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# EFFECT OF $GA_3$ ON THE GROWTH OF AXILLARY BUDS OF *HEVEA BRASILIENSIS IN VITRO*

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## ABSTRACT

Slow growth of axillary buds is one of the key reasons for the slow progress in micropropagation of clonal *Hevea*. Axillary buds induced in the presence of cytokinins were elongated satisfactorily in the presence of $GA_3$ at 2-4 mg/l. This effect was not observed when the medium contained thidiazuran. The effect of filter sterilized and autoclaved $GA_3$ on the elongation of *in vivo* grown seedlings showed no difference. However, in this study $GA_3$ was incorporated in to media prior to autoclaving.

**Key words:** $GA_3$, *Hevea brasiliensis*, micropropagation, rubber, thidiazuran (TDZ)

## INTRODUCTION

*Hevea brasiliensis* being the main source of natural rubber is an economically important tropical tree, belongs to the family Euphorbiaceae. Clonal multiplication by *in vitro* techniques was attempted by various methods over the past two decades or so (Paranjothi & Ghandimathi, 1975; Mascarehas *et al*, 1982 Carron and Enjalric, 1982, Carron *et al*, 1985, Chen *et al*, 1979, Chen 1984, Carron *et al* 1989). Use of thidizuran has remarkably increased the number of axillary buds produced (Seneviratna & Flegman, 1996) but satisfactory elongation of these buds would contributes to the proliferation rate. $GA_3$ has been used by some workers in the medium for both axillary bud proliferation and for somatic embryogenesis. However, any reason to use $GA_3$ or the effect of $GA_3$ in particular have not been discussed. In all reports $GA_3$ has been incorporated before autoclaving the medium.

## MATERIALS AND METHODS

The effect of autoclaved and filter sterilized $GA_3$ was first tested on rice plants grown on pots. In the second stage, 2 ppm and 4 ppm $GA_3$ solutions were sprayed with a hand sprayer on rubber seedlings grown *in vivo*. Autoclaved and filter sterilized solutions were tested with the control, the normal $GA_3$ solution.

## The effect of $GA_3$ on *Hevea in vitro*

Nodal explants, 3-5 cm long harvested from glass-house grown seedling plants were used as the starting planting material. After washing them thoroughly under the running water; surface sterilization was done by shaking in 70% ethanol solution for 1 min followed by 10 min immersion in 0.2% $HgCl_2$ solution to which a few drops of tween-80 were added.

Woody plant medium (Lloyid & Mc Cown, 1980) supplemented with 4% sucrose and 0.6% agar was used as the basic medium. Cultures were grown on an establishment medium with no growth regulators for 2 weeks before transferring them on to media containing growth regulators.

All the chemicals used were of analytical grade from BDH Chemicals U.K. Thidiazuran was supplied from Schering Agriculture, U.K. and other growth regulators were supplied by Sigma Chemicals, U.S.A. Media were prepared in 25 mm diameter boiling tubes, as slants in order to increase the surface area.

All growth hormones including $GA_3$ were incorporated to the medium prior to sterilizing the medium in an autoclave at 121°C and 15 lb/inch$^2$ presure for 20 min and slants were made soon after the medium was taken out from the autoclave.

After the establishment period of 2 weeks, all cultures were transferred to bud proliferating medium, which contained kinetin. BAP and $GA_3$ at 2.0,1.0 and 0.2 ppm respectively. After 16 weeks on this medium, the elongated primary and secondary axillary buds were transferred to $GA_3$ media. $GA_3$ was used at 0, 0.5, 1, 2, 4, 8, and 16 ppm. Cultures were transferred to fresh media every 4 weeks and the replication was ten.

After 12 weeks, all media were supplied again with kinetin, BAP and NAA at 2.0 ppm, 1.0 ppm and 0.2 ppm respectively in addition to the $GA_3$.

In the second phase of this experiment, the established cultures were transferred to thidiazuron containing medium at 0.002 ppm. After 16 weeks on this medium, while transferring to fresh media every 4 weeks, the cultures were transferred to media containing $GA_3$. Two levels of $GA_3$ were used, 2 and 4 mg/l with a control of 0 ppm $GA_3$.

Also, $GA_3$ was used along and in combination with thidiazuran at 0.002 ppm. Replication was 12 and the leaf growth and the length of axillary shoots were monitored for 16 weeks.

## RESULTS

### The effect of $GA_3$ on the growth of secondary and tertiary axillary shoots.

After 4 weeks of culture, no difference was observed in cultures grown on different media, and no elongation was seen in any of the cultures. Leaf growth was observed at 1 ppm $GA_3$. At the end of 8 weeks, a little apical elongation was observed in cultures at 8 ppm $GA_3$. The growth of cultures was generally poor. After 12 weeks cultures were transferred to media containing other hormones, *i.e.* kinetin 2 ppm, BAP 1 ppm and NAA 0.2 ppm; in addition to the $GA_3$. After 16 weeks, nodes on 1 ppm $GA_3$, produced good leaves and about 30% of culture showed axillary bud elongation of about 1 cm. After 20 weeks of culture, about 40% of the nodes on 16 ppm $GA_3$ medium showed axillary bud elongation of 3-5 mm.

Leaf growth wasseen in some cultures of most media. However, the axillary bud elongation rate was very poor and remained unchanged even after 24-28 weeks, where the number of cultures started to decline.

**The effect of $GA_3$ on the elongation of clusters of axillary buds**

The growth of axillary buds on $GA_3$ media is shown in Fig.1 and 2 and the results up to 12 weeks are summarized in Table 1.

Table 1. *The mean length of axillary buds (cm) on $GA_3$ and thidiazuron media*

| $GA_3$ | TDZ | Number ofweeks | | |
|---|---|---|---|---|
| | | 4 | 8 | 12 |
| | .0 | cluster | cluster | cluster |
| 0 | 0.002 | cluster | cluster | cluster |
| | 0 | 1.14 (.38) | 1.96 (.38) | 2.0 (.38) |
| 2 | 0.002 | .53 (.3) | .63 (.41) | .63 (.41 |
| | 0 | 1.38 (.42) | 2.78 (.46) | 3.1 (.55) |
| 4 | 0.002 | cluster | cluster | cluster |

Over the years it was experienced that $GA_3$ alone in the medium always caused yellowing and eventual death of the cultures in 8-10 weeks. Therefore, when thidiazuran was absent, the combination of hormones kinetin 2 ppm, BAP 1 ppm and NAA 0.2 ppm which was found best for normal axillary bud elongation & leaf growth was incorporated in to media. In some cases, about 5 cm long axillary shoots were seen at 4 ppm $GA_3$. However, when considering the quality of the axillary shoots produced, those produced at 2 ppm $GA_3$ were better than those produced at 4 ppm $GA_3$. As shown in Fig. 1b, at 2 ppm $GA_3$ the leaf growth was normal and satisfactory and also dark green in colour. From some cultures 3-4 axillary shoots elongated (Fig.1b). On the other hand, the axillary shoots elongated at 4 ppm $GA_3$ looked etheolated. Leaf growth was very poor and also they were pale yellow. Internodal lengths were higher and some elongation of secondary axillary buds was also observed (Fig.1 c).

When thidiazuran was present with $GA_3$, the effect of $GA_3$ was not prominent as in absence of thidiazuran. Some effect was seen in the leaf growth but most of the time the clusters of axillary buds remained as clusters (Fig.2 b & c). However, the clusters could be divided into 2-3 small clustes each containg one or two axillary shoots though these shoots are very short. In the absence of Thidiazuran , the elongation was not seen if the medium lacks $GA_3$ (Fig. 1 a).


Fig. 1. The growth of axillary buds in the presence of $GA_3$ (a) at 0 ppm, (b) at 2.0 ppm and (c) at 4.0 ppm

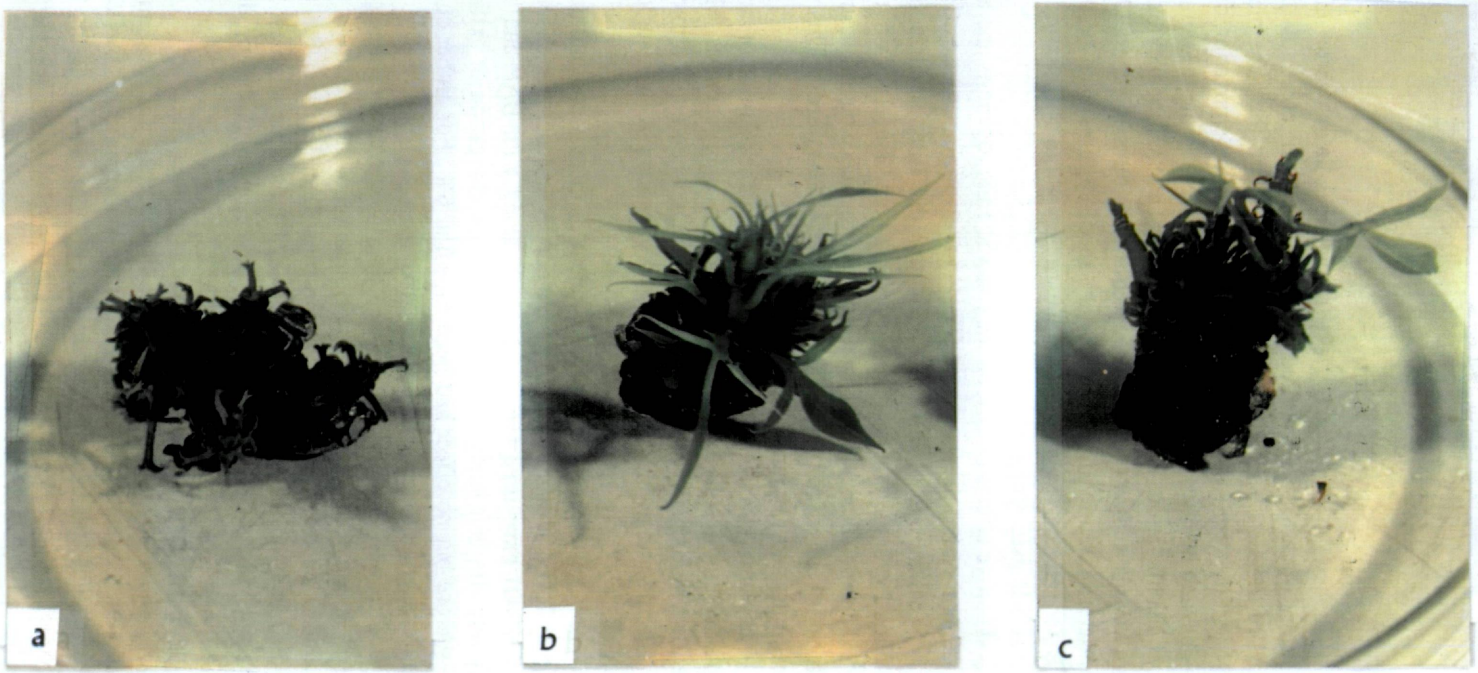


Fig. 2. The growth of axillary buds in the presence of thidiazuron at 0.002 ppm in combination of GA3 (a) at 0 ppm (b) at 2.0 ppm and (c) at 4.0 ppm

## DISCUSSION

Gibberellic acid ($GA_3$) is a growth regulator whose effect on the growth and morphology of the tissue cultured plants has been different for different species, different cultivars of the same species and different explants of the same plant *etc*.

In the present study, the effect of $GA_3$ on the elongation of clusters of axillary buds was visible and positive in the absence of thidiazuran in the medium. Also, for secondary and tertiary bud elongation, $GA_3$ in combination with conventional cytokinins, showed some effect but this was masked by the poor growth of the cultures in general. $GA_3$ alone in the medium always deteriorate the growth of axillary shoot cultures even before the effect of $GA_3$ was seen. Based on this experience, in the present study, a mixture of conventional growth hormones was used along with $GA_3$.

Positive effects of $GA_3$ on the somatic embryogenesis and also on the germination of somatic embryos has been reported for a number of plant species (Hunault & Maatar, 1995).

Sterilization of $GA_3$ has been done by filter sterilization by some workers and autoclaving by some others. Gibberelic acid being a heat sensitive compound can be partly destroyed during autoclaving. The personnel experience on this is such that the effect on shoot elongation is reduced in the autoclave up to about two thirds. This was tested by spraying on rice seedlings and also on *Hevea* seedlings.

Hunault & Maatar (1995) have found that the addition of autoclaved or filer sterilized $GA_3$ had similar results in stimulating somatic embryogenesis. However, $Ga_3$ has clearly reduced the frequency of somatic embryogenesis when applied into the medium after filter-sterilization, while no effect had been observed when $GA_3$ was autocalved with the rest of the medium.

$GA_3$ has been a medium component in many tissue culture media for rubber also. Paranjothi & Ghandimathi, (1975) have used $GA_3$ at 1 ppm for shoot tip culture. Enjalric and Carron (1982) have used $GA_3$ at 0.5 ppm along with BAP and 1BA at 0.5 & 0.25 ppm respectively. In their study, beneficial effect on elongation of axillary buds has been shown at 2% sucrose but no effect had been shown at 6%. For anther culture also GA3 has been used in regeneration medium (Chen, 1984).

Cresswell *et al* (1982) reported that the increase of the concentration of $GA_3$ from 1 ppm to 10 ppm lead to a considerable elongation of the internodes of shoots. The internodal length increased from 1 mm to 15 mm in less than 15 days. But after this the apex of the shoot died and the leaves became fragile and fell at the slightest shock. However, media containing 0.5 ppm $GA_3$, though causing less elongation, has produced healthier shoots and better roots.

In an experiment conducted to see the effect of $GA_3$ on the growth of *Chrysanthemum* shoot tips, it has been found that, the leaf and shoot development & elongation in the presence of $GA_3$ at 10 mg/l was significant (Earl & Langhans, 1974). As in the present study, for *Chrysanthemum* also, in the presence of 2 mg/l kinetin 0.02 mg/l NAA, the effect of $GA_3$ has been striking though it did enhance shoot development and elongation.

Economou & Spanoudaki (1986) have also used $GA_3$ in the medium for better growth of *in vitro* derived gardenia shoots. $GA_3$ has been used at 0, 0.5, 2.5 and 12.5 ppm. The number of shoots produced has been increased at both 2.5 and 12.5 ppm in combination with 2iP at 25 ppm. The number of shoots produced has been increased at both 2.5 and 12.5 ppm of $GA_3$. However, shoot length has been increased with all $GA_3$ concentration while the quality of the shoots has been highest at 0.5 and 2.5 ppm of $GA_3$. Continuous production of shoots from the original explant has also been improved with $GA_3$ in the medium at 2.5 & 12.5 ppm. Since 2iP was present in all media with $GA_3$, the effect of $GA_3$ along could not be seen. However, satisfactory shoot multiplication and elongation has been achieved in the presence of 2iP.

$GA_3$ has been usd to inhibit flowering and to induce juvenile morphological characteristics in Citrus, *Acacia* and some *Prunus* species (Hackett,1985). Faster growth, which is a juvenile characteristic has been observed in the presence of $GA_3$.

The effect of $GA_3$ on the elongation of micropropagated *prunus* shoots have been tested with 6 levels of GA3 (0,.05, .1, .5, 2.5 & 12.5 ppm) in combination with 0.2 ppm BAP. Shoot proliferation has been achieved at 1 ppm BAP (Reeves et al, 1983). Filter sterilized $GA_3$ dissolved in ethyl alcohol has been added to cooled solid medium after autoclving. Decline of cultures due to $GA_3$ treatment and increased variability have been reported for .5 & 2.5 ppm levels. Cultures grown at 12.5 mg/l $GA_3$ have shown elongation.

In the present study, the effect of $GA_3$ on shoot elongation is very clear in the absence of thidiazuron. Thidiazuron being a strong cytokining may not allow cell elongation but keeps on producing more and more new buds. However, both satisfactory proliferation and elongation of axillary buds is possible by using two media alternatively.

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