Minimal Inhibitory Concentrations of Kanamycin on
Melastoma malabathricum and Tibouchina semidecandra

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Abstract

Minimal inhibitory concentrations of kanamycin on shoots and nodes of both Melastoma malabathricum and Tibouchina semidecandra were assessed. Results were based on the percentage of surviving and regeneration of explants after four weeks of kanamycin treatments. The percentages of surviving and regeneration dropped as the kanamycin concentration increased until the optimal concentrations were reached to inhibit the regeneration or killed the tissues. Results showed that kanamycin at 500 and 400 mg/L were sufficient to inhibit the regeneration of Melastoma malabathricum shoots and nodes respectively, whereas 400 and 300 mg/L were inhibitory to the shoots and nodes of Tibouchina semidecandra, respectively.

Keywords: Melastoma malabathricum, Tibouchina semidecandra, kanamycin, inhibitory, surviving, regeneration.

Introduction

Selection of transformed cells is a key factor in developing successful methods for genetic transformation. There are various selective agents that are presently used in plant transformation experiments [7] such as antibiotics, herbicides and toxic levels of amino acids or amino acid analogues. Choosing the proper selectable marker gene is critical for the successful recovery of stably transformed tissues. The most effective selectable markers should allow the transformed cells to proliferate in the presence of selective agent while non-transformed cells will be killed [6]. Thus the optimal selection pressure will use the lowest level of selective agent needed to kill the non-transformed tissues. The most widely used antibiotic selectable marker gene has been the nptII coding for neomycin phosphotransferase, a type II (NPT-II) enzyme originally isolated from bacterial transposon Tn5 [5]. This enzyme detoxifies aminoglycoside compounds such as kanamycin, gentamicin (G418) and neomycin by phosphorylating the specific hydroxyl group of the antibiotics [4]. This nptII gene was first established in 1983 as a dominant selectable marker for higher plants [10]. Its usefulness also has been demonstrated with other plant species such as tobacco, barley and European chestnut [8, 9, 12]. Besides the selection of tissue cultures, in solium selection was also reported where the kanamycin was applied as a spray in selecting for kanamycin-resistant Arabidopsis transformants grown in soil [10]. The aim of this study is to determine the kanamycin sensitivity of Melastoma malabathricum and Tibouchina semidecandra at the levels of regenerating shoots and nodes. With the application of these minimal inhibitory concentrations on selection media, only the putative transformants with kanamycin resistant gene will be survived while the non-transformed tissues will be inhibited.

Materials and Methods

Kanamycin was purchased from Sigma Chemical Company. It was filter sterilized and added to sufficiently cooled (below 50°C) autoclaved media prior pouring onto Petri dishes. Two-leaf shoots and first-three nodes counting from shoot tips of 5-6 weeks old Melastoma malabathricum and Tibouchina semidecandra plantlets were subjected to various concentrations of kanamycin (0, 100, 200, 300, 400, 500, 600, 700 and 800 mg/L) on half-strength MS basal medium [3] supplemented with 6 µM 6-Benzaminopurine (BAP). Three replications with 20 explants per replicate were done for each kanamycin concentrations. The plates were cultured under 16 hours light / 8 hours dark photoperiod at 25 ± 2°C. Observation of colour changes, growth and regeneration of explants were done once a week for four weeks. Data were analyzed using one-way ANOVA and the differences contrasted using Tukey’s multiple comparison test. The statistical analyses were performed using SPSS 11.0 (SPSS Inc. USA) at 5% level.

Survived explants were determined based on the colour of explants that remained green. Early signs of explant death were observed when the colour turned yellow or brown. The calculation is shown as below:

\[
\text{% Survival} = \frac{\text{number of survived explants}}{\text{total explants cultured}} \times 100\%
\]

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The numbers of regenerated explants were calculated based on the number of explants that were capable of regeneration. The calculation is shown as below:

\[ \% \text{ Regeneration} = \frac{\text{number of regenerated explants} \times 100\%}{\text{total explants cultured}} \]

Results and Discussion

The sensitivity of different plant tissues and organs of different species to specific selective agent concentration may vary considerably. Previous researches used 100 mg/L of kanamycin to select callus transformants of actinorhizal tree (*Allocasuarina verticillata*) [2], 150 mg/L to select shoots of European chestnut (*Castanea sativa* Mill.) [8], 200 mg/L to select protocorm-like bodies (PLBs) of *Cymbidium* orchid [11], and up to 500 mg/L to select seeds of wheat cv. UP 2338 [1]. In this study, minimal inhibitory kanamycin concentrations for *Melastoma malabathricum* and *Tibouchina semidecandra* were determined at the levels of regenerating shoots and nodes. Results obtained were based on the percentage of surviving and regeneration of explants after four weeks of kanamycin treatments.

**Kanamycin Sensitivity Test on Melastoma malabathricum**

Figure 1 shows the kanamycin sensitivity level of *Melastoma malabathricum* shoot and node explants. The minimal inhibitory kanamycin concentration for both shoots and nodes was 800 mg/L, where the explants were totally killed. Kanamycin lower than 200 mg/L was not exhibited any toxic effect to *Melastoma malabathricum* shoots but inhibited 13.33% of the node explants. This suggested that *Tibouchina semidecandra* shoots were more susceptible than nodes to kanamycin treatments. Kanamycin at 500 mg/L (showed 60% shoots survival) is the suggested choice concentration to be used for selecting putative *Melastoma malabathricum* transformants using shoot explants as the target tissue, while 400 mg/L (showed 71.67% nodes survival) is to be used for nodes selection because at these concentrations, explants regeneration were significantly (p≤0.05) inhibited (Figure 3 & 4).

**Kanamycin Sensitivity Test on Tibouchina semidecandra**

Figure 2 shows the kanamycin sensitivity level of *Tibouchina semidecandra* shoot and node explants. Kanamycin concentration at 100 mg/L was not exhibited any toxic effect to *Tibouchina semidecandra* shoots and nodes. Kanamycin at 500 mg/L (showed 60% shoots survival) is the suggested choice concentration to be used for selecting putative *Melastoma malabathricum* transformants using shoot explants as the target tissue, while 400 mg/L (showed 71.67% nodes survival) is to be used for nodes selection because at these concentrations, explants regeneration were significantly (p≤0.05) inhibited (Figure 3 & 4).

Figure 1: Percentage of surviving and regeneration of *Melastoma malabathricum* explants after four weeks of kanamycin treatments. (1) Shoots; (2) Nodes. Error bars correspond to standard deviation (n=3). Different letters indicate values are significantly different (p≤0.05).

Figure 2: Percentage of surviving and regeneration of *Tibouchina semidecandra* explants after four weeks of kanamycin treatments. (1) Shoots; (2) Nodes. Error bars correspond to standard deviation (n=3). Different letters indicate values are significantly different (p≤0.05).
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Figure 3: Effect of different concentrations of kanamycin treatment on *Melastoma malabathricum* shoot explants. (a) Control at 0 mg/L; (b) 100 mg/L; (c) 200 mg/L; (d) 300 mg/L; (e) 400 mg/L; (f) 500 mg/L; (g) 600 mg/L; (h) 700 mg/L; (i) 800 mg/L.

Figure 4: Effect of different concentrations of kanamycin treatment on *Melastoma malabathricum* node explants. (a) Control at 0 mg/L; (b) 100 mg/L; (c) 200 mg/L; (d) 300 mg/L; (e) 400 mg/L; (f) 500 mg/L; (g) 600 mg/L; (h) 700 mg/L; (i) 800 mg/L.
Figure 5: Effect of different concentrations of kanamycin treatment on *Tibouchina semidecandra* shoot explants. (a) Control at 0 mg/L; (b) 100 mg/L; (c) 200 mg/L; (d) 300 mg/L; (e) 400 mg/L; (f) 500 mg/L; (g) 600 mg/L; (h) 700 mg/L; (i) 800 mg/L.

Figure 6: Effect of different concentrations of kanamycin treatment on *Tibouchina semidecandra* node explants. (a) Control at 0 mg/L; (b) 100 mg/L; (c) 200 mg/L; (d) 300 mg/L; (e) 400 mg/L; (f) 500 mg/L; (g) 600 mg/L; (h) 700 mg/L; (i) 800 mg/L.
percentages of survival dropped as the kanamycin concentration increased. *Tibouchina semidecandra* shoots and nodes were totally killed at 800 and 600 mg/L kanamycin, respectively. However, kanamycin at concentration 400 mg/L (showed 53.33% shoots survival) was suggested to be used in the selection medium for *Tibouchina semidecandra* shoots transformation, while 300 mg/L kanamycin (showed 31.67% nodes survival) is suggested for nodes selection because at these concentrations, explants regeneration were significantly (*p*≤0.05) inhibited (Figure 5 & 6).

**Conclusion**

Minimal inhibitory concentrations of kanamycin on *Melastoma malabathricum* and *Tibouchina semidecandra* were determined based on the regeneration of the explants. Kanamycin concentrations at 500 and 400 mg/L were suggested to be used in the selection media for *Melastoma malabathricum* shoot and node explants transformation, whereas 400 and 300 mg/L of kanamycin were suggested to select the shoots and nodes of *Tibouchina semidecandra*, respectively.

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**References**