

Full Length Research Paper

Influence of auxin and cytokinin on *in vitro* multiplication of *Ficus anastasia*

Ameena Abdulla H. S. Al Malki and Khaled M. Suliman Elmeer*

Biotechnology Centre, Ministry of Environment, Doha - Qatar.

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The role of cytokinin and auxin has been found to be effective in shoot multiplication of *Ficus anastasia*. When the multiplication medium contained 8 mg l⁻¹ of BA with 1 mg l⁻¹ of IBA, a mean of 20 lateral branches per stem was induced which was the best combination recorded. Even though IAA particularly combined with 2ip or kinetin, it had weak shoot multiplication influence resulting in only two lateral shoots which increased to 7 lateral shoots per stem when combined with BA. The plant height was negatively affected by the increase of cytokinin concentration. However, the control plant was more in length than any of the cytokinin treatments among combination with IBA or IAA.

Key words: *Ficus Anastasia*, multiplication, BA, IBA.

INTRODUCTION

Ficus anastasia is one of the most popular indoor plants native to India, Southeast Asia and Northern Australia. It is propagated either by air layering or rooting by stem cutting. Genus *Ficus* has more than 800 species and are used as foliage plants including different *Ficus* species which is propagated by shoot tips or axillary bud explants. The propagation however, is slow and limited. Hence, *in vitro* micropropagation of *Ficus* species has been widely studied as an alternate method for mass-scale production of high quality planting material (Rout et al., 2006). Many commercial ornamental plants are being propagated by *in vitro* culture on the culture medium containing auxins and cytokinins (Preil, 2003; Rout and Jain, 2004). Rzepka-Plevnes and Kurek (2001) regenerated multiple shoots from nodal explants of *Ficus benjamina* on MS medium supplemented with 3.0 mg/l BA. Plant hormones are among the most important physiological factors affecting the regeneration of plants *in vitro*. Clonal multiplication is a most reliable method for plant propagation, as it yields plants, true-to-type. Thus, it helps in maintaining uniform-

mity within the population. The objective of the present study was to develop an efficient reproducible protocol for the rapid propagation of *F. anastasia* L. using stem explants investigating the influence of BAP, Kinetin and 2-ip in addition of two auxins IAA and IBA on the development of proliferated plantlets of *F. anastasia*.

MATERIALS AND METHODS

Indistinguishable 3 cm long stem of *F. anastasia* selected from Murashig and Skoog (MS) were inoculated vertically on the MS multiplications media with either 1 mg l⁻¹ of IBA or NAA supplemented with (0, 4, 8 and 12 mg l⁻¹) of three cytokinins BA, 2ip and kinetin in addition of 30 g l⁻¹ sucrose. Other additives of the multiplications medium were (in mg l⁻¹) 100 myo-inositol, 0.1 thiamine- HCl, 0.5 nicotinic acid, 0.5 pyridoxine-HCl, 2 glycine. The medium was solidified with 7 g l⁻¹ agar (purified agar-agar/gum agar; Sigma), adjusted to pH 5.7 ± 0.1 dispensed in culture tube (15 ml per test-tube) capped with a closer and left for 8 weeks after autoclaving for 20 min at 121 °C and 1 × 10⁵ Pa (1.1 Kg cm⁻²). The cultures were incubated at 25 - 28 °C in 16 h photoperiods (50mmol-m⁻²s⁻¹).

The experiment consisted of factorial arrangements of treatments (auxin at two levels, IBA, NAA and Cytokinin at 3 levels, BA, 2ip and kinetin, with four concentration) in a completely random design. Twenty replicates (culture tube) were assigned per treatment with one stem per tube. Data were analyzed using the Statistical Analysis System, general linear model (GLM procedure, SAS Institute Inc., 2004) and means were evaluated by LSD (least significant difference).

The response was assessed 8 weeks later, on the number and

*Corresponding author. E-mail: elmeer@gmail.com.

Abbreviations: BAP, 6-Benzyl amino purine; IAA, indole-3-acetic acid; IBA, indole-butyric acid; 2IP, isopentenyl adenine; NAA, naphthalene acetic acid.

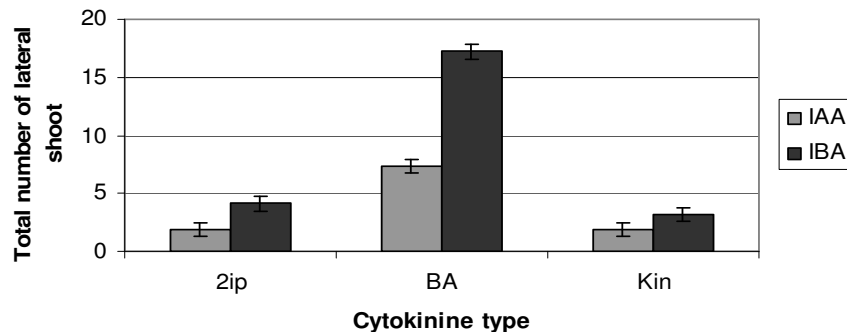


Figure 1. Effect of cytokinin and auxin type on *Ficus anastasia* total number of lateral shoot.

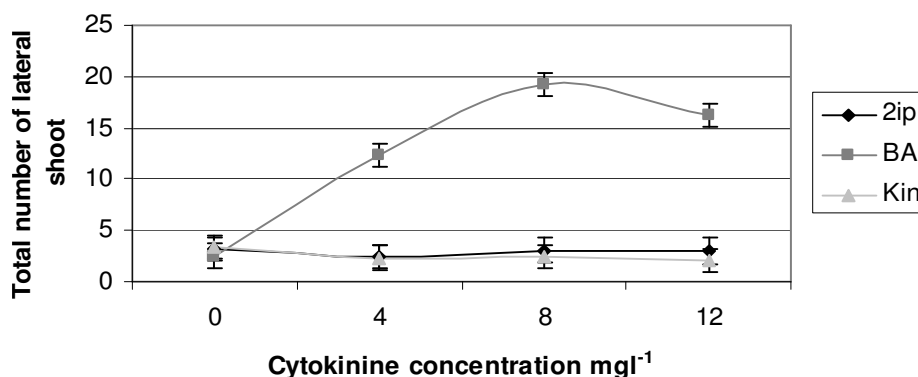


Figure 2. Effect of cytokinin type and concentration on *Ficus anastasia* total number of lateral shoot.

length of lateral branches and plants.

RESULTS AND DISCUSSION

Plant hormones are among the most important physiological factors affecting the regeneration of plants *in vitro* and the role of BA as an effective cytokinins in shoot multiplication has been established in many plants. Cytokinins are classified into two major groups (by their chemical structures): synthetic phenylurea derivatives and adenine derivatives, which may occur naturally.

The combination of cytokinins BA and auxin IBA had significant effect on the number of lateral shoot branches of *F. anastasia*, resulting to a mean of 17 branches (Figure 1) reaching up to 20 lateral branches specially in concentration of 8 mg l⁻¹ of BA (Figures 2-7), even though the effect of IBA declined when combined with either 2ip or kinetin. This indicates that BA is an important plant growth regulator in the multiplication phase of *in vitro* micropropagation of *F. Anastasia*.

Whereas IAA particularly combined with 2ip or kinetin, it showed weak shoot multiplication influence resulting in only two lateral shoots (Figure 8); at the same time it was seem to have increased to about 7 when combined with

BA (Figure1) which points to strong stimulating effect of BA on IAA.

Generally, the presence of BA had superior influence over kinetin and 2ip with varieties of concentration. When the concentration of BA was increased, the shoot number also increased up to an optimum concentration of 8 mg l⁻¹ beyond which the number of shoot declined to 15 per stem at 12mg l⁻¹. These results are in accordance with those obtained by Lobna et al. (2008) They noticed that increasing BA concentration from 1.0 to the other concentrations (2.0, 4.0 or 6.0 mg l⁻¹), generally had a depressive effect on the morphogenesis characteristics of *Paulownia kawakamii* and BA was also more effective for *Lilium* bulblets development. However, high BA had an inhibiting effect on further bulblet growth. These results emphasized that cytokinins have important physiological effects, as they have been seen to stimulate cell division as well as cell elongation, activate RNA synthesis, stimulate protein synthesis and enzyme activity. The use of high cytokinin levels was one of the most effective methods to reduce shoot and leaf growth and promote the formation of meristematic clusters (Lobna et al., 2008). On the other hand the increase of Kinetin and 2ip concentrations had no significant effects in *F. anastasia* multiplication (Figure 2).

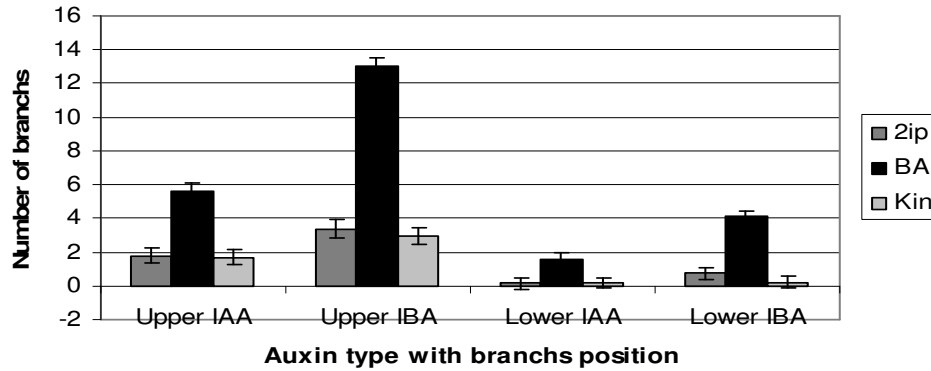


Figure 3. Effect of auxin and cytokinin type on *Ficus anastasia* total number of upper and lower branches.

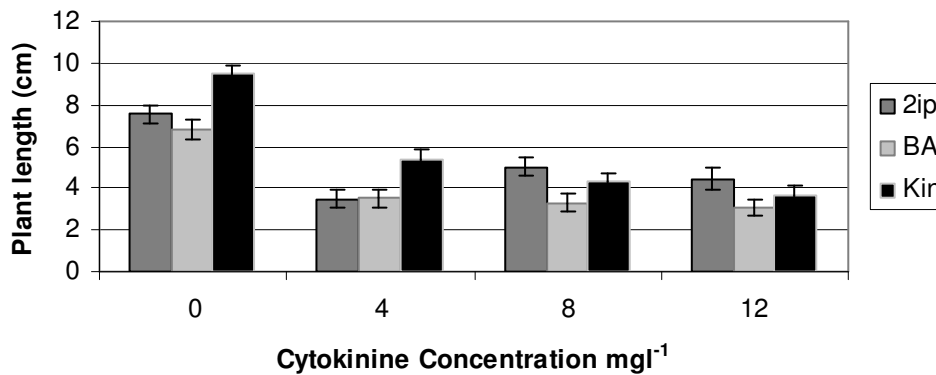


Figure 4. Effect of cytokinin type and concentration on *Ficus anastasia* length.

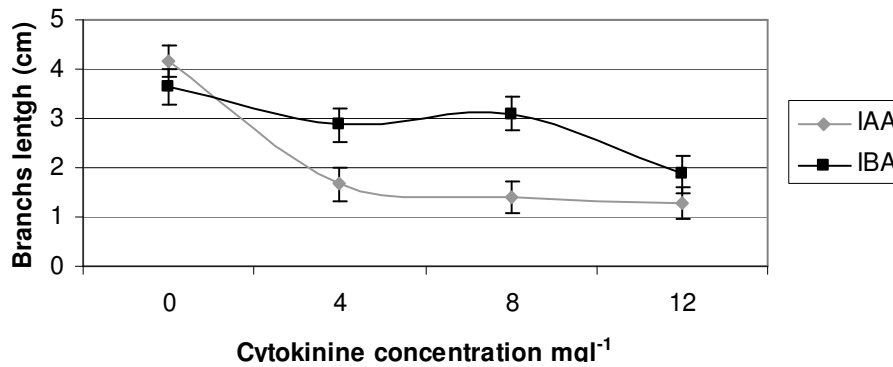


Figure 5. Effect of auxin type and cytokinin concentration on *Ficus anastasia* branch length.

In general, the lateral branches raised from the base of stem and from upper side, proved to be more in number than the base branches, IBA produce more branches than IAA in the upper and lower position of the stem given a mean of 6.4 branches when compared with 3 branches in the upper side, while it gave a mean of 1.6 when compared with 0.6 branches in the lower side.

The best combination was BA with IBA in the upper

side, while the lowest combination was 2ip with IAA in the lower side of stem (Figure 3).

The plant height was negatively affected by the increasing cytokinin concentration, the control plant was more in length than any of the cytokinines treatments among combination with IBA or IAA (Figure 3), indicating the inverse proportions between number induction and plant length. Similar proportions occurred with branches

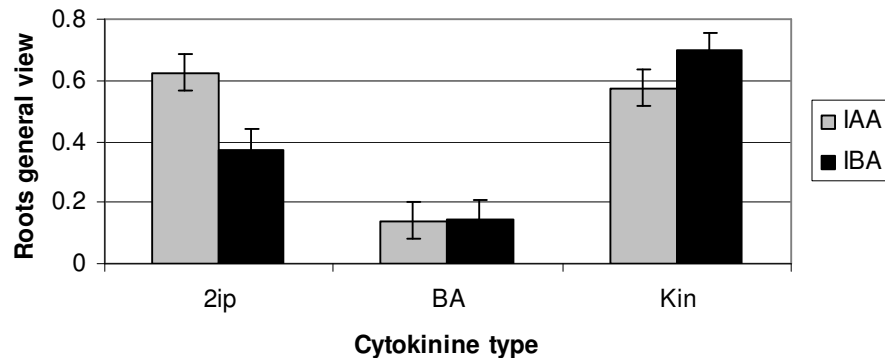


Figure 6. Effect of auxin and cytokinin type on *Ficus anastasia* roots general view.



Figure 7. *Ficus anastasia* lateral branches reaching up to 20 in concentration of 8 mg^{-1} of BA.



Figure 8. *Ficus anastasia* weak shoot multiplication in IAA particularly combined with 2ip or kinetin.

length (Figure 4) which came to pass in the propagation of arbutus andrachne reported by Bertsoouklis and Papafotiou (2009) who found that BA and kinetin were the least effective as they could not induce elongation of the shoots produced.

Ružić and Vujović (2008) suggested that the choice of cytokinines for the phase of multiplication was limited to BA for more rapid micropropagation, through joining rooting and multiplication phases, kinetin and 2ip may be applied. The latter two may also be used to obtain sturdy

shoots (elongation phase, prior to rooting).

As shown in Figure 5, the root general view showed poor percentage with the BA combined with IBA which was the best treatment for inducing more branches number as mentioned previously (Figure 1), whereas kinetin was better than BA in root percentage (Figure 5). Dragan (1989) showed that shoots were differentiated on media

containing BA, the same media were suitable for shoot multiplication and shoot elongation, and rooting were strongly inhibited by BA and stimulated by auxins IBA and NAA.

Plantlets were successfully acclimatized and grown in the greenhouse. These results are a promising step in the direction of *in vitro* cloning of valuable genotypes directly from field-grown plants and the conservation of plant genetic resources.

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