.6 – 5.2 | 3.13 |

* Rooting medium was consisted of Murashige and Skoog basal medium 1962 supplemented with 0.5 mg/l indolebutyric acid.

In general, such proposed technique could be successfully used for *in vitro* rapid clonal propagation of *Ficus lyrata*. Survival percentage was sufficient in producing enough survived explants can be used in shooting step. Proliferation rate at a concentration of 2.0 mg/l benzylaminopurine (6.32 shoot/explant) can produce theoretically a tremendous amount of proliferated shoots within one year with no rooting problems.

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# الإكثـــار المعمـــلي لنبـــات الفيكس لـــيراتا

**سمير زكي العجمي و محمد الفاتح مصطفى زويل**
كلية الأرصاد والبيئة وزراعة المناطق الجافة ، جامعة الملك عبد العزيز
جـــدة - المملكة العربية السعودية

المستخلص . كانت نسبة الحياة للقمم المرستيمية لنبات الفيكس ليراتا عالية (٨٢,٢٢٪) عنـــدمـــا عقمت بالكحـول الإثيلي ومحلول هيبـوكلوريت الكـالسيوم وزرعت على بيئة موراشيجي وسكـوج المضـاف إليها (مجم/لتر) ٠,٠٢ إندول بيوتريك ، ١,٠ كينتين ، ١,٠ حمض الجبرليك ، تباين معدل التفريع - حسب نوع السيتوكينين وتركيزه - من ١,٢٥ إلى ٦,٣٣ فرع/منفصل نباتي (متوسط عام ٣,٦٢ فرع/منفصل نباتي) . وكانت بيئة التفريع تتكون من بيئة موراشيجي وسكوج مع ٠,٥ ، ١,٠ ، ٢,٠ مجم/لتر من أيٍّ من الكينتين أو البنـزيل أمينوبيورين . كان التجذير ١٠٠٪ عندما أضيف للبيئة ٠,٥ مجم/لتر إندول بيوتريك . عدد الأوراق والجذور وكذلك متوسط طول النباتات والجذور تم حسابهم .