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Shoots Induction from *Hibiscus Rosa-sinensis* Nodal Explant Using N^6-benzylaminopurine (BAP)

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Abstract: *Hibiscus rosa-sinensis* is a profusely flowering, widely cultivated woody ornamental shrub belonging to the family of Malvaceae. The objective of the present study was to determine the effects of exogenous BAP supplemented at different concentrations in MS medium of varying strengths on the induction of viable shoots from nodal *Hibiscus rosa-sinensis* explants. It was shown that increasing the concentration of BAP on all MS medium strengths resulted in a decline in shoot viability for *in vitro* micropropagation. Of the three medium strengths tested, MS full strength was observed to attenuate the negative effects of increasing BAP supplementation. MS half strength yielded the most healthy and vibrant shoots without BAP in the medium. The results also suggest that the concentration sensitivity narrow-range of BAP in *Hibiscus rosa-sinensis* is between 0µM to 5µM BAP for optimum *in vitro* growth.

Key words: *Hibiscus rosa-sinensis*, shoot induction, nodal explant, N^6-benzylaminopurine

INTRODUCTION

The charismatic genus *Hibiscus* is familiar, particularly in the tropical and sub-tropical regions of the world where its diversity is especially concentrated. Perhaps the best known species of the genus is the widely-cultivated ornamental shrub *Hibiscus rosa-sinensis* with its bewildering array of cultivars [22]. It is a profusely flowering woody species in the southern United States of America [6]. It is the national flower of Malaysia and known locally as *Bunga Raya* and also the state flower of Hawaii.

The commercial cultivation of *Hibiscus rosa-sinensis* is hindered by pathogenic invasions by nematodes and also by competition from weeds at the earlier stages. Although the commercial value of *Hibiscus rosa-sisnensis* as cut flowers is somewhat limited also due to the fact that its flower blooms only few days, between one and three at the most, but it has high commercial value as a landscaping plant. In spite of its landscaping and symbolic value, *Hibiscus rosa-sinensis* has received very limited attention in the field of horticulture, especially in terms of its *in vitro* micropropagation. This may be attributed to the fact that *Hibiscus rosa-sisnensis* is a woody species, despite, tissue culture and micropropagation protocols having been described for a number of woody species [21,25,28], The rates of development *in vitro,* shoot proliferation and root induction of woody species have always been low and erratic [12,13]. Furthermore, *in vitro* cultures of woody species have low rates of bud opening[15] and high rates of ethylene-induced leaf abscission[15,24]. Browning of media is also a serious concern in micropropagation for woody species [24].

The long-term use fulness of tissue culture for woody species has been described as limited [17]. Nevertheless, it is important to determine empirically the utility of tissue culture for various woody plants that have been deemed commercially valuable. The benefits of a successful tissue culture protocol would allow the deemed commercially valuable woody species such as *Hibiscus rosa-sinensis* to be propagated and thus commercialised, much more rapidly than other more traditional methods. However, very few studies exist on the *in vitro* regeneration of the Malvaceae family.

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In this paper, we report the effects of exogenous supplementation of N^6-benzylaminopurine (BAP), a cytokinin phytohormone, at different concentrations in the Murashige and Skoog [18] medium high mineral salts components (MS medium) of full, half and quarter strengths on the induction of viable shoots by direct regeneration from nodal explants of *Hibiscus rosa-sinensis in vitro*. Explant source is one of the most important factors in the induction of morphogenetic response of *in vitro* culture, especially in dicot plants. This is in order to optimise the shoot induction and multiplication step in the establishment of a plant tissue culture protocol as described by the general five-stage approach by Lindsey and Jones [16] after the selection and preparation of mother plants and the establishment of aseptic cultures. The optimisations of shoot induction and multiplication step continue with the preparation for transfer to the natural environment, that is, acclimatization of the plantlets. Hence, development of *in vitro* regeneration methods would be important for the improvement of the existing cultivars of *Hibiscus rosa-sinensis* using genetic transformation systems.

MATERIALS AND METHODS

Explant Preparation: Excised single nodal segments of *Hibiscus rosa-sinensis* with dormant axillary buds were used as explant materials from mature disease-free shoots of *in vitro* plants bearing similar phenotypic expressions with red non-frizzy flowers of five petals with non-variegated leaves. Excision was carried out when the stem was green and at its relatively less lignified (non-woody) stage of maturation. Single nodal cuttings from stems were standardised to about 1.0-1.5 cm length and roughly 0.3-0.5 cm of stem circumference. All leaves and stipules were detached. Following excision, the nodal segments were placed under running tap water for about an hour, after which the container was changed and the explants washed with a few drops of detergent for about one minute with continuous agitation. The explants were then rinsed thoroughly with distilled water. In the laminar flow, a diluted solution of 2.63% (v/v) of sodium hypochloride was prepared from Clorox® (Unilever, Malaysia.) in a sterile container. Two drops of the surfactant polyoxyethylenesorbitan or Tween® 20 (Merck & Co., U.S.A.) were then added to a 100mL solution of the diluted sodium hypochloride. The explants were then transferred into sterile container containing the mixture and agitated continuously for about 15 minutes. Agitation should be stopped if extensive tissue whitening is observed especially at the edges of the nodal explants. The explants were then rinsed off three times with sterile distilled water for about ten minutes each time and subsequently placed in sterile Petri dishes.

Shoots Induction: Single nodal segment as explants was deliberately selected against using shoot tips or other possible explant types since nodal explants have been shown to be highly responsive and this would aid in reducing the duration for micropropagation of *Hibiscus rosa-sinensis*. Its high responsiveness compared to shoot tips as explants has been shown in mulberry [3]. Nodal explants also showed direct regeneration in MS medium without BAP being added to the medium unlike stem and leaf explants in *Scaevola aemula* R.Br. or also known as the Australian fan flower [2]. The differential response of nodal explants and shoot tip explants has been attributed to the differences between physiological states of the buds on different regions of the stem [32]. Shoot tips are known to exert strong apical dominance even in the presence of BAP [14]. It was anticipated that axillary bud break would occur within a week in the present study and this was later confirmed. Other explant types such as leaf and internodal segments would required longer duration for callus induction and additional plant growth regulator treatments to induce adventitious shoots.

Since the objective of the this study was to obtain viable shoots induction from nodal explants of *Hibiscus rosa-sinensis* by determining the best cytokinin concentration with good shoots induction effects in a chemically-defined medium of varying strengths, an appropriate micropropagation medium and cytokinin need to be selected.

MS medium was selected since it is the most widely used medium for plant tissue culture and is generally easily prepared. MS medium has also been indicated as more suitable than Gamborg's B5 medium and Woody Plant Medium (WPM) for shoot induction from nodal explants in mulberry [3, 20]. In this experiment, MS medium strength ranged from full, half (1/2) and quarter (1/4) strengths. The rational of this selection are based on two premises, the first being the fact that by varying the strengths of the MS medium, the interaction of the MS medium and the varying concentrations of BAP supplementation on shoots induction can be observed. This allows for the further understanding of the interrelationship of the two factors on shoots induction. Secondly, the optimal MS medium strength necessary for viable shoots induction can be determined, and if it is lower than the full strength MS medium, a saving in material cost could be derived especially with regards to the large scale commercial *in vitro* micropropagation.

BAP is known to be the most effective cytokinin compared to the cytokinins, thidiazuron (TDZ) and kinetin, for shoots induction in the nodal explants of the woody species *Feronia limonia* (L.) Swingle [10]. Similar results were reported in the woody species such as guava [1] and mulberry [3, 19]. BAP was also shown to give the best results in the establishment stage of aseptic cultures of nodal shoots of *Citrus limon* (L.) Burm, f. cv. *Interdonato* seedlings [13]. Elangomathavan *et al.* [7] showed that earlier bud break, higher frequency of bud break and various degree of multiple shoot formation on nodal explants occurred in MS medium fortified with BAP than with other cytokinins, N_6–isopenyladenine (2iP) and kinetin in *Orthosiphon spiralis* (Lour.) Murr. (Lamiaceae), or commonly know as the Kidney Tea Plant.

Similar stimulatory effects of BAP on bud break and multiple shoot induction form nodal explants have also been reported in *Ocimum* spp. [19]. BAP concentrations of 0, 5, 10, 15 and 20µM were selected, as the step-up increase of 5µM units would allow a greater degree of differences. Optimisation of BAP concentration most propitious for shoots induction would lead to similar savings in material cost as with the strength of the MS medium described earlier. Shoot formation resulting from organogenesis is enhanced by the presence of BAP, which is concordant with previous reports on plant regeneration from leaf explants of *Iris pumila* shoots [11].

Treatments and Parameters: The experimental treatments consisted of four levels of BAP concentrations (5, 10, 15 and 20µM) and three levels of media strengths (full, half and quarter). Experimental controls consisted of the three levels of media strengths without any BAP application. Each treatment consisted of 40 replicates. All cultures were maintained at 27 °C ± 1°C with a photoperiod regimen of 16 hours daylight and 8 hours darkness in a culture room illuminated using cool-white fluorescent lamps.

The experimental parameters observed were shoot numbers at weekly intervals for four weeks After four weeks incubation, the percentage of tissue necrosis, number of leaves on explants, number of abscised leaves, number of leaf primodia on explant and number of abscised leaf primodia, percentage of the presence of callus on cut edges and callus intensity were determined.

Statistical Analysis: Data were expressed as mean ± standard deviation. Continuous variables were analysed using one-way ANOVA. Two tailed p-value of less than 0.05 was considered significant. Pair wise comparison was done using Dunnett's T3 test at $\alpha = 0.05$.

RESULTS AND DISCUSSION

When cultured on MS medium of full, half and quarter strengths, single nodal explants from mature disease-free plants harbouring pre-existing meristems or dormant axillary buds sprouted dense chlorophyll-containing protuberances within 3-4 days, which later differentiated into shoots. One of the two developing buds present at the node developed into a shoot with the other bud showing only limited growth. Similar observations were made by Tiwari *et al.* [31] on the *Bacopa monniera* plant. The single nodal explants inoculated on MS medium responded differently to increasing BAP concentrations on varying MS medium strengths.

BAP application did result in increased shoots induction in single nodal segments of *Hibiscus rosa-sinensis* when compared to controls. This is especially true for MS full strength where the mean shoot number recorded was 1.94 ± 0.54 shoots on 15µM BAP (Table 1). However, increasing concentrations of BAP did not sustain shoot numbers on MS half strength (Table 1) and quarter strength (Table 1 and Fig. 1) over a four-week period but showed a declining shoot number trend throughout the four weeks. MS half strength without BAP concentration demonstrated viable shoot induction with sustainable shoot numbers over a period of four-weeks. In all cases the mean number of shoots did not reach two per explant.

Continued exposure of explants to high concentrations of BAP during shoot induction caused high accumulation of cytokinin, which inhibited further shoots from developing. Bhau and Wakhlu [3] observed that high concentration of BAP resulted in a decrease in shoot multiplication rate of mulberry. High concentrations of cytokinin also have been reported for the reduction of shoot-bud induction frequency in *Bacopa Monniera* nodal explants [31]. The failure of shoots to develop on half strength MS medium and full strength MS medium could be attributed to the increasing tissue vitrification (Fig. 1d) and necrosis (Fig. 1e) of the shoots and the nodal regions, observed as the concentration of BAP applied increased progressively. Zaid *et al.* [33] defines the word *vitrified* as having a glassy, transparent or wet and often swollen appearance. The process of vitrification is a general term for a variety of physiological disorders that lead to shoot tip and leaf necrosis.

MS medium full strength, however, was able to mitigate the effects of increasing BAP concentrations not seen in both half strength and quarter strengths. High tissue vitrification and tissue necrosis percentages were not observed for MS full strength after four weeks in incubation. However, with high BAP concentrations, there was a propensity for the shoot growth to become stunted but remain green and

Table 1: Effects of different MS medium strengths with different BAP concentrations from single nodal segment of *Hibiscus rosa-sinensis***.** Mean values followed by same alphabet are not significantly different at p=0.05 level.

MS medium strength	BAP concentration (µM)	Mean number of shoots per explant ± SD			
		Week 1	Week 2	Week 3	Week 4
Full	0	1^{a}	1	1	1.14^{d} + 0.36
	5	1^{a}	1.5^{b} + 0.53	1.50^{c} + 0.53	1.50^{d} + 0.53
	10	1.21 + 0.42	1.37^{b} + 0.49	1.26^{c} + 0.56	1.74 + 0.65
	15	1.28 + 0.46	1.39 + 0.50	1.56 + 0.51	1.94 + 0.54
	20	1.28^{a} + 0.57	1.22 + 0.43	1.11^{c} + 0.58	1.67 + 0.84
Half	0	1.05^{a} + 0.23	1.05^{b} + 0.23	1.56^{c} + 0.37	1.68 + 0.48
	5	1.32 + 0.48	1.26^{b} + 0.56	1.05^{c} + 0.85	0.68^{d} + 1.23
	10	1.13^{a} + 0.34	1.13^{b} + 0.34	0.50 + 0.73	0.38 + 0.81
	15	1.16^{a} + 0.37	0.89^{b} + 0.57	0.89^{c} + 0.81	0.47 + 0.77
	20	1.19^{a} + 0.40	1.19^{b} + 0.40	0.25 + 0.77	0.25 + 0.77
Quarter	0	1.08^{a} + 0.27	1.12^{b} + 0.33	1.69 + 0.47	1.50 + 0.76
	5	1.04^{a} + 0.20	1.35 + 0.63	1.49 + 0.90	0.88^{d} + 1.07
	10	1.04^{a} + 0.20	1.44 + 0.58	1.04^{c} + 1.02	0.60^{d} + 1.35
	15	1^{a}	1.58 + 0.69	1.21^{c} + 1.08	0.37 + 0.90
	20	1^{a}	1.18^{b} + 0.81	0.12 + 0.49	0^{d}

[a] testing if the mean is away from 1, across MS Medium strengths for leaves.
[b] testing if the mean is away from 1, across MS Medium strengths for abscised leaves
[c] testing if the mean is away from, across MS Medium strengths for primodia leaves
[d] testing if the mean is away from1, across MS Medium strengths for abscised premodia leaves

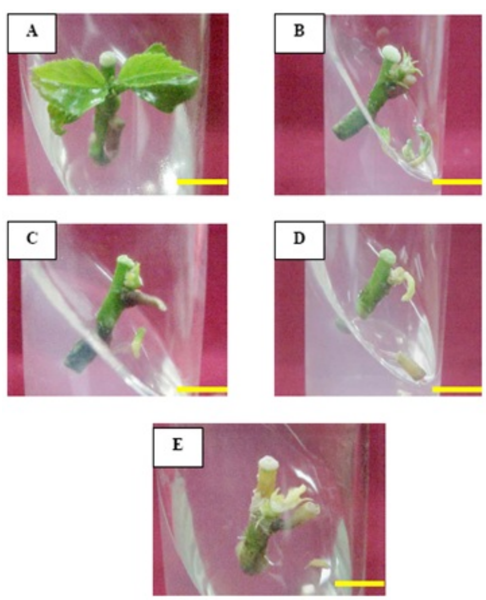

Fig. 1: Single nodal culture on MS medium with half (1/2) strength after four week in incubation on different BAP concentrations. (a) 0µM BAP, healthy growth. (b) 5µM BAP, leaf primodia abscission occurs on non-necrotic explants. (c) 10µM BAP, leaf primodia abscission occurs on non-necrotic explants. (d) 15µM BAP, tissue vitrification occurs. (e) 20µM BAP, tissue necrosis occurs. The bar in the bottom of each image represents 1.5 cm.

leafless with multiple leaf primodia, as seen in this study at 20μM BAP on MS medium full strength. Similar results were seen by Chengalrayan and Gallo-Meagher [4] in sugarcane where shoot elongation decreased with increasing BAP concentration. Kotsias and Roussos [13] also showed that shoot elongation of nodal shoots of *Citrus limon* (L.) Burm, f. cv. *Interdonato* seedlings did not increase with higher BAP application and Tawfik and Noga [29] showed that higher concentrations of BAP (more than 2.5μM) did not increase the numbers of shoot-buds per explant in *Cuminum cyminum* (L.). This observation is in line with present knowledge of BAP hormone that can delay senescence by slowing down vibrant growth. Delaying senescence does not produce viable shoots for micropropagation since the shoots produced will be stunted. The most viable explants were on MS medium half strength, showing healthy growing explants with multiple leaves (Fig. 1a).

Tissue Necrosis: Figueiredo[8] reported that the use of high levels of BAP resulted in browning of tissues and colouration of dark-brown medium, ultimately resulting in complete death of explants. Tissue necrosis for nodal explants in this experiment was defined as visible tissue browning of any part of the explant including browning of callus on the cut edges of the explant but excluding the nodal branch, shoots, leaf and leaf primodia.

Tissue necrosis was determined to be a problem with increasing BAP concentrations especially with both half and quarter strengths of MS medium (Fig. 2). MS medium full strength was observed to show the lowest tissue necrosis percentages (below 20% for all BAP concentrations) when compared to MS half strength and MS quarter strength. Tissue necrosis was absent in MS full strength with 0μM and 5μM BAP concentrations and also in MS half strength without BAP supplementation. MS half strength with the addition of 5μM BAP into the culture medium resulted in a significant increased in tissue necrosis percentages (73.7%) as cultures became necrotic after four weeks incubation period. MS quarter strength showed the highest tissue necrosis percentages with the highest seen for 20μM BAP with 100% of cultures being necrotic after four weeks. MS medium full strength somehow was able to attenuate the effects of BAP concentration increases, mitigating tissue necrosis, resulting in consistent low tissue necrosis percentages.

Leaf and Leaf Primodia: The quality of leaves produced on shoots would be an indicator of shoot viability for micropropagation. Healthy profuse leaves without any malformation are the standard indicator for viable shoot induction. Other standards include low turnover of leaves due to senescence, low numbers of leaves and leaf primodia abscission and high counts of leaves and leaf primodia on the explant. In this study, the number of leaves on explants, number of abscised leaf from explants, number of leaf primodia on explant and number of abscised leaf primodia from explants were counted visually after four weeks of incubation.

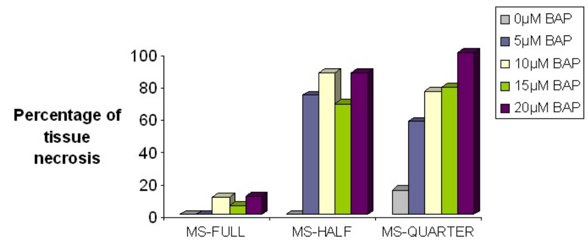

Fig. 2: Shows the percentage tissue necrosis versus MS medium strength after four weeks in incubation.

The mean number of leaves was highest in explants cultured on MS medium full strength with 1.43 ± 0.85 leaves and MS medium half strength with 1.63 ± 1.12 leaves without supplementation of BAP (controls) after four weeks in incubation (Table 2). Once BAP was introduced into the medium, the number of leaves on explants declined drastically for both the medium strengths. MS medium quarter strength showed negligible numbers of leaves on explants after four weeks in incubation (Table 2). It was observed that the rate of senescence of leaves on both MS medium full strength and MS medium quarter strength without BAP supplementation differed within the four-week period. On MS full strength most of the leaves had already gone through a cycle of senescence and new leaves were developing which is a completely natural process. On MS medium half strength the leaves were largely healthy, and green and fewer leaves had gone through senescence (Fig. 1a). This is also indicated from the number of abscised leaves, MS medium full strength showed the highest numbers, followed by MS medium half strength and MS medium quarter strength (Table 2). No leaves were present on explants after 10μM BAP supplementation for both MS medium full and half strengths as all leaves were abscised after four weeks in incubation (Table 2).

Table 2 shows that on MS medium full strength leaf primodia numbers on explants increased from 0μM BAP to 5μM BAP and declined drastically only after 10μM BAP addition into the medium. This was unlike MS medium at both half and quarter strengths, which registered a decrease in leaf primodia numbers after addition of BAP at 5μM. Leaf primodia abscission was highest for MS medium quarter strength at 0μM BAP, while MS medium full and half strengths showed very low leaf primodia abscission. Leaf primodia abscission

Table 2: Effects of *Hibiscus rosa-sinensis* leaf and leaf primodia after 4 weeks incubation period. Mean values followed by same alphabet are not significantly different at p=0.05 level.

MS medium strength	BAP concentration (µM)	Mean number of leaves per explant ± SD	Mean number of abscised leaves per explant ± SD	Mean number of leaf primodia per explant ± SD	Mean number of abscised leaf primodia per explant ± SD
Full	0	1.43 + 0.85	0.86 + 0.95	0.86 + 0.77	0.07 + 0.27
	5	0.20[a] + 0.63	0.10 [b] + 0.32	1.90 + 0.99	1.10[d] + 0.88
	10	0[a]	0.32 [b] + 0.82	1.89 + 1.49	0.95[d]+ 1.03
	15	0[a]	0 [b]	0.50 + 0.79	2.50 + 1.69
	20	0[a]	0 [b]	0.33 + 0.59	0.56 + 0.70
Half	0	1.63 + 1.12	0.63 + 0.68	0.68 + 1.06	0.16 + 0.37
	5	0.16 [a] + 0.69	0 [b]	0.11[c] + 0.46	1.53 + 1.31
	10	0 [a]	0 [b]	0.13[c] + 0.34	0.75[d] + 0.77
	15	0 [a]	0 [b]	0	0.74 + 0.45
	20	0 [a]	0 [b]	0	0.69 + 0.60
Quarter	0	0.08 [a] + 0.27	0.31 + 0.55	0.65 + 0.89	1.15[d]+ 0.83
	5	0 [a]	0.08 [b] + 0.27	0.35 + 0.56	0.88[d]+ 0.65
	10	0 [a]	[b] 0	0.08[c] + 0.40	0.56 + 0.65
	15	0 [a]	0 [b]	0.05[c] + 0.23	0.68 + 0.75
	20	0 [a]	0 [b]	0	0.24 + 0.56

[a] testing if the mean > 0, across MS Medium strengths for leaves.
[b] testing if the mean > 0, across MS Medium strengths for abscised leaves
[c] testing if the mean > 0, across MS Medium strengths for primodia leaves
[d] testing if the mean away from1, across MS Medium strengths for abscised premodia leaves

for MS medium half and quarter strengths only begun to occur at higher numbers following addition of BAP.

Generally, increasing BAP concentrations on MS medium full strength resulted in the abscission of green leaves without going through senescence and at 15µM BAP leaf primodia abscission occurred on non-necrotic explants. Leaves did not grow on 20µM BAP supplementation on non-necrotic explants as the growth of shoots was stunted. On most explants, leaf primodia abscission occurred on non-necrotic explant tissues following addition of 5µM BAP and 10µM BAP on MS medium half strength (Fig. 1b and 1c). Further increases in BAP concentration caused tissue vitrification and subsequent tissue necrosis (Fig. 1d and 1e). In addition, using MS medium quarter strength, leaf primodia abscission occurred on non-necrotic explants even without BAP supplementation. At 5µM BAP, leaf primodia abscission still occurred. However, at concentrations beyond 5µM BAP, tissue vitrification and necrosis resulted. It therefore can be concluded that increasing of BAP concentration, resulted in the decrease in the number of leaves on explants and an increase in the number of leaf abscissions on all medium strengths. Similarly, increasing BAP concentration reduced the number of leaf primodia on explants and made it more prone to abscission on all medium strengths. Similarly, a promotive effect at low BAP concentrations and toxic effect effect at higher concentrations has also been described in the literature for explants from different citrus genotypes [5].

Anxillary Observations: Callus formation at the cut ends of the nodal explants of *Hibiscus rosa-sinensis* was observed. It is known that callus development is a natural plant response to injury in which cells around the areas of damage divide rapidly so that a layer of cells forms over the wound. Callus growth and development involve a complex relationship between the explant used to initiate the callus, the constituents of the medium and the environmental conditions during the culturing period [23,30]. Srivatanakul *et al.* [27] observed that high concentrations of BAP resulted in callus formation from the shoot apical meristems in *Hibiscus cannabinus* (kenaf). Herve *et al.* [9] reported that shoot generation was associated with variable callus formation at the cut end of the nodal segment of *Eucalyptus gunnii*, especially when the segments were grown in a horizontal position. It was also noted that a vertical orientation of the nodal segments reduced callus formation, which tended to be limited to the cut end of the explant positioned in the medium. Tiwari *et al.* [31] indicated that in *Bacopa monniera* higher concentrations of cytokinins supported profuse callusing. In the present study of *Hibiscus rosa-sinensis*, callus was formed on the cut ends of the single nodal explant grown vertically. The callus formed on explants was greenish-white, non-nodular and compact. This was seen throughout all concentrations of BAP and MS media strengths. Callus on cut edges can be excised and subcultured with addition phytohormone treatment to produce an entire plantlet.

MS media browning was observed in all treatments. Media browning is believed to be caused by the exudation of phenolic substances by injured tissue of the cut ends of explants and although the colour of the oxidation of phenolics distracts from the beauty of tissue culture, its greater danger lies in the toxicity associated with some of these substances [26]. Figueiredo *et al.* [8] reported that the presence of phenolic compounds and high polyphenol oxidase activity cause explant browning in *Rollinia mucosa* (Jacq.) Baill., which affects vegetative propagation and limits morphogenetic responses. In this study phenolic browning of medium occurred after approximately three weeks in culture on all BAP concentrations and MS medium strengths. Therefore, the sub-culture period or intervals for *Hibiscus rosa-sinensis* would be about two and a half weeks in culture.

Root formation was observed on MS medium full and half strengths without the presence of plant growth regulator after three months in incubation period. This is an ideal situation since rooting by phytohormonal induction is not necessary and the plantlet with fully formed leaves and roots can develop to the last stage of its *in vitro* micropropagation, acclimatizing the plantlet to the natural environment by optimizing conditions for survival in a glasshouse.

Conclusion: In conclusion, increasing the concentration of BAP on all MS medium strengths resulted in a decline in shoot viability for micropropagation of *Hibiscus rosa-sinensis* nodal explants. These effects of this was more pronounced on MS half and quarter strengths, with MS quarter strength being the most affected. MS full strength was able to attenuate the effects of increasing BAP supplementation, resulting in low tissue necrosis percentages when compared to other medium strengths. It was observed that of the media without BAP, MS half strength yielded the most healthy and vibrant shoots followed by MS full strength , albeit with a higher degree of leaf senescence. MS quarter strength was not viable for shoot induction, even with no BAP supplementation. The effects on shoots, leaves and leaf primodia of BAP were seen following addition of 5μM BAP. This would suggest that the concentration sensitivity narrow-range of BAP in *Hibiscus rosa-sinensis* is between or at 0μM to 5μM BAP range.

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