



Micropropagation and the development of an *in vitro* method for long term storage of *Justicia cooleyi*

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I. Introduction

Justicia cooleyi, a rare plant native to Florida from the Acanthaceae family is a rhizomatous perennial herb, also known as water willow. It grows about 40 centimeters tall, has upright square-edged stems with oppositely arranged thin leaves and is a member of the flora in hardwood forests with sandy substrates over limestones. Like for many other endangered species, the main threat to *J. cooleyi* is habitat destruction due to residential and agricultural development and limestone mining. Other factors that affect their habitat is the presence of "invasive" plant species which grow over them, preventing their growth. One important strategy for the conservation of *J. cooleyi* and other endangered species is the development of tissue culture methods that allow the *ex-situ* long-term storage of existing genotypes.

This study was conducted with the aim of developing tissue culture methods for *J. cooleyi*.

II. Materials and Methods

Twenty *Justicia. cooleyi* seeds collected at Bok Tower Gardens in 2007 were surfaced decontaminated with a 15% sodium hypochlorite solution for 15 min and incubated in test tubes containing Murashige and Skoog (MS) media at 25°C in the dark. After germination, test tubes were transferred to light conditions of 16h/8h photoperiod at 25°C.

Two-month old seedlings were removed from test tubes and cut into explants consisting of stem segments containing two axillary buds. Two explants/tube were incubated in MS media supplemented with 2 plant growth regulators (PGRs): 6-benzylaminopurine (BAP) and thidiazuron (TDZ). Treatments consisted on MS media with 0.5 mg/L (2.22 µM) BAP, 0.1 mg/L (4.44 µM) BAP, 0.5 mg/L (4.27 µM) TDZ or 1.0 mg/L (8.54 µM) TDZ. A control group on MS media without PGRs was included.

With the aim of developing a media for long-term storage of *in vitro* cultures of *Justicia cooleyi*, shoots induced on 0.5 mg/L BAP were transferred to MS media containing 0, 1 or 2 mg/L IAA for rooting.

With the purpose of reducing the amount of callus formation in slow-growing shoots by reducing the amount of BAP in the media, explants obtained from plantlets grown on MS media without PGRs were incubated on MS media supplemented with 0, 0.1, 0.2, 0.3 or 0.4 mg/L BAP.

III. Results

1. Micropropagation

Effect of Plant Regulators BAP and TDZ on shoot Length

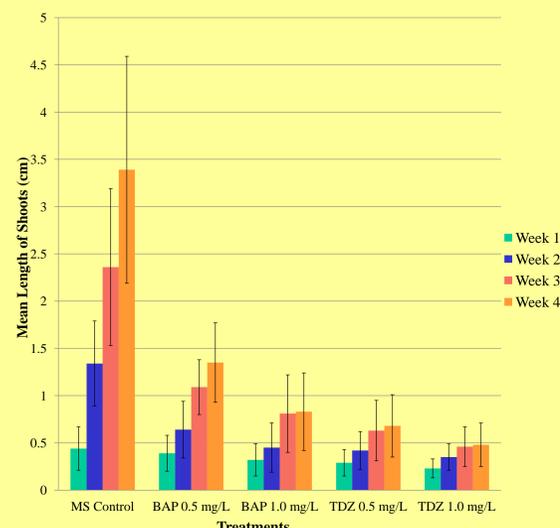


Figure 1. Mean length of axillary shoots/tube. All plant growth regulator treatments produced shorter shoots than those obtained in the control treatment. The 0.5 mg/L BAP treatment was more effective in reducing shoot length, while maintaining minimal callus formation.



Figure 2. Plantlets growing from axillary buds in MS media without PGRs (control)

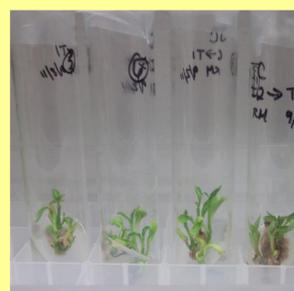


Figure 3. MS media with 0.5 mg/L BAP treatment produced short shoots with minimal callus formation

2. Developing a long-term storage protocol

Effect of Plant Growth Regulator IAA on Rooting of Short Shoots Induced with 0.5 mg/L BAP

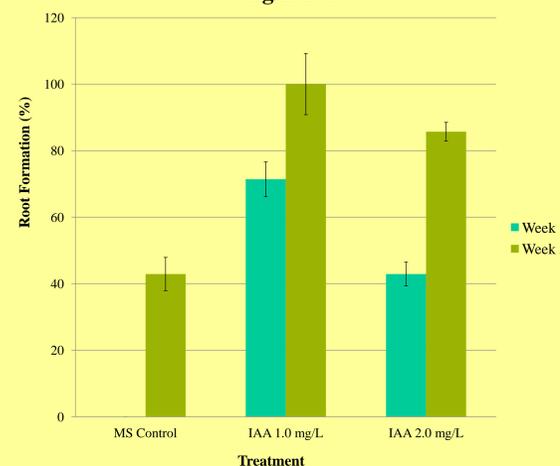


Figure 7. IAA treatment at 1 mg/L produced the highest number of rooted shoots.



Figure 8. Induction of a well-developed rooting system with 1.0 mg/L IAA on shoots grown in MS media with 0.5 mg/L BAP

IV. Conclusions:

1. Slow-growing shoots with roots and minimal callus formation can be induced in *Justicia cooleyi* in MS media supplemented with 0.1 mg/L BAP and 1.0 mg/L IAA. This is the first step towards the development of a long-term storage method for this endangered species.
2. *J. cooleyi* can be micropropagated from axillary shoots in MS media without growth regulators.
3. BAP and TDZ significantly decreased shoot length but did not affect the number of axillary shoots per explant.

Effect of Plant Growth Regulators BAP and TDZ on Number of Shoots

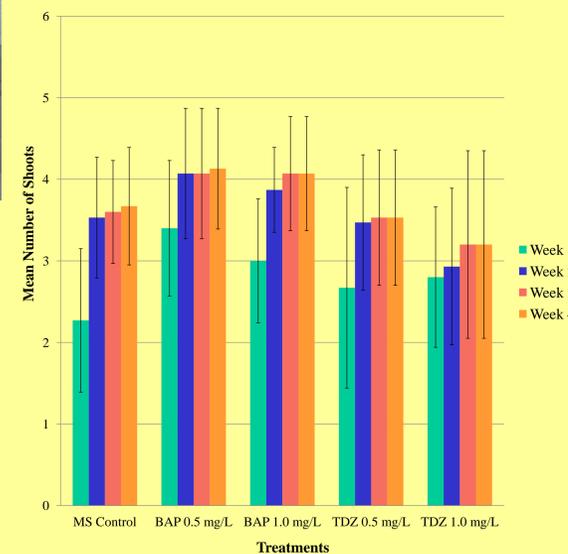


Figure 4. Mean number of axillary shoots/explant. Neither BAP nor TDZ caused a significant increase in the number of shoots produced by axillary buds. However, plant architecture and the development of callus at the base of the explants were different.



Figure 5. MS media with 1.0 mg/L BAP treatment produces short shoots, but some callus formation



Figure 6. MS media with 1.0 mg/L TDZ treatment produced shorter shoots, but induced excessive callus formation.

Effect of BAP on Shoots

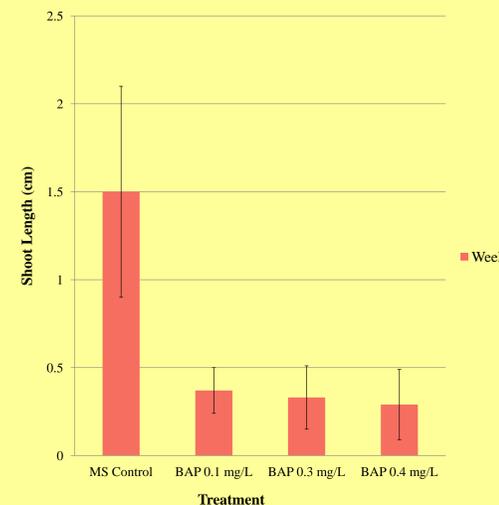


Figure 9. BAP 0.1 mg/L treatment produced minimal callus while still significantly reducing the length of shoots compared to the control after 3 weeks of incubation



Figure 10: Plantlets in MS media without PGRs



Figure 11. Plantlets in MS media with 0.1 mg/L BAP

V. Future Steps

1. In vitro long-term storage requires cultures to withstand long months or years in a single test tube with good survival rates. Further experiments and data collection for *Justicia cooleyi* under in-vitro long-term storage conditions are needed.

VI. Acknowledgements

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VII. References

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